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The Associations Between Motor Corticospinal Excitability and Neuromechanics of the Paretic Soleus and Tibialis Anterior in People Post-Stroke

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THE ASSOCIATIONS BETWEEN MOTOR CORTICOSPINAL EXCITABILITY AND NEUROMECHANICS OF THE PARETIC SOLEUS AND TIBIALIS ANTERIOR IN PEOPLE POST-STROKE

BY

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A dissertation submitted to the faculty of the Medical University of South Carolina
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in the College of Health Professions

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DEDICATION

To
Costa and Dora

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This project would not have been completed without the valuable assistance and support of numerous people.

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The corticospinal drive to the paretic soleus (SOL) and tibialis anterior (TA) is degraded, but whether it changes and is task-dependent remains unclear. We examined the relationships between corticospinal drive and muscle-specific neuromechanics. We collected eight measures of corticospinal drive to SOL and TA in healthy and stroke participants, and muscle-specific neuromechanics during walking and isolated task in stroke participants. We examined the reliability, and the inter-group differences in variance and mean for each corticospinal measure, and the correlations between corticospinal drive and neuromechanics of each muscle in both tasks. Only certain corticospinal measures were simultaneously reliable and had inter-group differences in variance and mean. SOL resting latency was not associated with any neuromechanical measure in either task, whereas TA resting and active latencies were associated with only the ankle angular velocity during walking. In conclusion, TA latencies may strongly indicate an impaired mechanical pattern in the ankle during walking.

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ABBREVIATIONS

6MWT	6 minute walking test
AH	abductor hallucis
aMT	active motor threshold
AAV	ankle angular velocity
CHP-C	College of Health Professions C building
CI	confidence interval
CM	corticomotoneuronal
CNS	central nervous system
COM	center of mass
CV	coefficient of variation
CV_{ME}	coefficient of variation of method error
cSP	contralateral silent period
CST	corticospinal tract
DF	dorsiflexors
EMG	electromyography
FM-LE	Fugl-Meyer lower extremity
FNL	Functional Neurostimulation Laboratory
GAS	gastrocnemius
GC	gait cycle
GRF	ground reaction force
GRF_{AP}	anterior-posterior ground reaction force
GRF_{ML}	medio-lateral ground reaction force

GRF_V	vertical ground reaction force
Hz	Hertz
ICC	intraclass correlation coefficient
ISI	inter-stimulus interval
iSP	ipsilateral silent period
LEA	Locomotion Energetics and Assessment Laboratory
LED	light-emitting diode
LG	lateral gastrocnemius
LRL	Locomotor Rehabilitation Laboratory
MC	motor cortex
MCE	motor corticospinal excitability
MEP	motor evoked potential
MG	medial gastrocnemius
µH	microhenry
MNI	Montreal Neurological Institute
MPL	Motor Performance Laboratory
MSO	maximal stimulator output
MT	motor threshold
MUSC	Medical University of South Carolina
MVIC	maximum voluntary isometric contraction
nTMS	neuronavigated transcranial magnetic stimulation
PCSA	physiological cross sectional area
PF	plantarflexors

PI	propulsive impulse
ppTMS	paired pulse transcranial magnetic stimulation
RC	recruitment curve
r	Pearson product-moment correlation coefficient
r_s	Spearman rank correlation coefficient
rMT	resting motor threshold
ROM	range of motion
SCI	spinal cord injury
SD	standard deviation
sEMG	surface electromyography
SICF	short interval intracortical facilitation
SICI	short interval intracortical inhibition
SOL	soleus
SP	silent period
SSWS	self-selected walking speed
T	Tesla
TA	tibialis anterior
TES	transcranial electric stimulation
TIA	transient ischemic attack
TMS	transcranial magnetic stimulation
TUG	Timed Up and Go

CHAPTER ONE

INTRODUCTION

1.1 Background and Need

The motoneurons of plantarflexors (PF) and dorsiflexors (DF) have connections with their respective areas in the primary motor cortex (MC). Early work using transcranial magnetic stimulation (TMS) suggested that corticomotoneuronal (CM) connections (i.e., fast corticospinal projections that are monosynapticaly connected with spinal motoneurons) to tibialis anterior (TA), which is the primary DF, are numerous and strong whereas the CM connections to PF, gastrocnemius (GAS) and soleus (SOL), are few and weak (Brouwer & Ashby, 1992; Brouwer & Qiao, 1995). Contradicting the assumption that PF had fewer CM connections than DF, subsequent evidence showed that PF, in particular SOL, had as many CM connections as TA, yet the strength of the input-output relations was found to be weaker in SOL than in TA (Bawa, Chalmers, Stewart, & Eisen, 2002). The weaker CM connections to the PF (SOL) led to the suggestion that other descending pathways than corticospinal might contribute to the control of these muscles (J. Nielsen & Petersen, 1995). This discrepancy in the strength of CM connections might be due to the different functional role that each muscle group plays. The PF, as high force generating muscles (e.g., active during the stabilization and push off periods during the stance phase of walking), may have strong reliance on

subcortical pathways (i.e., corticoreticulospinal, corticorubrospinal) (J. Nielsen & Petersen, 1995). In contrast, the DF, as fine motor skill muscles (e.g., foot clearance during the swing phase of walking), may rely more on cortical control.

Regardless of the neurophysiological and functional differences between PF and DF, the motor corticospinal excitability (MCE) of both muscle groups can be quantified by using TMS (Ackermann, Scholz, Koehler, & Dichgans, 1991). Typically in those without neurologic impairment, the resting motor threshold (rMT) is higher for PF than DF, the motor evoked potential (MEP) amplitude is smaller in PF than in DF, and the MEP latency is similar between PF and DF (Rossini et al., 1999). Furthermore, the MCE of PF and DF may be task dependent. The PF (SOL) stimulus response curve was less during the stance phase of walking than in maximum voluntary isometric contraction (MVIC), whereas the DF MCE was high and similar in the swing phase of walking and MVIC in early studies with healthy controls (Capaday, Lavoie, Barbeau, Schneider, & Bonnard, 1999). Therefore, MC via the CM connections may strongly contribute to the control of DF during the swing phase but not during the stance phase for PF control. During MVIC, however, control of both PF and DF may rely heavily on motor cortical input.

Though some evidence of the motor cortical control of PF and DF in healthy adults does exist, only a few studies have examined the PF and DF MCE after stroke. In order to detect real changes in MCE after stroke, the measure used to quantify MCE should meet certain psychometric prerequisites such as reliability and validity. These studies, one for each muscle group, reported good reliability for certain PF (SOL) and DF MCE measures (Cacchio et al., 2011; Lewis, Signal, & Taylor, 2014). In addition to the

reliability studies, few studies tested the changes in PF and DF MCE after stroke using several MCE measures. The focus in those studies was on the paretic side in which the greatest changes in PF and DF MCE occur. Compared to healthy controls, the active MT (aMT) of PF (SOL) increases (i.e., greater stimulus intensity is required to elicit MEP) (Lewis et al., 2014) while the MEP latency and amplitude of DF increase and decrease, respectively (Beaulieu, Masse-Alarie, Brouwer, & Schneider, 2014). Nevertheless, a few gaps still exist in the current knowledge regarding the measurement of PF and DF MCE after stroke. No study has investigated systematically the reliability of the most commonly used PF and DF MCE measures in either healthy controls or stroke participants. In addition, little is known about which PF and DF MCE measures detect the differences in central nervous system (CNS) function between individuals with stroke and healthy controls.

After stroke, the PF and DF function during motor tasks is degraded like their motor cortical control is degraded. However, it is not well understood whether the control of each muscle group is task-specific and whether these alterations are similar between PF and DF. An approach to elucidate these gaps is to investigate the associations between the PF or DF MCE and specific measures of PF or DF neuromechanical function (i.e., muscle activity, joint kinetics and kinematics) during a functional task (e.g., walking) and voluntary motor task (e.g., MVIC). In people with chronic stroke, a positive relationship was reported between the amplitude of DF MEP and DF range of motion (ROM) and strength during MVIC (Beaulieu et al., 2014). In addition, strong relationships were found between the amplitude and latency of DF MEP and forefoot elevation (kinematic measure that quantifies indirectly the function of TA during swing) during walking in

people with spinal cord injury (SCI) (Barthelemy et al., 2013; Barthelemy, Willerslev-Olsen, Lundell, Biering-Sørensen, & Nielsen, 2015; Barthelemy et al., 2010). Such evidence strengthens the notion that investigating the associations between MCE and neuromechanics of PF and DF may contribute to a better understanding of the motor cortical control of PF and DF during different tasks in people post-stroke.

1.2 Problem Statement

Among the measures used in these associations, the PF and DF MCE measures are the least well understood, especially in people post-stroke. Therefore, it is crucial to use an MCE measure that has low methodological error (i.e. reliable) and detects the differences in both central tendency (i.e., mean) and dispersion measures (i.e., variance) between an intact and lesioned CNS (i.e., stroke). Though a few studies reported good reliability of certain PF and DF MCE measures in people post-stroke (Cacchio et al., 2011; Lewis et al., 2014), there is no clear understanding as to which measure clearly elucidates changes in PF and DF MCE that result from damage to the nervous system. This understanding is essential prior to conducting any sort of correlational analysis to determine the relationships between the MCE and the neuromechanics of PF and DF.

Investigating the associations between MCE and neuromechanics of PF and DF during functional and isolated tasks in people post-stroke may reveal the underlying motor cortical control, which, as implied in the early control studies, may not be the same for each muscle group. This evidence may provide vital information on how PF and DF are controlled after a brain lesion and influence rehabilitation practices to help people post-stroke optimally gain functional recovery. Unfortunately, such potential associations

have not been well investigated in stroke. Limited evidence exists during walking for PF and DF; the only existing evidence is for DF during MVIC (Beaulieu et al., 2014).

1.3 Specific Aims and Hypotheses

Specific aims for this dissertation were as follows:

Aims 1 and 2:

The overall goal of Aims 1 and 2 was to quantify eight MCE measures of SOL and TA in people post-stroke and in healthy controls. For thorough explanation on the justification for Aims 1 and 2 and their hypotheses, see section 3.1.

Specific Aim 1: To determine which of the eight SOL MCE measures was reliable both in healthy controls and in people post-stroke yet differed in both variance and mean between groups.

When SOL was at resting state:

Hypothesis 1.1.a: SOL rMT, latency, and normalized latency of SOL would be the most reliable in both groups

Hypothesis 1.1.b: SOL rMT would be significantly higher and more variable in people post-stroke than in healthy controls.

Hypothesis 1.1.c: SOL latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.1.d: SOL normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

When SOL was at active state:

Hypothesis 1.2.a: SOL latency, normalized latency, and contralateral silent period

(cSP) would be the most reliable in both groups.

Hypothesis 1.2.b: SOL latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.2.c: SOL normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.2.d: SOL cSP would be significantly longer and more variable in people post-stroke than in healthy controls.

Specific Aim 2: To determine which of the eight TA MCE measures were reliable both in healthy controls and in people post-stroke yet differed in both variance and mean between groups.

When TA was at resting state:

Hypothesis 2.1.a: TA rMT, latency, and normalized latency of SOL would be the most reliable in both groups.

Hypothesis 2.1.b: TA rMT would be significantly higher and more variable in people post-stroke than in healthy controls.

Hypothesis 2.1.c: TA latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 2.1.d: TA normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

When TA was at active state:

Hypothesis 2.2.a: TA latency, normalized latency, and cSP would be the most reliable in both groups.

Hypothesis 2.2.b: TA latency would be significantly longer and more variable in

people post-stroke than in healthy controls.

Hypothesis 2.2.c: TA normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 2.2.d: TA cSP would be significantly longer and more variable in people post-stroke than in healthy controls.

Aims 3 and 4:

The overall goal of Aims 3 and 4 was to investigate the associations between the descending drive, quantified by MCE measures determined in the two previous aims, and the specific ankle neuromechanics of the paretic SOL and TA during walking and MVIC in people post-stroke. For thorough explanation on the justification for Aims 1 and 2 and their hypotheses, see section 3.1.

Specific Aim 3: To investigate the associations between the MCE measures determined in Aim 1 and the neuromechanics of the paretic SOL during walking and MVIC in people post-stroke.

For the functional task (i.e., walking):

Hypothesis 3.1.a: SOL MCE would not be significantly associated with SOL electromyography (EMG) in stance phase.

Hypothesis 3.1.b: SOL MCE would not be significantly associated with propulsive impulse (PI).

For the isolated voluntary task (i.e., MVIC):

Hypothesis 3.2.a: SOL MCE would be significantly and negatively associated with SOL EMG.

Hypothesis 3.2.b: SOL MCE would be significantly and negatively associated

with SOL isometric torque.

Specific Aim 4: To investigate the associations between the MCE measure(s), as determined in Aim 2, and the neuromechanics of TA during walking and MVIC in people post-stroke.

For the functional task (i.e., walking):

Hypothesis 4.1.a: TA MCE would be significantly and negatively associated with TA EMG in the first half of swing phase.

Hypothesis 4.1.b: TA MCE would be significantly and negatively associated with ankle angular velocity (AAV).

For the isolated voluntary task (i.e., MVIC):

Hypothesis 4.2.a: TA MCE would be significantly and negatively associated with TA EMG.

Hypothesis 4.2.b: TA MCE would be significantly and negatively associated with TA isometric torque.

CHAPTER TWO

REVIEW OF THE LITERATURE

2.1 Introduction

After a stroke, two muscle groups in the lower limb that are impaired significantly are PF and DF. Since PF and DF play a significant role in various motor tasks, especially in upright activities (e.g., walking), their impairments due to stroke detrimentally influence the motor performance in these tasks. Though several rehabilitation strategies exist, none can fully restore either PF or DF function or the performance of the specific task. A possible reason might be the insufficient understanding of the underlying neurophysiological mechanisms of the motor control of these two muscle groups. The motor cortical input, which is one of the neural inputs to alpha motoneurons of the target muscle during a task, can be quantified using TMS in both healthy adults and individuals with neurological injury.

A potential approach is the investigation of the association between the motor cortical control and specific neuromechanics of PF and DF. The motor cortical control of PF and DF can be quantified using measures of MCE, while the task neuromechanics quantify indirectly the contributions of each muscle group during a specific task. To determine whether there are true associations between brain (i.e., MC) and motor behavior, the measures used in these associations must truly identify the construct of interest.

This chapter reviews the main characteristics of PF, in particular SOL, and DF, in particular TA, in terms of motor behavior, the basic principles and main measures of TMS related to SOL and TA, the primary effects of stroke on SOL and TA function, the principal measures that quantify indirectly the SOL and TA neuromechanics during walking and MVIC, and the importance of using MCE-neuromechanics associations for understanding the contributions of motor cortical output to SOL and TA during walking and MVIC. Lastly, certain existing gaps in the current literature are identified.

2.2 Plantarflexors and Dorsiflexors

2.2.1 Anatomical and Physiological Characteristics

The PF and DF are muscles located at the posterior and anterior compartment of the lower leg, respectively (Figure 2.1). The main function of the posterior muscles is to

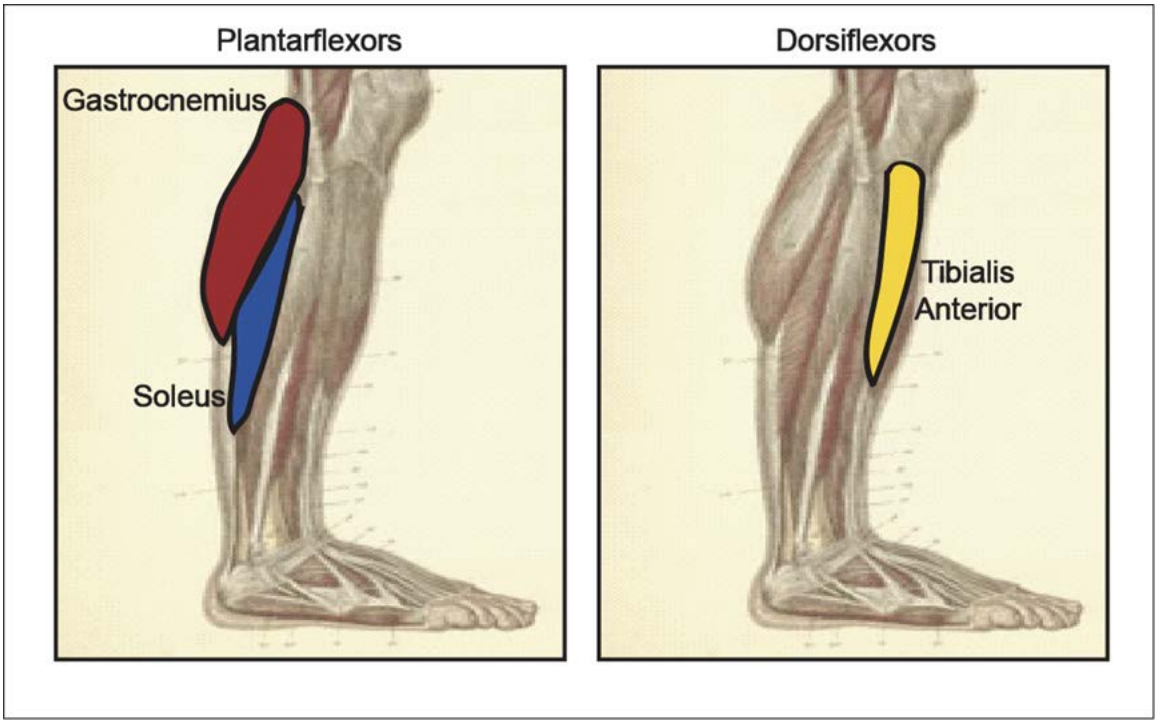

Figure 2.1: Anatomical location of the plantarflexors and dorsiflexors

plantarflex the talocrural joint, whereas the main function of anterior muscle is to dorsiflex the talocrural joint (Schünke, Schulte, Ross, Schumacher, & Lamperti, 2006). The primary PF are GAS that has two heads, medial gastrocnemius (MG) and lateral gastrocnemius (LG), and SOL. The primary muscle of DF is TA.

MG and LG have an origin at the medial and lateral epicondyle of the femur, respectively, while both heads are inserted at the calcaneal tuberosity via the Achilles tendon (Schünke et al., 2006). Due to its origin and insertion characteristics, GAS is a biarticular muscle (i.e., crossing two joints: knee and talocrural at the ankle). On the other hand, SOL is a uniarticular muscle, it has an origin at the posterior surface of the head and neck of the fibula, it is attached to the soleal line of the tibia via the tendinous arch, and its insertion is the same as GAS (Schünke et al., 2006). The architectural properties differ for GAS and SOL. GAS, in particular the MG, is composed mainly of fast and relatively short fibers, whereas the SOL is composed only of slow and short fibers (R. L. Lieber & Friden, 2000). On the other hand, the physiological cross sectional area (PCSA) of the SOL is higher than MG (R. L. Lieber & Friden, 2000). PCSA and fiber length are proportional to maximum muscle force and excursion, respectively. Regardless of the few anatomical and physiological differences within PF, both GAS and SOL are antigravity muscles designed to generate high force with small excursion of the muscle, and they are innervated by the tibial nerve, which is derived from nerve roots of S1 and S2.

Like the SOL, TA is also a uniarticular muscle. TA originates from the two upper thirds of the lateral surface of the tibia, the crural interosseous membrane, and the highest part of the superficial crural fascia. It is inserted at the medial and plantar surfaces of the

medial cuneiform and the medial base of the first metatarsal (Schünke et al., 2006). Compared to MG and SOL, TA weighs less, has larger fibers, is composed primarily by slow twitch fibers, and has smaller PCSA (R.L. Lieber, 2010). A general conclusion is that TA is more functional for long muscle excursions and less important for force production (R. L. Lieber & Friden, 2000). It is innervated by the deep fibular nerve, which is derived from the nerve roots of L4 and L5 (Schünke et al., 2006).

2.2.2 Muscle Activity during Walking

In intact human walking, PF and DF are active at different phases of the gait cycle (GC) (Figure 2.2 A), and thus they have different functional roles. PF are mainly active during the stance phase of gait (Figure 2.2 B), while DF are mainly active during early stance and throughout swing (Figure 2.2 C).

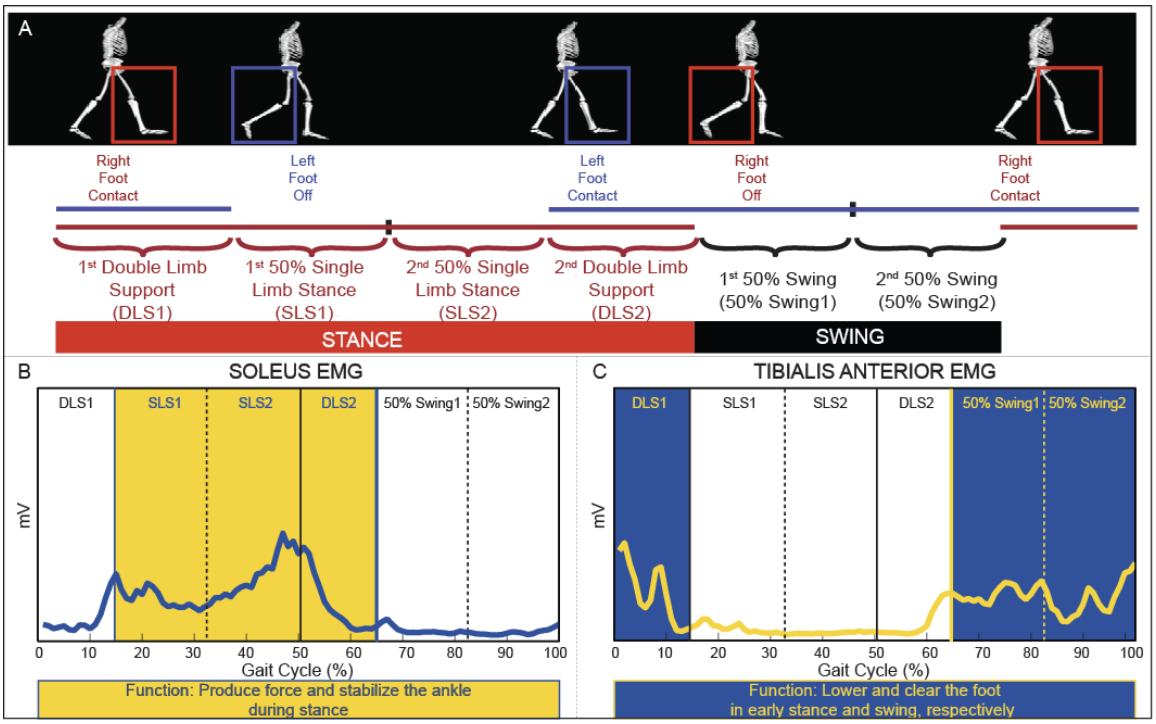

Figure 2.2: Phase descriptions of the gait cycle, and muscle activity of Soleus and Tibialis Anterior during gait cycle. (A) Depiction of a typical right gait cycle, and muscle activity of (B) Soleus and (C) Tibialis Anterior during gait cycle. (A) Modified by Bowden et al. (2010).

Just prior to heel strike, at the onset of the GC, only the GAS is activated (among PF muscles). As the leg rotates forward (5-40% of GC) PF contract mostly in isometric mode (mainly the muscle fascicle) (Cronin, Avela, Finni, & Peltonen, 2013; Panizzolo, Green, Lloyd, Maiorana, & Rubenson, 2013), while during the push-off phase (40-60% of GC), PF shorten to plantarflex the foot and produce a high impulse of energy. After toe off, GAS is slightly active due to its function as knee flexor, whereas SOL is silent. On the other hand, DF has a big burst of muscle activity at early stance to produce forces that isometrically (Chleboun, Busic, Graham, & Stuckey, 2007) control the lowering of the foot to the ground. After the forefoot touches the ground, DF is less active and has a minor role in pulling the leg forward over the foot. After the heel is off the ground, the muscle activity of DF begins to rise again due to DF lengthening, whereas after toe off, DF shorten and still increase muscle activity (Chleboun et al., 2007) to dorsiflex the foot in order to clear it during mid-swing (Winter, 1987).

2.2.3 Corticomotoneuronal Connections

The motoneurons of PF and DF have connections with their respective areas in the MC. Early work using TMS postulated that DF was more cortically controlled than PF for three reasons. First, it was suggested that DF had more CM connections than any other leg muscle (Brouwer & Ashby, 1992). Second, DF responses were less variable than SOL and MG responses (Brouwer & Qiao, 1995). Third, PF responses were weak, and other pathways (e.g., corticorubrospinal, corticoreticulospinal) might contribute to their control (J. Nielsen & Petersen, 1995). Contradicting the assumption that PF had fewer CM connections than DF, subsequent evidence showed that SOL has as many CM connections as TA, yet the strength of the connections is weaker in SOL than in TA

(Bawa et al., 2002). This discrepancy in the strength of CM connections might be due to the different functional role that each muscle group plays. The PF, as high force generating muscles (e.g., active during the stabilization and push off periods during the stance phase of walking), may have strong reliance on subcortical mechanisms, whereas the DF, as fine motor skill task muscles (e.g., foot clearance during the swing phase of walking), may rely more on cortical control. Regardless of these neurophysiological and functional differences between PF and DF, the MCE of SOL and TA, PF and DF muscles that we investigated in this project can be quantified using TMS (Figure 2.3). In this project, MCE is an umbrella term that reflects the excitatory and inhibitory characteristics of the neuromotor axis (motor cortical areas, crossed descending motor pathways, alpha motoneurons, and their corresponding muscles) when a stimulus via TMS is applied over the MC.

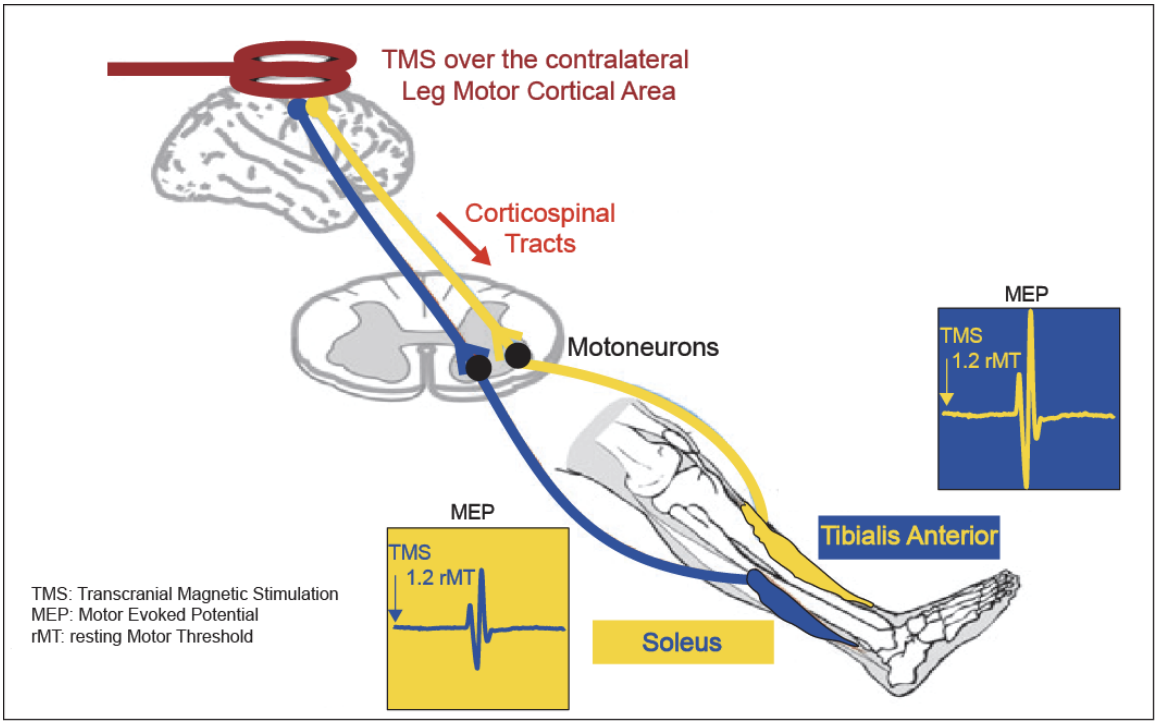

Figure 2.3: Depiction of Soleus and Tibialis Anterior MCE assessment using TMS. Modified by Geertsen et al. (2010)

2.3 Transcranial Magnetic Stimulation

2.3.1 Technical Principles

TMS is a non-invasive brain stimulation technique that can quantify the corticospinal connectivity to muscles, as well as the intracortical and intercortical excitability. Briefly, TMS was the successor of transcranial electric stimulation (TES), which was developed in the early 1980's (Merton & Morton, 1980). A few years later, Antony Barker et al. successfully elicited an MEP in healthy adults by using a magnetic stimulator and a coil that was placed on the person's contralateral MC (Barker, Jalinous, & Freeston, 1985). In contrast to TES, which can be uncomfortable and painful to the participant due to the high intensity required, TMS can elicit an MEP to a target muscle by applying a magnetic stimulation over the motor representation of that muscle. The main fundamental principle of TMS is the use of electromagnetic conduction. Simply, when an electric current passes through a metal (e.g., a stimulation coil) a magnetic field is produced, and subsequently this changing magnetic field elicits a flow of an electric current in the nearby environment (e.g., brain tissue) (Wasserman, Epstein, & Ziemann, 2008). When a stimulating coil is applied over the MC, the pulse generated from that coil stimulates intracortical neurons that propagate a neural impulse from the MC to the target muscle on the contralateral side through the crossed corticospinal tract (CST)

2.3.2 Stimulating PF and DF Motor Cortical Areas

TMS testing of the PF and DF MCE involves a few minor limitations associated with the anatomical properties of the PF and DF motor cortical areas. First, the motor areas of PF and DF are located adjacent to the interhemispheric fissure at approximately 3-4 cm below the scalp surface (Alkadhi et al., 2002; Conti et al., 2014; Terao & Ugawa,

2002) (Figure 2.4), while the axons of the corticospinal neurons are oriented perpendicular to the medial cortical surface. Second, the size of these areas is relatively small, in particular when they are compared with hand and facial muscles (Conti et al., 2014) (Figure 2.4). Third, these areas are not clearly segregated (Saisanen et al., 2010) (Figure 2.5); therefore, accurate stimulation of the target motor cortical areas requires cautious selection of certain stimulation parameters (i.e., coil type, current direction, and optimal stimulation site).

2.3.3 Stimulation Parameters

Use of the correct coil type for stimulating the PF and DF motor cortical areas is very important because different types of coil are capable of inducing different electric fields in the brain. Historically, the circular/round coil was the first to be used for testing PF and DF MCE. This type of coil has a diameter of 80-150 mm and 5-20 turns of wire (Wasserman et al., 2008) and is typically placed usually a few centimeters (e.g., 4 cm) anterior and lateral to vertex, the virtual intersection of the inter-aural and nasion-inion lines (Meyer, Britton, Kloten, Steinmetz, & Benecke, 1991). Subsequently, two types of figure-of-eight coil (i.e., two round coils together) were developed, flat and double cone, which typically are positioned either over the vertex or over 1-2 cm posterior. Terao et al. (1994) compared the effect of three coil types, large figure-of-eight with various angles, small figure-of-eight, and round coil, on the TA MCE. The large figure-of-eight coil with posterior current and round coil with lateral current induced the largest TA MEPs and used the lowest MT. A large figure-of-eight coil was more efficient than the round coil when TA was relaxed, whereas both coils elicited TA MEPs from all subjects when TA

was active (Terao et al., 1994).

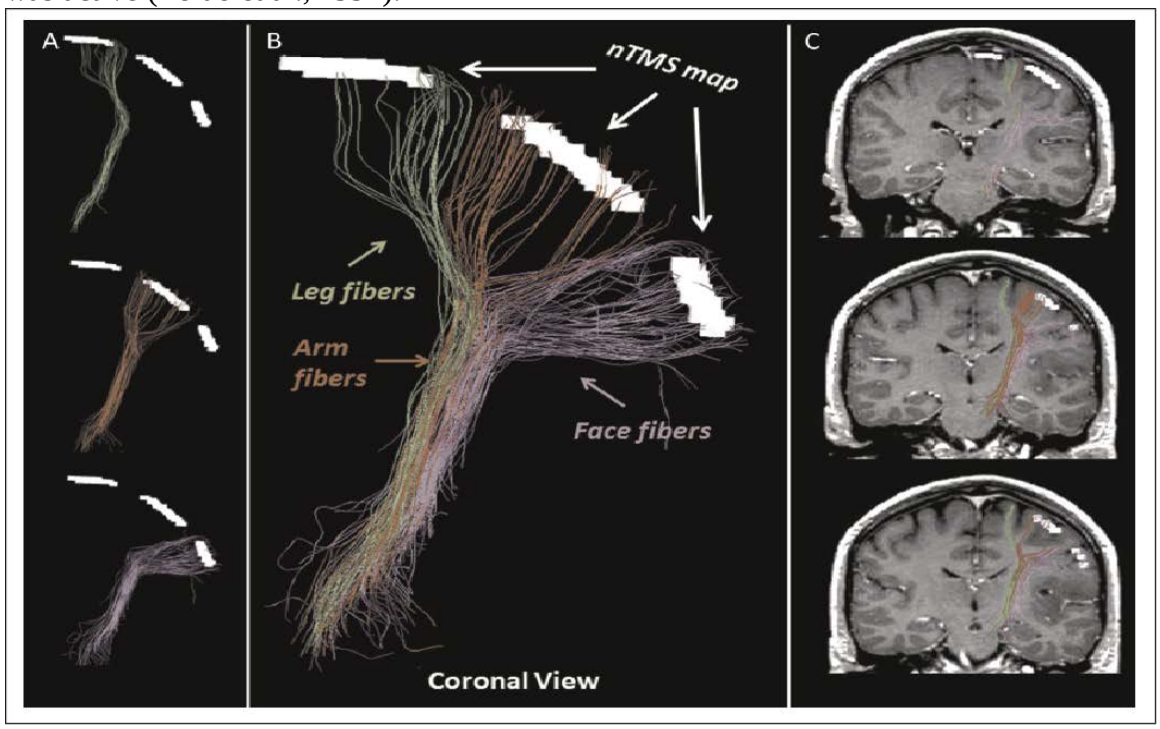

Figure 2.4: Neuronavigated TMS-based DTI tractography of leg, arm, and face muscle CST. Adapted from Conti et al. (2014)

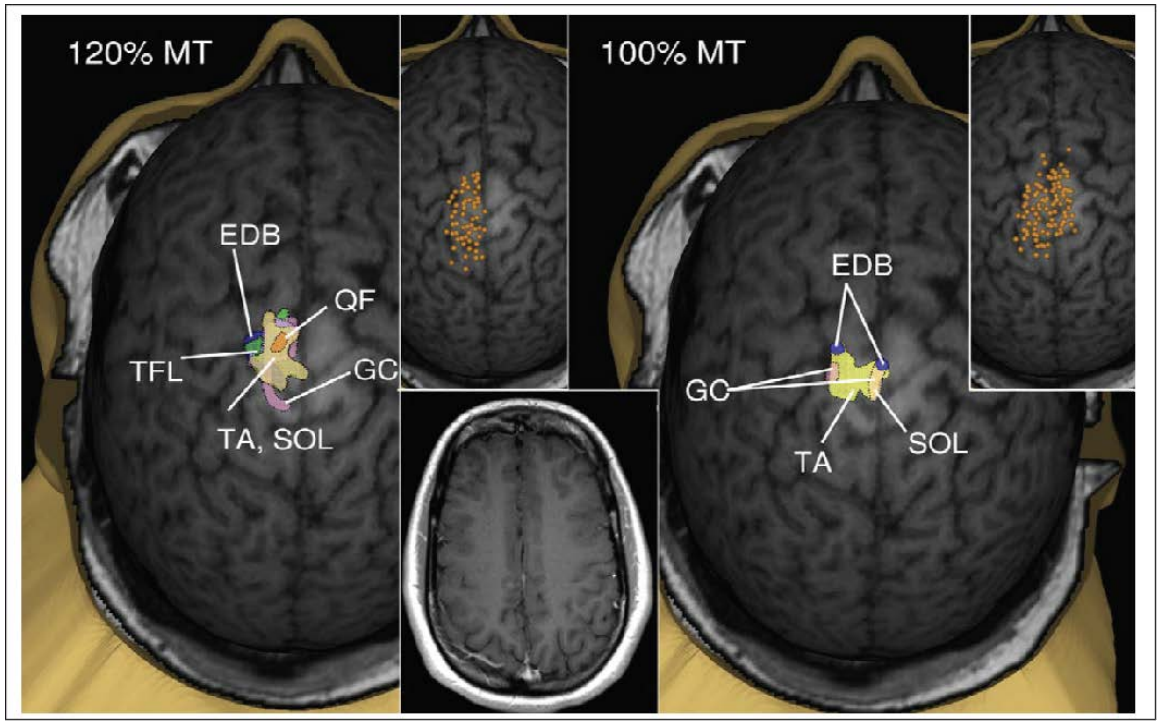

Figure 2.5: Neuronavigated TMS mapping of leg muscles. Mapping of right leg muscles was carried out on a patient with cortical dysplasia. EDB: extensor digitorum brevis (blue); QF: quadriceps femoris

(orange); GC: gastrocnemius (pink); TA: tibialis anterior (yellow); SOL: soleus (light yellow); TFL: tensor fasciae latae (green). Adapted from Saisanen et al. (2010)

Other differences between the round and figure-of-eight coils are the location, the area, and the amplitude of the electric field produced by the magnetic stimulation. In round coils, the maximum induced electric field is below the outer edge of the coil, and its area is diffuse (Cohen et al., 1990). In contrast, the maximum electric field induced by figure-of-eight coils is exactly below the junction of the two round coils, and its area is focal (Cohen et al., 1990; Toleikis, Sloan, & Ronai, 1991). These two characteristics of the figure-of-eight coils increase the user's ability to identify the stimulated site by placing the center of the coil (intersection of the two round coils) over that site. Another advantage of this type of coil is that only the center of the coil is close to the stimulated site because the outer parts of the coil (flat) are farther from the brain tissue (Cohen et al., 1990). Due to these characteristics, figure-of-eight coils are widely preferred and used for testing the MCE of the lower extremities.

Several differences exist between the flat and double cone figure-of-eight coils. The main difference is that the flat coil has no angle between the two round coils, whereas the double cone coil has an angle (e.g., 100°). The outer diameter of the former is usually smaller (e.g., 70 mm) than that the latter (e.g., 120 mm). The flat figure-of-eight coil can be pitched, yawed, and rolled, and the current can be manipulated in numerous directions; the maximal magnetic field strength is usually around 2 Tesla (T). On the other hand, a double cone coil can be pitched and rolled only because its angulation does not allow yaw, the current can be manipulated only in two directions, and the maximal magnetic field strength is usually less than 2 T. Both coils can induce mainly indirect waves (I1-wave: the shortest in latency among the three I-waves that occur

because of the indirect excitation of the CST via intracortical neurons (Maeda & Pascual-Leone, 2003), resulting from transynaptic activation of the neurons in the leg motor areas. Yet only the double cone coil can induce direct waves (D-waves) that result from direct activation of the CST neurons at high stimulus intensity (Terao et al., 2000). Despite these differences, the double cone coil is preferable to stimulate the leg motor area because it fits more securely on the head, and it has deeper stimulation strength than the flat figure-of-eight coil (Deng, Lisanby, & Peterchev, 2013, 2014; Lontis, Voigt, & Struijk, 2006; Terao et al., 1994).

The type of coil used dictates the optimal stimulation site of the PF and DF motor cortical areas that are typically located around the vertex. The optimal stimulation site is the coil location that elicits the largest response at the contralateral target muscle with the lowest intensity. When a round coil is used, the optimal site is usually a few centimeters lateral (contralateral to the target muscle) and anterior to vertex (Meyer et al., 1991). When figure-of-eight coils are used, the optimal site is usually closer to the vertex (e.g., 1-2 cm lateral and posterior to vertex). Because of the close proximity between the PF and DF cortical areas, a common procedure is to determine the site that elicits the largest response in DF and then to use that site to test the MCE of both muscle groups. Although this procedure is often used and might be valid, two alternative approaches may determine more accurately the optimal sites of PF and DF.

One approach to distinguish PF and DF MC is to examine the MCE (e.g., amplitude of MEP) of each muscle group on separate days (Geertsen, Zuur, & Nielsen, 2010; Obata, Sekiguchi, Nakazawa, & Ohtsuki, 2009). The advantage of this approach is that the number of stimulations applied in a single day on two proximal cortical areas is

reduced; the disadvantage is that the participants must visit the lab twice. Furthermore, a common characteristic of the two approaches is that they rely on locating vertex (i.e., virtual intersection of the inter-aural and nasion-inion lines). Although these approaches are relatively simple, they do not consider the differences among individuals in the size, anatomy, and morphology of the brain, or the thickness of the skull (Wasserman et al., 2008).

The second approach to differentiate PF and DF MC is to use the neuronavigation system with TMS (nTMS), which may solve the aforementioned issues. The nTMS is an image-guided TMS; either structural or functional MRI images can be used (Krings et al., 2001; Ruohonen & Karhu, 2010; Sparing, Hesse, & Fink, 2010). The basic function of nTMS is to act as a positioning system to locate different anatomical landmarks in the brain. An optical camera, which acts as a position sensor, detects signals that are transmitted by reflective spheres placed on a stimulation coil, a pointer, and the stimulated head. A registration matrix obtained by identifying homologous anatomical landmarks on both images, which can be either the subject's images or a representative set of images, is utilized to find the location of the coil from the real world to the image space. Then the coil can be displayed on the screen, and the target areas can be accurately stimulated. The main advantage of using nTMS is that it assures constant stimulation parameters within individuals across trials (Krings et al., 2001; Ruohonen & Karhu, 2010; Sparing et al., 2010), and the primary disadvantage is its high cost (~$50,000-250,000). In relation to testing the PF and DF MCE, nTMS might be a useful tool to eliminate the limitations of stimulating these areas. Several studies have used nTMS (Eximia, Nexstim Ltd.; Helsinki, Finland) to test the MCE of PF (Saisanen et al., 2010)

and DF (Forster, Limbart, Seifert, & Senft, 2014; Niskanen et al., 2010; Saisanen, Julkunen, et al., 2008; Saisanen et al., 2010; Thordstein, Saar, Pegenius, & Elam, 2013; Vaalto et al., 2013); those studies have found that nTMS has good reliability (TA only).

2.4 PF and DF MCE

2.4.1 Measures

The motor cortical control of the PF and DF can been quantified by several measures, and each reflects a different physiological property of the MCE. MT reflects the membrane-related neuronal excitability while MEP derived measures (i.e., the electrical potential recorded by a surface EMG (sEMG) placed over the belly of the target muscle in response to TMS stimulation of the MC) reflect the integrity and excitability of the whole CST (George & Belmaker, 2007). Center of gravity and map size, measures of cortical mapping, reflect the cortical representation of the target muscle. Recruitment curve (RC) derived measures likely represent the strength of the corticospinal projections (e.g., steeper curve requires low MT) (Chen, 2000). Another MCE measure is the silent period (SP), which can be measured on either the contralateral (cSP) or the ipsilateral target muscle (iSP). The cSP reflects the long-lasting motor cortical inhibition, which might be mediated via $GABA_b$ receptors, whereas the iSP reflects the functional integrity between the homologous motor areas (George & Belmaker, 2007). Lastly, measures of intracortical activity represent either corticocortical inhibitory mechanisms that are mediated by GABA receptors or corticocortical excitatory mechanisms that are mediated by glutamate receptors (Rossini & Rossi, 2007). For both muscle groups, MT, MEP, and RC derived measures are more commonly reported than others are.

MT can be determined either during muscle relaxation (i.e., rMT) or during slight tonic contraction of the target muscle (aMT). The MT of a muscle is defined as the lowest stimulation intensity required for inducing a distinguishable MEP on the target muscle (Groppa et al., 2012). Compared to the MT of distal hand muscles, the MT of distal leg muscles is greater (Forster et al., 2014; Lotze et al., 2003; Rossini et al., 2015; Saisanen, Julkunen, et al., 2008). In healthy adults, the PF MT (SOL) is usually higher than the DF MT, meaning that the current required to stimulate the PF cortical area is greater than DF (Needle, Palmer, Kesar, Binder-Macleod, & Swanik, 2013; Rossini et al., 1999; Rossini et al., 2015). This difference in MT between PF and DF may be caused by several potential factors. One factor is that the PF CM connections might be weaker than DF CM connections (i.e., similar number of peak responses but lower responses to different stimulus intensities) (Figure 2.6) (Bawa et al., 2002; Brouwer & Ashby, 1992). Other factor might be that the PF cortical areas may be smaller than DF (Figure 2.5) (Saisanen et al., 2010).

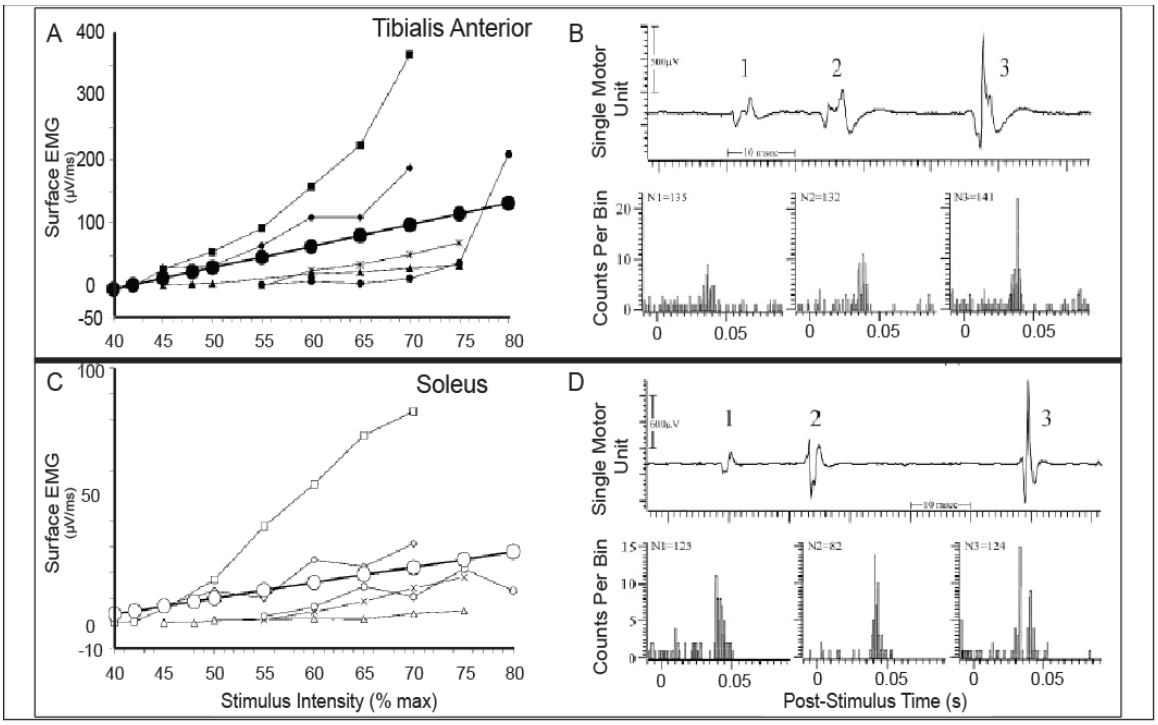

Figure 2.6. Comparisons of mean muscle activity responses of TA and SOL and responses of three concomitantly firing single motor units from TA and SOL. A and C: Thin lines with different symbols represent the EMG responses to different TMS stimulus intensities for each participant. Thick lines with large circles show the group means. The slope of the stimulus-response curve is higher for the TA (A) than for SOL (C). The same can be osberved in the mean stimulus-response curve. B and D: The top figure in each panel illutsrates the shapes of three concurrently recorded single motor units from TA (B) and SOL (D) while at the bottom of each panel the peristimulus time histograms, which show the discharge of each single motor unit of TA (B) and SOL (D), are illustrated. Modified from Bawa et al (2002)

MEP size parameters and latency are the other commonly used MCE measures. The assumption is that the MEP size parameters (amplitude: peak-to-peak; area: the integral of the rectified EMG between MEP onset and offset; duration: the time between MEP onset and offset) may reflect the number of the activated motoneurons (Wasserman et al., 2008). Among the three size parameters, amplitude is reported the most. The latency is defined as the time from the stimulus onset to the MEP onset and reflects the conduction time from MC to the target muscle. Latency may depend on two factors, the location of the muscle and the presence of a lesion in the neuromotor axis (i.e., MC, CST, alpha motoneuron pools, final common pathway, and muscle). Latency increases from proximal (quadriceps and hamstrings) to distal (PF and DF) muscles (Dimitrijevic et al., 1992), while the PF and DF latency are similar since both muscle groups are located at the same body segment (Wochnik-Dyjas, Glazowski, & Niewiadomska, 1998). For this reason, MEP latency is usually normalized by the person's height. After a stroke, the latency of the paretic DF increases, whereas the latency of the non-paretic DF is similar compared to healthy controls (Beaulieu et al., 2014; Cacchio et al., 2011). Conversely, the changes in the PF latency are unclear after stroke. A few studies have also investigated the PF and DF MEP area. A study (Soto, Valls-Sole, Shanahan, & Rothwell,

2006) reported a task (rest vs. voluntary contraction vs. standing) effect on the MEP amplitude, latency, and area for TA and SOL in healthy adults. During rest, the amplitude and area of both muscles were smaller than active tasks (Soto et al., 2006). Compared to TA amplitude and area, SOL amplitude and area were smaller during rest yet larger during voluntary and standing tasks (Soto et al., 2006). This evidence indicates that comparisons between PF and DF MCE, in particular MEP derived measures, should occur within the same task.

Cortical mapping of the area for each target muscle is often used as a measure of MCE since each muscle has a unique representation within the cortex. The motor cortical representation (i.e., cortical mapping) is the number of motor cortical sites that elicit MEPs to a target muscle, and either the optimal stimulation site (i.e., hot spot or center of gravity) or its extent (i.e., map size) can characterize it. Only a few studies have mapped the motor cortical areas of PF (Saisanen et al., 2010) and DF and reported good test-retest reliability (Forster et al., 2014) and low variation when nTMS is used (Niskanen et al., 2010). Furthermore, TMS and functional MRI (fMRI) can complement each other , and be equally reliable for mapping DF (Lotze et al., 2003). Since the stimulation site is vital for reliable TMS application, findings from these studies provide normative information about the exact location of both PF and DF.

RC (input-output or stimulus-response curve) has been commonly used to quantify the change in MEP size as a function of the stimulus intensity during resting (Devanne, Lavoie, & Capaday, 1997) and active tasks, such as voluntary contraction (Sandbrink, Syed, Fujii, Dalakas, & Floeter, 2000), standing (Obata et al., 2009), and walking (Capaday et al., 1999). The stimulus intensity (percentage of maximal stimulator

output; % MSO) ranges from sub- to supra-threshold MT values with increments of 5%, while the defined number of stimulations (e.g., 5) is applied at each intensity. The common shape of the RC is sigmoidal, which is a result of the Boltzman equation (Press, Flannery, Teukolsky, & Vetterling, 1986). The threshold (i.e., the x-intercept of the regression line flitted to the rising section of the RC), slope, and plateau level (MEP_{max}) (Devanne et al., 1997) are derived from RC and characterize the stimulus-response parameters of the CST as a whole. Obata et al. (Obata et al., 2009) investigated the input-output relation of both PF (SOL) and DF (TA) during sitting and standing in 14 healthy adults. Despite similar background EMG, slope and plateau level were significantly greater during standing versus sitting, whereas threshold was similar between conditions for both muscles (Obata et al., 2009). During standing, the plateau value and threshold of SOL and TA were similar, while the TA maximum slope was greater than SOL (Obata et al., 2009). These findings demonstrate that use of RC derived measures also can decouple the motor cortical control between PF and DF during different tasks.

In contrast to the aforementioned MCE measures that characterize primarily excitatory mechanisms, certain measures of MCE also can quantify inhibitory mechanisms. One example is the SP, which is defined as the suppression of the EMG following MEP during tonic voluntary contraction. This phenomenon of EMG interruption can be observed when either the contralateral MC (cSP) or the ipsilateral MC (iSP) is stimulated. Nearly all studies that have investigated the SP of PF and DF have reported cSP. The first report on cSP was by Inghilleri et al. (1993). These authors argued that cortical inhibitory mechanisms might contribute to the latter part of hand muscle cSP. In the same year, Ziemann et al. (1993) investigated the SOL cSP and concluded

that cortical inputs might also contribute to the latter part of the SOL cSP. The TA cSP has been examined as well, mainly in studies that investigated the reliability and variability of TA MCE measures (Cacchio, Cimini, Alosi, Santilli, & Marrelli, 2009; Tallent et al., 2012; van Hedel, Murer, Dietz, & Curt, 2007). TA cSP is a reliable measure with low variability (Cacchio et al., 2009; Tallent et al., 2012). Though these studies provided normative data of TA cSP, it is not clear what the cSP implies in terms of function. In addition, it is not well understood whether there are differences between the cSP mechanisms of PF and DF or whether PF cSP is also a reliable measure with low variability.

It is important to distinguish iSP from the measures of inter-hemispheric inhibition. Though both measures reflect inhibitory effects, it has been suggested that they are mediated via different mechanisms (Chen, Yung, & Li, 2003). No study has investigated PF and DF iSP, yet one study recorded the iSP of a toe flexor muscle, abductor hallucis (AH) (Lo & Fook-Chong, 2004). Use of posterior-anterior and latero-medial current directions induced by a figure-of-eight coil (70 mm diameter; 2.2 T) elicited the most AH iSPs, while age and leg side did not have any effect on iSP (Lo & Fook-Chong, 2004). iSP was recorded from the majority of the participants who all were neurologically healthy adults (age range: 20-80), 100% of the MSO was required (Lo & Fook-Chong, 2004). However, that high level of intensity might not be tolerable by every participant; therefore, the use of this measure may have some limitations. Thus, future studies should investigate whether iSP can be measured in PF and DF. If iSP of either PF or DF can be quantified, this metric might be used to determine the contribution of the ipsilateral MC to the target muscle, a phenomenon that is present in people post-stroke.

Both the inhibitory and excitatory intracortical neuronal circuits that converge on the CST can influence MEP. Using a paired pulse TMS (ppTMS), subthreshold condition stimulus following by suprathreshold test stimulus with an inter-stimulus interval (ISI) less than 6 ms, Kujirai et al. reported a suppression in hand muscle MEP (1993). They suggested that this suppression might have cortical origin. A few years later, Stokic and colleagues utilized a similar approach to characterize the intracortical inhibition in DF motor areas (Stokic, McKay, Scott, Sherwood, & Dimitrijevic, 1997). They tested a wide range of ISIs (1-15 ms). The MEP of the relaxed TA was suppressed when ISI was less than 5 ms, whereas facilitated MEP was observed when the ISI was between 9-10 ms. This study was the first to show that both inhibitory and excitatory intracortical circuits in the leg motor areas can modulate the responses of the leg muscles. Since then, numerous studies have examined both intracortical inhibition, either short interval (SICI) or long interval, and facilitation (SICF) during different tasks (e.g., voluntary contraction, cycling) and postures (e.g., seated, standing) in various populations (healthy young and old adults, SCI, stroke, Parkinson disease) (Beaulieu et al., 2014; Mileva, Bowtell, & Kossev, 2009; Oliveri et al., 2012; Roy, Zewdie, & Gorassini, 2011; Soto et al., 2006; Stokic et al., 1997; Yamaguchi, Fujiwara, Liu, & Liu, 2012; Yang et al., 2013). The cumulative evidence from these studies indicates that using ppTMS over the leg motor area can elucidate the characteristics of the intracortical mechanisms during control of PF and DF in various tasks and populations.

2.4.2 Effect of Posture during Testing

The motor cortical control of PF and DF MCE is posture-dependent. In most studies, PF and DF MCE were tested while the subject was sitting on a chair and the foot

fixed in a footrest with either relaxed or slightly contracted muscle. MEP is facilitated when the target muscle is voluntarily contracted, even slightly. However, only a few studies have examined the effect of posture (seated vs. lying vs. standing) on the SOL and TA MCE, and their results were inconclusive. This inconsistency might be due to several methodological differences among the studies. Ackermann et al. (1991) found that the PF (SOL) and DF (TA) MEP size was larger in standing than in sitting or lying supine. Similarly, Obata et al. (2009) found that maximum slope and plateau value of both SOL and TA RC were significantly larger during standing than in sitting. Despite similar findings, a main difference between the two studies was that the background EMG was not matched among tasks in the former study, whereas it was matched in the latter study. Conversely, two studies (Lavoie, Cody, & Capaday, 1995; Soto et al., 2006) showed no difference in SOL MEP size between standing and sitting. The main reason for this discrepancy was stimulus intensity, which was lower in Lavoie et al. (1995) (125% MT) and Soto et al. (2006) (120% MT) than the intensity used in Obata et al. (2009) (40-90% MSO). Additionally, SOL and TA responses were elicited from the same stimulus site in the former studies (Lavoie et al., 1995; Soto et al., 2006), whereas the optimal site of SOL was determined separately from TA in the study by Obata et al. (2009). Another study examined the effects of voluntary contractions (rest, dorsiflexion, and plantarflexion in lying supine) and postural tasks (standing on the soles, standing on the heels, and standing on the toes) on the SOL and TA MCE while using 100% MSO (Valls-Sole, Alvarez, & Tolosa, 1994). SOL MEPs were elicited 100% of the time during the active conditions but were elicited only 61% of the time during rest. SOL MEP latency was shorter during dorsiflexion and plantarflexion and during standing on the

heels and toes. On the other hand, TA MEP latency was shorter only during voluntary dorsiflexion and standing on the heels. Both SOL and TA MEP amplitudes were larger in all voluntary and standing conditions compared to rest (Valls-Sole et al., 1994). The findings from these studies indicate that posture may have an important effect on both PF and TA MCE; therefore, the task used during MCE testing should be thoughtfully selected. Among the different postures, sitting on a chair with fixed positions of the hip, knee, and ankle joints while the target muscle is slightly contracted might be the most feasible and reliable posture to use for testing PF and DF MCE across populations.

2.4.3 During Walking

Walking is a complex behavior resulting from integration of multiple structures and functions of the musculoskeletal and nervous systems. In humans, walking is controlled by three different subsystems of the nervous system (i.e., sensory receptors in the skin, muscles, and joints; the spinal cord; and the brain). The integration of these systems is responsible for the activation and modulation of motoneuron pools that innervate the active muscles during walking (J. B. Nielsen, 2003). All three subsystems are important during the GC, and the difference among them is their specific function during walking. For instance, the input from the supraspinal areas (e.g., MC, brain stem, cerebellum, visual cortex, etc.) (Figure 2.7) contribute to walking in several ways: triggering walking-related spinal systems, regulating walking speed, fine-tuning motor patterns in response to feedback from the moving limbs, and steering limb actions in response to visual input (Armstrong, 1988; Kandel, Schwartz, & Jessell, 2000). One of these supraspinal areas is the MC, the major motor cortical area responsible for the motor output to the periphery. Initially, much of what we knew about the role of MC in walking

was derived from experiments in quadrupedal mammals (Drew, 1988, 1991; Drew, Andujar, Lajoie, & Yakovenko, 2008). These studies demonstrated that MC contributes significantly to gait modifications, especially when precise end-point control is required (e.g., paw placement and trajectory during stepping over obstacles) (Drew, 1988, 1991; Drew et al., 2008; Drew & Marigold, 2015).

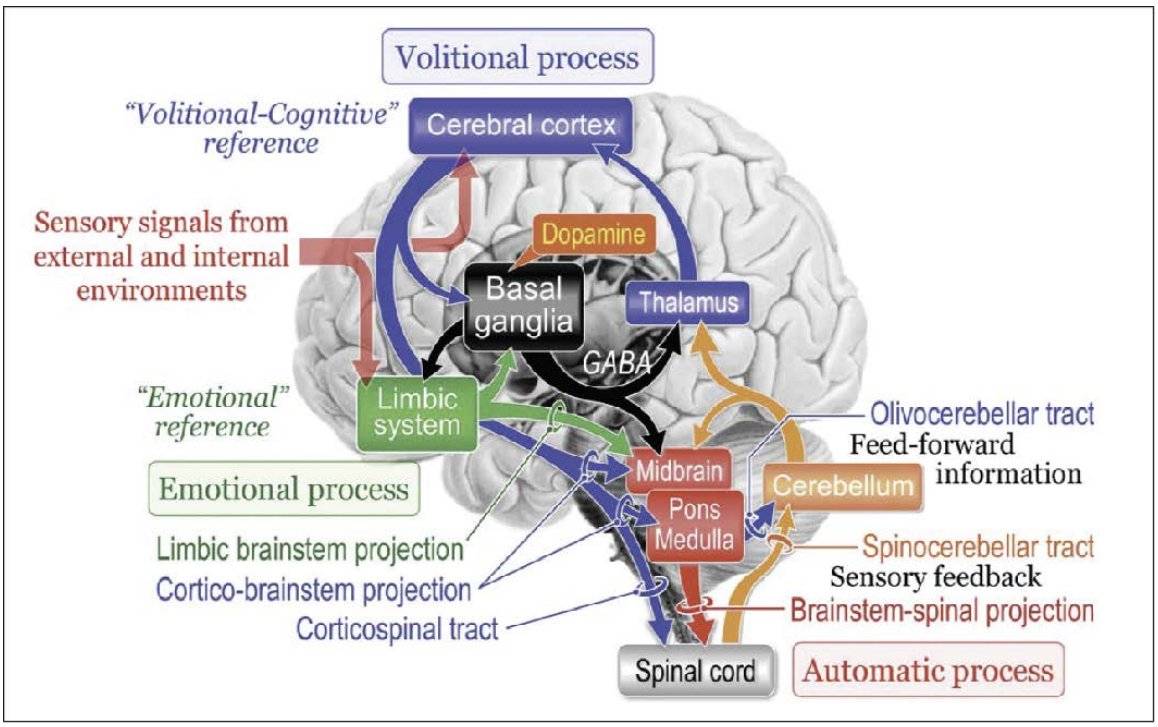

Figure 2.7. Schematic of the signal flows within and from supraspinal substrates. Adapted from Takakusaki (2013)

In humans, it has been postulated that the MC is a prerequisite for normal walking (J. B. Nielsen, 2003), yet the role of MC and CST during walking is not fully elucidated (Barthelemy, Grey, Nielsen, & Bouyer, 2011). The existing evidence has been derived from studies that used non-invasive techniques including TMS (Capaday et al., 1999; Schubert, Curt, Colombo, Berger, & Dietz, 1999; Schubert, Curt, Jensen, & Dietz, 1997), fMRI (Dobkin, Firestine, West, Saremi, & Woods, 2004), diffusion tensor imaging (Seo et al., 2014), single photon emission tomography (Fukuyama et al., 1997),

encephalography (Gwin, Gramann, Makeig, & Ferris, 2011; T. H. Petersen, Willerslev-Olsen, Conway, & Nielsen, 2012), and near-infrared spectroscopy (Miyai et al., 2001). Among these techniques, the most evidence comes from studies that applied TMS over the PF and DF cortical areas during walking on a treadmill. Those TMS studies tested the effect of TMS on PF and DF muscle activity and reflexes during walking in healthy adults.

An obvious limitation of using TMS during dynamic actions, such as walking on a treadmill, is to keep the position of the TMS coil constant and, thus, maintain the stimulation site in spite of rhythmic body actions. All studies that have applied TMS during walking on a treadmill used the following methods to solve this issue. The coil was mounted to a helmet by using Velcro® tapes that fix the inside of the coil to the outer surface of a linen cap. The helmet was attached to a halo vest system. The coil cable was held by an elastic restraint that could be fixed either to the ceiling or to a body weight support system (Figure 2.8). This technique allowed the participant to walk in

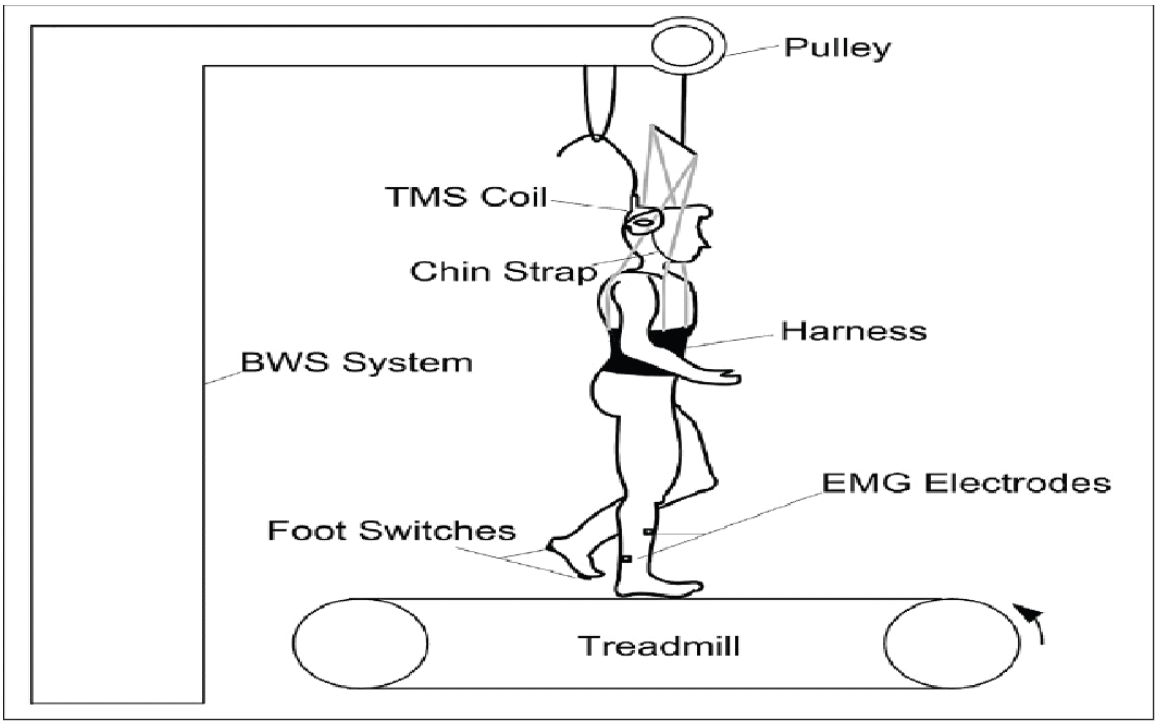

Figure 2.8. Schematic depiction of the experimental set-up of TMS application during treadmill walking. Adapted from Knikou et al. (2013)

natural cadence and freely move the head without affecting the coil's location.

The first study that stimulated the leg motor cortical areas in humans during walking was by Schubert et al. (1997), who examined the effect of cortical input by using TMS on PF (MG) and DF EMG during treadmill walking. Thirteen young healthy adults walked on the treadmill with an average walking speed of 4.27 km/h (1.19 m/s). The TMS coil was fixed with the approach described in the previous paragraph. Because the results showed constant MEPs across strides that indicated stable stimulation, subsequent studies adopted those techniques. TMS stimuli were applied at different moments during GC, which was divided into 16 periods. The main finding was that there was a modulation (i.e., changes relative to muscle activity during tonic voluntary muscle contraction) of TA and MG MEP amplitudes during walking. This modulation was predominant for TA MEP during pre-swing and swing phases in which TA is typically active, whereas the modulation was minor for MG MEP during mid-stance in which MG is mainly active. Between muscles, the response to TMS was greater in TA than MG. This was the first study to suggest a potential difference in the motor cortical control between PF (MG) and DF during walking in humans (Schubert et al., 1997). The same authors also found an effect of visual input on the motor cortical control of PF (MG) and DF during walking in humans (Schubert et al., 1999). Eleven healthy adults walked on the treadmill in two conditions: normal (walking without visual feedback of foot placement) and precision stepping (hit a colored spot on the treadmill). The main finding was that visual feedback of foot placement facilitated MG MEP and inhibited TA MEP during swing phase, whereas it facilitated only TA MEP during stance (i.e., pre-swing).

The results from both studies indicate that the motor cortical control of PF and DF differ during walking and can be modulated by visual feedback.

In addition to muscle responses, stimulation of the cortex can modulate the PF and DF reflexes during walking. In one study, 17 healthy adults walked on a treadmill while subthreshold TMS stimuli were applied over the leg motor area at different instances during the stance phase (N. Petersen, Christensen, & Nielsen, 1998). Recordings of the SOL H-reflex during stance showed a large short-latency facilitation due to TMS. Based on these results, the authors argued that SOL CM neurons are highly excitable during walking. In addition, they found no difference in the excitability of SOL CM cells between the stance phase of walking, tonic, and dynamic plantarflexion (N. Petersen et al., 1998). A subsequent study from the same lab examined the effect of TMS during stretch of the DF during walking (Christensen, Andersen, Sinkjaer, & Nielsen, 2001). Seventeen healthy adults walked on a treadmill while DF was stretched by imposing a quick plantarflexion and suprathreshold TMS was applied. The researchers found that the combined response to stretch and TMS increased as the interval between the two stimuli increased (Christensen et al., 2001). This finding provided further evidence on the cortical control of DF during walking. Though the findings from both studies are crucial for understanding the neural control of PF and DF during walking, their results do not determine the actual activation of corticospinal cells of these two muscle groups during walking (J.B. Nielsen, 2002).

To clarify this issue, the same group conducted a study in which subthreshold TMS was applied during walking in 19 healthy adults while PF (SOL) and DF EMG were recorded (N. T. Petersen et al., 2001). A suppression of SOL EMG (300 ms after

heel contact) and DF EMG (700 ms after heel contact) was found, yet neither muscle was suppressed when TESn was applied. The authors suggested that this TMS-specific suppression of PF (SOL) and DF EMG was probably restricted to the cortical level. This study was one of the first to indicate that PF (SOL) and DF muscles receive cortical contributions during walking.

Around the same time, another seminal study examined the extent to which CSTs are associated with PF (SOL) and DF spinal circuits during walking and tonic voluntary tasks (Capaday et al., 1999). The methodology of TMS application during walking was similar to the aforementioned studies. One of the main differences between this study and the others was the use of RC instead of reflexes or just MEPs. Utilization of the RC allowed the quantification of MEPs across several stimulation intensities, whereas the other studies used a single stimulation intensity. To compare the SOL and TA MCE between tasks, the prescribed level of SOL and TA EMG activities during the voluntary task were matched with their equivalent level of EMG activities during walking (e.g., SOL: early part of stance; TA: early part of swing). Twenty healthy adults participated in the study. The results showed that SOL MCE was less during walking (stance phase) than in MVIC, whereas the DF MCE was high and similar during walking (swing phase) and MVIC (Capaday et al., 1999). In addition to these findings, the results showed something that was not anticipated; TA MCE during the stance phase of walking was similar to SOL MCE during MVIC (Capaday et al., 1999). The authors suggested that during walking, the MC via the CM connections may strongly contribute to the control of TA during the swing phase and to a lesser extent during the stance phase, yet it has weak control over the SOL during the stance phase. During MVIC, however, motor cortical input is heavily

required for the control of both PF and DF. Similar to other studies, this study pointed out the extent to which SOL and TA are cortically controlled, and how this control differs for each muscle group between tasks (Figure 2.9).

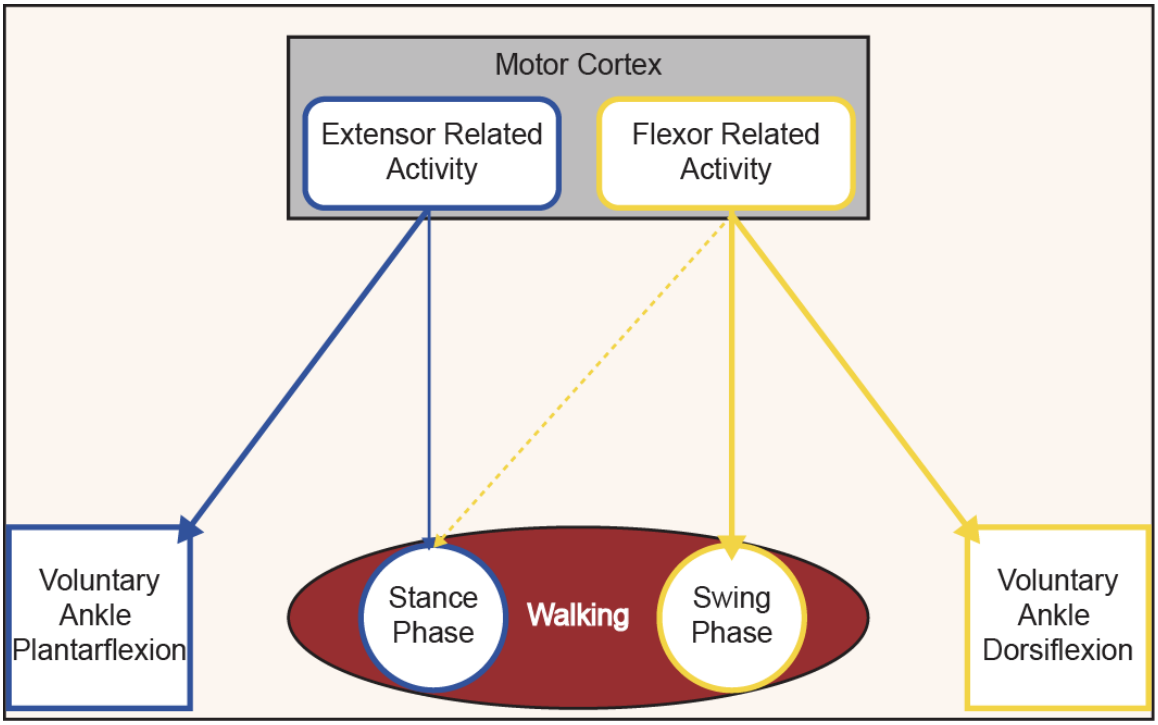

Figure 2.9. Schematic depiction of the strength of the linkage between the motor cortical areas and the muscle activities of SOL and TA during walking and MVIC. Modified from Capaday et al (1999).

2.4.4 Reliability of PF and DF MCE Measures

Consistent measurement of MCE requires the minimization of error (e.g., noise) to demonstrate that these metrics (e.g., MEP amplitude) reflect the actual MCE property (e.g., the number of the activated corticospinal motoneurons) measured. Though there is enough evidence to verify the investigation of PF and DF MCE using different MCE measures during multiple tasks, only a few studies have investigated the reliability for PF (SOL; one study) and for DF (three studies).

The intra- and inter-session reliability of three SOL MCE measures were tested in 13 neurologically healthy participants who were seated on a chair with the test leg in a

fixed position (Lewis et al., 2014). During TMS stimulation, which was applied via double cone coil at 120% of aMT, participants contracted their SOL (electrode was placed at the medial site) at 10% of maximal voluntary contraction (MVC: voluntary contraction in which the maximal force of a muscle group is tested (Winter, 1991)). The MCE measures tested were the amplitude and area of the MEP and aMT, and reliability was quantified using the intraclass correlation coefficient (ICC). The results showed that amplitude and area had good (ICC>0.75) intra- and inter-session reliability when using as few as six MEP responses. In addition, aMT had high inter-session reliability. This is the only study that has examined the reliability of SOL MCE measures. Further studies should investigate the reliability of the other PF (i.e., LG and MG) MCE measures during resting and active conditions of the target muscle.

The reliability of several TA MCE measures was also found to be very good to excellent. Van Hedel et al. (2007) investigated the test-retest reliability (14.0 ± 11.4 days) of DF MCE measures (amplitude and latency of the MEP, and cSP) during static and dynamic tasks at different levels of MVC (10, 20, 40, and 60%). Twenty healthy adults lay in a supine position while biphasic TMS stimuli were applied via a figure-of-eight coil at 120% of aMT. Findings showed that the amplitude was the most reliable (ICC: 0.77) during static condition at 40% MVC, the latency was reliable during static and dynamic conditions at both 40% and 60% MVC (0.74 < ICC < 0.81), and cSP was not reliable (van Hedel et al., 2007). Cacchio et al. (2009) investigated the intra- and inter-investigator, and test-retest reliability (within-session with a 1.5-h interval; between-session with a 4-week interval) of TA MCE measures (rMT, three RC measures, latency, and cSP) in 50 healthy adults. TMS was applied via circular coil while participants were

seated on a chair. For all three types of reliability the ICC of rMT, latency, and cSP was higher than 0.75; among the three measures, rMT always had the highest ICC. On the other hand, the RC measures had only moderate to good reliability; therefore, these measures should be used for quantifying the DF MCE with caution. Tallent et al. (2012) investigated the repeatability of TA MCE measures (rMT, amplitude of MEP, and cSP) during shortening and lengthening of TA at different levels of MVC (15, 25, 50, and 80%) on three consecutive days. Experiments took place at the same time of the day. Twenty healthy adults were seated on a dynamometer while stimulations were applied via double cone coil. The ICC of rMT, amplitudes during shortening at 25% MVC, and cSP during shortening and lengthening were very good to excellent across 3 days. The cumulative evidence from these three studies indicates that a few TA MCE measures are very reliable especially during muscle contraction. None of these studies used nTMS, which may increase reliability, or investigated the reliability of SICI or SICF.

In healthy adults, PF and DF are cortically controlled, and this control depends on several factors. Use of TMS can reliably measure the MCE of these muscle groups. After a stroke, however, these two muscle groups are impaired while there is a lesion in the brain. Therefore, stroke can alter the motor cortical control to PF and DF, but its ramifications during a motor task are not well understood.

2.5 Stroke

2.5.1 Categories

Stroke is a sudden neurologic deficit, either focal or global, due to occlusion (ischemic stroke) or rupture (hemorrhagic stroke) of blood vessels that supply the brain

blood circulation (Corbyn, 2014; Cuccurullo, 2004). To be classified as stroke, symptoms and signs of this deficit must last more than 24 hours; otherwise it is classified as a transient ischemic attack (TIA) or mini stroke (Aminoff, Greenberg, Simon, & Greenberg, 2005). Ischemic stroke accounts for 85% of all strokes, and it is classified either as thrombotic (~35%), embolic (~30%), or lacunar (~20%) (Cuccurullo, 2004). Hemorrhagic stroke is less prevalent, accounts only for 15% of all strokes, and can be caused by either intracerebral (hypertensive; ~10%) or subarachnoid (ruptured aneurysms; ~5%) hemorrhage (Cuccurullo, 2004).

2.5.2 Epidemiology of Stroke

In the U.S. in 2010, an estimated 6.8 million people, older than 20 years of age, had experienced a stroke. The prevalence rate of stroke is 2.8 %. Stroke is less prevalent in men than women, and it is most prevalent in African Americans, for both genders. Each year, nearly 795,000 people have a stroke, either new (610,000) or recurrent (185,000). The incidence is lower in men than women, and it is higher in whites than in African Americans. Stroke is the fourth leading cause of death, and a person dies from stroke every four minutes. The stroke death rate is the highest in the Southeastern states. Furthermore, stroke is a leading cause of chronic disability, and the combined direct and indirect costs of stroke in 2010 were $36.5 billion (Mozaffarian et al., 2015). The most common impairments after stroke are motor-related and include detrimental alterations in muscle strength, tone, and activation. One of the most common limitations is impaired walking.

2.5.3 PF and DF Impairments

Typically, the main walking impairments after stroke are limited joint motion, altered muscle activity, and impaired force production, which contribute to the reduction of walking speed and the alteration of spatiotemporal measures (Olney & Richards, 1996). These impairments are observed in all three lower extremity joints causing different gait patterns to emerge (Mulroy, Gronley, Weiss, Newsam, & Perry, 2003). The ROM, torque, and power of the ankle joint in the sagittal plane as well as PF and DF activity are smaller in the paretic side than in the non-paretic (Kim & Eng, 2004). The main impairment in the ankle sagittal motion is the reduced dorsiflexion motion during the swing phase, in particular during the mid-swing in which the foot clearance is a crucial gait event to advance the swing limb (Winter, 1992). The PF EMG is reduced during stance, while DF EMG is reduced during swing (Lamontagne, Malouin, Richards, & Dumas, 2002). The ankle plantarflexion torque is reduced during the push off phase; also decreased are the ankle power absorption during the first half of stance and the power generation during push off. Certain aforementioned ankle neuromechanics (muscle activity, joint kinematics, and kinetics) are positively correlated with gait speed. On the paretic side, only ankle power absorption and generation are associated with gait speed, whereas non-paretic ankle ROM, plantarflexion torque, and power absorption and generation are associated with gait speed (Kim & Eng, 2004). This evidence indicates that gait speed improvement is multifactorial, and it may have different contributions for each side's neuromechanics.

These impairments have an effect on the overall function of people post-stroke. Six months after stroke onset, 1 of 4 people require assistance with activities of daily living and one of three is unable to ambulate independently (Mozaffarian et al., 2015).

Two-thirds of people after stroke may experience critical limitations in functional walking and may be at risk for additional declines in physical mobility and independent walking (Jorgensen, Nakayama, Raaschou, & Olsen, 1995; Pouwels et al., 2009; Weerdesteyn, de Niet, van Duijnhoven, & Geurts, 2008). Impaired locomotion restricts a person after stroke from both performing mobility activities (e.g., walking) and participating in vocational and avocational activities.

2.5.4 PF and DF Rehabilitation

Improving walking is one of the main goals in stroke rehabilitation (Bohannon, Andrews, & Smith, 1988) while numerous rehabilitation strategies have been developed to improve the functionality of walking and to promote participation in social activities in people post-stroke (Bowden, Embry, Perry, & Duncan, 2012).

Among all the rehabilitation strategies, impairment-based and task-specific approaches are the two most common. The main principle of the impairment-based approach is that the focus of the rehabilitation should be on a specific impairment. For example, DF are weak after a stroke; therefore, rehabilitation should focus on strengthening this muscle group. On the other hand, a task-specific approach is used to improve a certain activity. Since walking after stroke is limited, people post-stroke should be trained during walking activities. Strength/power training is an example of an impairment-based rehabilitation approach, whereas treadmill training, either with or without a body weight support system, is an example of a task-specific approach. Yet, neither approach has been found to be capable of fully restoring walking after stroke (Dickstein, 2008), and neither has demonstrated superiority over other interventions.

Research in this area is limited for many reasons, two of which we will highlight here. First, there is insufficient understanding of the underlying neurophysiological mechanisms of the motor control of walking and how to apply specific rehabilitation techniques to engage these mechanisms. Second, it is not well understood what the relationships are between the neural control (e.g., motor cortical control) and neuromechanics (muscle activation, joint kinematics, and kinetics) of walking. Therefore, a potential area of study to understand the recovery mechanisms of walking after a stroke is to focus on a specific neural mechanism (i.e., motor cortical input) of the motor control of walking and then to examine the association between that mechanism and the neuromechanics of walking. Such a research focus is warranted for both PF and DF, since those two muscle groups play a significant role during walking.

2.5.5 PF and DF MCE after Stroke

Since stroke affects brain areas and potentially the descending pathways, TMS can be generally used to test the integrity of connectivity between MC and the target muscle after a stroke. One of the uses of TMS after stroke is to predict recovery. Several studies have showed that testing the DF MCE after stroke could predict recovery of mobility as measured by clinical evaluation tests (Arac, Sagduyu, Binai, & Ertekin, 1994; Hendricks, Pasman, van Limbeek, & Zwarts, 2003; Piron, Piccione, Tonin, & Dam, 2005). Yet no study has examined whether PF MCE can be a strong predictor of mobility recovery after a stroke. Another use of TMS after stroke is to assess the effect of a rehabilitation strategy on the MCE of a certain muscle. For example, 4 weeks of treadmill training enhanced certain DF MCE measures in people with chronic stroke, whereas general physical therapy of the same duration did not have the same effects on DF MCE

(Yen, Wang, Liao, Huang, & Yang, 2008). Furthermore, TMS can be used as a research tool to elucidate the residual motor cortical control (i.e., functional integrity of CST) of the target muscle.

Compared to the existing evidence on the motor cortical control of PF and DF in healthy adults, only a few studies have examined the PF and DF MCE after stroke, and in those studies, the greatest changes in PF and DF MCE occurred on the paretic side. Compared to DF MCE, there is minimal evidence regarding PF MCE. To elicit an MEP in PF (SOL), relatively greater stimulus intensity is required as compared to that in healthy controls (i.e., an increase in aMT) (Lewis et al., 2014). For DF, latency increases and the amplitude and SICF decrease compared to healthy controls (Beaulieu et al., 2014). Although these studies provided some evidence on changes in PF and DF MCE, further research should be conducted to elucidate clearly the changes in PF and DF MCE.

2.5.6 Reliability of PF and DF MCE Measures after Stroke

The reliability of measuring the PF and DF MCE has been minimally investigated in stroke. There is one study for each muscle group. Lewis et al. (2014) investigated the intra- and inter-session (7 days apart) reliability of PF (SOL) MCE measures (area and amplitude of MEP, and aMT) in 13 people with chronic stroke. TMS stimuli (120% of aMT) were applied via double cone coil while participants were seated on a chair with the test leg in a fixed position, and they contracted their SOL at 10% of MVC; only the paretic side was assessed. The intra-session reliability was excellent for the area and amplitude. This reliability increased as the MEP responses increased (from 4 to 10) and was similar for both methods used for calculating the MEP measures (average trace vs. single traces). Conversely, the inter-session reliability of both MEP measures was poor,

but the inter-session reliability of aMT was good (ICC: 0.82). This evidence indicates that certain PF MCE measures are very reliable within a single session, but for assessing PF MCE between sessions, only aMT is highly reliable. However, not all PF MCE measures were tested, and testing occurred only during tonic contraction. Therefore, further studies should examine the reliability of other PF (MG) and MCE measures (e.g., latency, MEP duration, cSP, RC derived measures, SICI, SICF) during both resting and active muscle contraction in people post-stroke.

The test-retest reliability (4 weeks apart) of DF MCE measures (rMT, amplitude, and latency) was tested in 16 people post-stroke (Cacchio et al., 2011). Both paretic and non-paretic legs were assessed. TMS stimuli were applied via circular coil while participants were seated on a chair. For the paretic leg, the inter-session reliability was excellent for rMT (ICC: 0.90) and latency (ICC: 0.85), yet poor for the amplitude (ICC: 0.38). In contrast, all measures on the non-paretic leg had excellent inter-session reliability. It is unclear whether these results would be different if figure-of-eight coils, either flat or double cone, were used in combination with nTMS. Similar to these findings, the inter-session reliability (7-10 days apart) of MCE measures of the quadriceps (vastus medialis and lateralis) was high on the paretic leg (rMT) and non-paretic leg (rMT and amplitude) in 23 people with chronic stroke (Wheaton, Villagra, Hanley, Macko, & Forrester, 2009).

Based on the existing evidence, MT, either resting or active, might be the most reliable measure for quantifying both PF and DF MCE measures on the paretic side in people post-stroke, while latency might be also a reliable MCE measure for the paretic DF only.

2.6 PF and DF Neuromechanics

Due to their respective locations in the lower leg and to their muscle properties, PF and DF have different functions during walking. One may argue that the use of clinical tests may not elucidate specifically and precisely the PF and DF contributions during a task. On the other hand, the function of PF and DF can be quantified using neuromechanical measures, either muscle activity, which is quantified using EMG, or body segment motions, which can be quantified by either kinematics or kinetics.

2.6.1 Electromyography

EMG characterizes the amplitude and the temporal characteristics of the electric signal associated with the muscle contraction during a motor task (Winter, 2009). EMG is quantified using either sEMG electrodes or fine wire electrodes. The former electrodes are easy to place and handle, but they measure the activity of surface muscles only. The latter type of electrodes is used when the electrical activity of deep and small muscles must be measured. The main limitation of fine wire electrodes is that they are invasively applied; this application is not simple and easy to handle, and it may cause discomfort to the participants. Since both PF and DF are relatively superficial muscles, their activity can be accurately quantified using sEMG electrodes.

Using EMG has a few strengths and drawbacks. The main strength of EMG is that it records the electrical activity from individual muscles; therefore, comparisons in muscle activity can be done between unilateral muscles and bilateral muscles. Another advantage is that EMG provides information about the timing and coordination of the muscle activity during a task (e.g., during intact gait the TA EMG profiles has two prominent bursts, early stance, and swing). One clinical application of EMG is in people

with hemiparesis whose bilateral EMG recordings elucidate the integrity of muscle activity in both paretic and non-paretic muscles. However, EMG has a few inherent weaknesses. EMG can be influenced by multiple factors (Farina, Merletti, & Enoka, 2004). Physiological cross talk (i.e., sEMG detection from neighboring muscles) and external noise (e.g., power line noise) are a few of those factors, which can be eliminated by using the right methodology. Similarly, EMG can have high variability due to factors such as adipose tissue, oily skin, electrode placement, and artificial movement of the electrode during the task. Of course, the experimenter can control nearly all these preceding factors. Furthermore, EMG does not provide direct information about the type of muscle contraction (e.g., eccentric vs. concentric). For example, TA is active during early stance and swing phase of GC, yet the type of contraction of the TA is not the same during these phases (Chleboun et al., 2007). By combining EMG with ultrasound and kinematic recordings, muscle contraction can be determined during specific subtasks of a motor task (e.g., walking). Lastly, EMG is not a direct measure of motion, which can be fully characterized using both kinematics and kinetics.

2.6.2 Kinematics

Kinematics describe bodies in motion using the linear and angular positions and their temporal derivatives (i.e., velocity and acceleration), without regard to the motion's cause (forces at work) (Robertson, 2004). The kinematics of dynamic tasks can be quantified either in two dimensions (planar) or in three dimensions (spatial). Via modern motion capture systems, three dimensional body kinematics can be characterized from the data collected from spatial kinematics (position) of reflective markers (passive markers), or light-emitting diodes (LEDs; active markers) positioned on specific body landmarks of

the participant. Then the three dimensional joint angles (sagittal, frontal, transverse) can be calculated offline using several methods, most commonly the Cardan/Euler approach; and subsequently, both joint linear and angular velocities and accelerations are quantified (Robertson, 2004). During walking, joint ROM and joint angular velocities are usually used to quantify joint kinematics, especially in the sagittal plane, which characterize joint flexion and extension. At the case of the ankle joint, which consists of two joints (talocrural and subtalar), the sagittal ankle kinematics reported in gait studies quantify the motion at the talocrural joint, which GAS/SOL plantarflexes and TA dorsiflexes. In addition to motion capture systems, simple goniometers can be used to measure the active joint moved voluntarily by the participant or passive joint moved by the examiner without assistance from the participant in order to determine joint ROM (i.e., the arc of joint motion) in static conditions (Norkin & White, 2003).

Although the kinematic analyses of certain joints during specific tasks can answer questions related to the speed of motion and the ROM, kinematics do not describe directly the forces that cause the motion; however, kinetics do. Nevertheless, kinematics can be used as one of the inputs in inverse dynamics modeling to estimate the joint torques and powers of a linked system.

2.6.3 Kinetics

Kinetics describe the forces that cause a motion in a rigid body and their resultant energetics. The three laws of motion can explain the relationship between the forces and the motion (1st: Law of inertia; 2nd: Law of acceleration; 3rd: Law of action-reaction). The motion of the rigid body can be either linear (caused by force) or angular (caused by

torque); the motion is caused either by intrinsic musculoskeletal forces or forces present in the interaction with the surroundings (e.g., ground reaction forces; GRFs).

Intrinsic musculoskeletal forces include the joint reaction forces and muscle forces (Enoka, 2008). Muscle forces can be measured in vivo using buckle transducers that are implanted invasively. Although few studies have measured these forces (e.g., force exerted at Achilles' tendon) in humans (Komi, Fukashiro, & Jarvinen, 1992), direct force measurement is limited due to the invasiveness of the technique. However, the net force, the sum of all these forces, can be indirectly calculated with the inverse dynamics approach. Three sets of data are used as inputs in the inverse dynamics: body segment kinematics (position, velocity, acceleration), anthropometric measures (mass and mass distribution of body segments), and external forces (e.g., GRFs) (Winter, 2009). Two of the main outputs of this modeling are joint torques and powers. Joint torque is the net result of all forces (e.g., from muscles and ligaments.) acting internally upon that joint, while joint power is the product of joint torque and angular velocity and quantifies the rate of energy change (Winter, 1991). To calculate joint torques and powers during walking, kinematics collected via motion capture system, anthropometric measures, and GRFs measured by force plates are required. During an isolated task, joint torques and powers, if the task is isokinetic or isotonic, are calculated using just a dynamometer. Lastly, both joint torques and powers can be used when the level of interest is at the joint.

In addition to intrinsic musculoskeletal forces, external forces can cause a motion. Such external forces are the GRFs, which are examples of the 3rd law of motion that states that "when one object (e.g., human body) applies a force to another object (e.g., surface of the earth), the latter exerts an equal and opposite reaction force on the former"

(Robertson, 2004). GRFs have three components to the force vector, vertical (GRF_V), anterior-posterior (GRF_{AP}), and medio-lateral (GRF_{ML}). Each component (i.e., the algebraic summation of the mass-acceleration products of all body segments during foot contact (Winter, 1991)) represents the reaction of the support surface on which the task is performed (e.g., treadmill's surface) to body motions (e.g., walking) that exert forces through the feet to the ground (Enoka, 2008). GRFs are present only during the stance phase of GC in which the foot is in contact with the ground; therefore, GRFs can be used to quantify kinetics only in stance. All three components of GRFs during walking can be measured by force plates that are located underneath the walking surface. Modern instrumented treadmills (e.g., split-belt) can measure the GRFs exerted on each leg. Among the three GRF components, the GRF_V and GRF_{AP} are the largest and most commonly used during walking. During stance, the GRF_V has a typical bimodal profile (two modes representing peak vertical forces at heel strike/weight acceptance and toe off/late stance), whereas the GRF_{AP} has negative and positive phases. In GRF_{AP}, the negative phase in the first half of stance demonstrates a net braking of the whole body, while the positive phase in the second half of the stance demonstrates the forward acceleration of the body, which is the force that moves the body anteriorly over the ground. Although GRFs are limited to stance phase only, they provide great information about the whole body's kinetics during stance and can be used to calculate the joint torques with inverse dynamic calculations.

Both kinematic and kinetic data during a task should be acquired because they provide a comprehensive description of the motion during that task. Otherwise, description of the motion will be incomplete. A perfect example is walking. Using just

kinematics to describe the motion of the leg joints during walking omits the significant contributions of GRF during the stance, yet this kinematic analysis occurs throughout the GC. Conversely, capturing only kinetic data during gait can quantify causes of motion mainly during stance because joint torques and powers during swing are low (especially at the ankle joint) compared to stance. Therefore, if the goal is to capture fully the motion of certain joints during a task, both kinematic and kinetic data should be collected. A limitation of this approach is the high cost of acquiring the tools that collect this data; however, currently nearly all motion analysis laboratories obtain all the required tools for collecting both kinematic and kinetic data.

2.6.4 Quantification of PF and DF Function

All aforementioned neuromechanical measures indirectly describe the body motion during a task. However, not all measures represent the specific contribution of a certain muscle during a motor task. In both walking and isolated tasks, certain neuromechanical measures can be used to quantify indirectly the function of a specific muscle.

Plantarflexors: During walking, PF have three functions. First, PF stabilize and control the ankle joint as the lower leg rotates forward during the early part of single leg stance (Winter, 1991) while they contract isometrically (Cronin et al., 2013; Panizzolo et al., 2013). Second, PF move the body's center of mass (COM) forward by major energy generation during the single leg stance (push-off phase; ~40-60% of GC in healthy controls) (Neptune, Kautz, & Zajac, 2001; Winter, 1983) while contracting concentrically. Third, PF contribute to swing initiation during the pre-swing phase of GC (second double limb support) (Neptune et al., 2001). SOL and GAS are synergists, yet

their contributions in these three functions differ. SOL may contribute more than GAS to ankle stabilization throughout single leg stance (Neptune et al., 2001), whereas GAS (i.e., MG) may be the strongest contributor during push-off (Gottschall & Kram, 2003; Neptune et al., 2001) and pre-swing (Neptune et al., 2001). Among all three functions of PF, the second function might be the most crucial. Therefore, EMG and GRFs can quantify this function of PF because during the push-off phase in stance, PF, both SOL and GAS, are concentrically active, and the positive phase of GRF_{AP} occurs.

Since in push-off, PF shorten and contribute to a PI (the time integral of the positive GRF_{AP}), any impairment to PF will have an impact on both muscle activity and PI. After stroke, the push-off phase of stance is limited due to impairments in PF whose muscle activity decreases compared to healthy controls (Knutsson & Richards, 1979; Peat et al., 1976). The PI reflects the forward acceleration of the body. In an intact system, PI results from a large ankle power burst, which occurs while PF are active during the push off phase of stance, and from the angle of the trail leg (i.e., the angle between a line from the pelvis COM to the foot COM and vertical) during the second half of the single leg stance and the second double limb support (Peterson, Cheng, Kautz, & Neptune, 2010). After stroke, in which one side is more affected than the other, the PI generated from each leg has been found to be asymmetrical (Bowden, Balasubramanian, Neptune, & Kautz, 2006; Sousa, Silva, Santos, Sousa, & Tavares, 2013), and it can be influenced by joint torques other than ankle joint torque (Peterson et al., 2010). One of the advantages of using PI is that only force plates are required to calculate directly PI, whereas ankle joint torques and powers can be calculated only using inverse dynamics, which require

multiple sets of different data. Therefore, PI may provide a quantitative measure of the coordinated output from PF either in healthy controls or people post-stroke.

Dorsiflexors: During walking, DF have two distinct functions. First, just prior to and just after heel contact, DF are isometrically active (largest DF burst during GC) (Chleboun et al., 2007) in order to lower the foot to the ground and to avoid slapping the foot. Second, DF is concentrically active immediately after toe off to clear the foot sufficiently during mid-swing. Between the two functions, the second holds more significance, especially in people post-stroke. The main goal in swing is to move the unloaded limb from behind the body to the front of the body with minimum foot lift and to optimize energetic efficiency. In normal walking, the minimum toe clearance has been reported to be less than 20 mm with low variability (Moosabhoy & Gard, 2006; Winter, 1992), and it occurs typically at about mid-swing (Moosabhoy & Gard, 2006), which is preceded by DF EMG burst at the time point when the ankle joint is dorsiflexing with peak AAV (Winter, 1991).

Toe clearance in swing is partly controlled by DF. This function of DF is considered to be cortically driven because of the strong CM connections. In addition, in contrast to stance in which the leg is in contact with the ground, the swing limb is not affected by the environmental surroundings. Therefore, this precise motor task (clear the foot with minimum lift) may rely on input from supraspinal centers (e.g., MC) (Capaday et al., 1999; Schubert et al., 1997). This cortical reliance of the DF during swing may explain the decreased dorsiflexion ROM (drop foot) during swing in people post-stroke. Further evidence for the cortical control of DF is that the PF and DF isometric strength both decrease in people with incomplete SCI and stroke. However, unlike in people with

incomplete SCI, ankle motor skill quantified by the root mean square error during a visuomotor ankle torque-tracking task is deteriorated in people post-stroke (van Hedel, Wirth, & Curt, 2010). Since toe clearance is a type of fine motor skill, this evidence may explain why people post-stroke are usually unable to clear the foot during swing without using compensatory patterns generated in other joints.

Toe clearance is an important kinematic measure that describes quantitatively the ability of the swing limb to be advanced and positioned forward by clearing the floor. In two studies, toe clearance (toe elevation) was employed to quantify foot drop in people with incomplete SCI; the authors suggested that toe elevation during swing is more valid and reliable than angular changes (Barthelemy et al., 2013; Barthelemy et al., 2010). Nevertheless, toe clearance may not fully quantify the function of DF during swing. A reason may be that the angular changes in other joints, both ipsilateral and contralateral, can contribute to toe clearance as well (Winter, 1992). For example, activating DF immediately after toe off achieves toe clearance in healthy adults, yet in people post-stroke, whose DF are impaired, toe clearance can be accomplished by either increasing the knee and hip flexion during swing or through circumduction (greater than normal frontal thigh ROM during mid-swing (Kerrigan, Frates, Rogan, & Riley, 2000)). Therefore, improving toe clearance after stroke does not necessarily imply improvement in DF function.

Other measures than toe clearance should be used to quantify the contribution of DF during swing. Reliance on DF EMG has the same limitations that have been discussed in a previous section. In contrast to stance phase, in which the foot is in contact with the ground and GRFs contribute to joint torques and powers, ankle torque, and ankle

power in swing are minimum. Thus, the ankle motion during swing is primarily quantified by the ankle kinematics, joint ankle angle and angular velocity. Between the two, ankle joint angular velocity might be the most appropriate to characterize the contributions of DF during swing (the peak AAV when the ankle joint is dorsiflexing during the first half of swing); because the ankle joint torque is so low in swing, the AAV during swing can be used as a surrogate of mechanical power, which is the product of joint torque and joint angular velocity. Therefore, ankle joint power during swing primarily represents the AAV.

Compared to research on ankle joint angle, few studies have used ankle joint angular velocity to quantify local kinematics at the ankle during walking. Granata et al. (2000) investigated whether joint angular velocities were influenced by muscle-tendon lengthening in children with spastic diplegia (cerebral palsy). They reported that joint angular velocity was a better discriminator than joint angle for gait patterns between clinical and normal populations (Granata et al., 2000). Similarly, another study showed no differences in ankle excursions (i.e., the difference between the maximum dorsiflexion and plantarflexion during gait) between healthy individuals and people with incomplete SCI whereas ankle joint angular velocity during the second double limb support differed between groups (Krawetz & Nance, 1996). Findings from these studies indicated that using joint angular velocities might help to discriminate gait changes between healthy people and neurologically impaired people more accurately than the typically reported joint angles. Granata et al. (2000) proposed two potential reasons for the superiority of joint angular velocity to discriminate gait patterns. First, joint angular velocities may have less variability between individuals than joint angles. Second, compared to joint

angles, which are prone to bias errors (e.g., incorrect marker placement and calibration procedures), joint angular velocities are not influenced by those bias errors because they characterize the relative change in joint angles.

Regardless of the measure used to quantify PF or DF during walking, we should bear in mind that joint kinematics and kinetics can be influenced by other factors (e.g., walking speed). Conversely, during MVIC, which is a closed chain task, PF and DF neuromechanics may be less influenced by external factors. Therefore, measuring both muscle activity and torques during MVIC may accurately quantify the contributions of each muscle group.

2.7 Associations between PF and DF MCE and Motor Behavior

Associations between the motor cortical input to a leg muscle and motor behavior of that muscle during a motor task have been found in both healthy controls and neurologically disabled individuals. In nine people with chronic stroke, Jayaram et al. (2012) investigated the relationship between an MCE measure of the vastus lateralis (knee extensor) and two commonly used clinical measures, Fugl-Meyer lower extremity (FM-LE) score and walking speed. The authors used the functional connectivity ratio to assess the corticospinal connectivity from each MC to both the paretic and non-paretic vastus lateralis muscles. This functional connectivity measure represented the ratio between the slope of ipsilateral and contralateral RC. Values of the functional connectivity ratio less than 1 indicated predominant contralateral connectivity (normal state). Results showed negative relationships between the functional connectivity ratio and both FM-LE score and walking speed. These findings demonstrated that greater

reliance from the ipsilateral MC was associated with lower FM-LE score and slower walking speed. This study was the first to show that the functional integrity of the bilateral CST that innervates a leg muscle was associated with clinical measures in people post-stroke. Despite the clinical importance of this study, its results should be interpreted with caution. Walking speed is a global measure that can be influenced by multiple factors. Therefore, the significant relationship between the cortical control of the vastus lateralis and walking speed does not explicitly explain the contributions of the motor cortical input to vastus lateralis during walking after stroke. In addition, a recent study that applied TMS during treadmill walking in 13 healthy adults showed that the vastus lateralis was less cortically driven than ankle muscles, suggesting weak motor cortical contributions to the vastus lateralis during walking (Iglesias, Lourenco, & Marchand-Pauvert, 2012).

For a better understanding of how a certain muscle is cortically controlled during a task, the association should be between the MCE of that muscle and a specific neuromechanical measure that quantifies its function during a specific task. Few studies utilized that approach. Barthelemy et al. (2010) tested whether there was a relationship between the TA MCE and foot drop in people with chronic SCI. As a measure of foot drop and function of TA, Barthelemy and colleagues used toe elevation; the largest distance between the marker placed on the 5th metatarsophalangeal joint and the ground during swing. Their results showed a negative relationship between MEP latency and toe elevation and a positive relationship between MEP amplitude and toe elevation. The findings suggested that adequate toe elevation during the swing phase requires sound TA MCE in people with SCI. Barthelemy et al. (2013) also investigated whether TA MCE

measures (amplitude and latency of MEP) were associated with clinical measures such as walking speed, the 6 minute walking test (6MWT), and Timed Up and Go (TUG). The results demonstrated no relationship between any TA MCE measure and clinical tests, yet toe elevation was strongly associated with all clinical tests. In contrast to Jayaram et al. (2012), the two studies by Barthelemy et al. (2013; 2010) showed that both amplitude and latency of TA MEP were associated with the toe elevation, a kinematic measure that indirectly quantifies the function of TA during swing, but not with any clinical tests. Therefore, this evidence strengthens the argument that to investigate the motor cortical control of a muscle during a task, a neuromechanical measure that quantifies the function of that muscle should be used in the association instead of a clinical measure.

The existence of this type of association between MCE of either PF or DF and a neuromechanical measure in people post-stroke is limited. A recent study (Beaulieu et al., 2014) examined whether the impairment of voluntary dorsiflexion motion was associated with the functional integrity of CST in 18 people with chronic stroke. The DF MCE measures used were the aMT, latency, amplitude, cSP, SICI, and SICF. Ankle dorsiflexion was quantified using the neuromechanical measures of active dorsiflexion ROM, MVICn, and TA EMG in both tasks (i.e., ROM and isometric muscle strength). The rresults demonstrated only two significant relationships among all the relationships examined between the DF MCE measures and the neuromechanical measures (six TMS measures x four clinical measures: 24). The amplitude of DF MEP was positively associated with DF ROM and strength during MVIC.

The cumulative evidence from these studies supports the notion that investigating the association between MCE and neuromechanics of DF can contribute to a better

understanding of the motor cortical control of DF during different tasks in people post-stroke. Such potential associations have not been widely investigated in stroke or for PF. The only existing evidence is for DF during MVIC, whereas none exists during walking.

2.8 Remaining Gaps

The measures used in these associations must be fully understood for the associations to be scientifically and clinically important. Among the measures used in these associations, the PF and DF MCE measures are the least well understood, especially in people post-stroke. First, the reliability of the PF and DF MCE measures has not been adequately investigated either in neurologically intact adults or in people post-stroke. Within the last decade, only a few studies have tested the reliability of certain MCE measures in healthy controls. These studies focused primarily on TA (Cacchio et al., 2009; Tallent et al., 2012; van Hedel et al., 2007); only one study investigated a few MCE measures of SOL (Lewis et al., 2014). In people post-stroke, only two studies have reported good reliability of certain PF (only for SOL) (Lewis et al., 2014) and DF (Cacchio et al., 2011) MCE measures, even though all possible MCE measures have been used in stroke studies that have investigated the motor cortical control of PF and DF.

Although the findings from these studies are important, several gaps in knowledge regarding reliability still exist. First, both physiological (state of muscle contraction during stimulation) and methodological (type of coil, use of nTMS) factors that may influence the reliability of PF and DF MCE measures have not been thoroughly investigated. Secondly, the reliability has not been established for all potential measures that quantify the MCE of SOL and TA. In addition to lack of certain knowledge about reliability, no consensus exists regarding which MCE measure of PF and DF should be

used to examine comparisons across persons. The MCE measure used in the brain and leg motor behavior associations in people post-stroke must detect the differences between an intact and lesioned CNS (i.e., stroke). In addition, it may be inaccurate to assume that the measure used to characterize the best PF MCE is the same for DF MCE. These brain and leg motor behavior associations in people post-stroke can be useful only after the characterization of MCE measures for each muscle.

Investigating the associations between MCE and the neuromechanics of PF and DF during functional and isolated tasks in stroke has the potential to elucidate the extent to which deficits in post-stroke motor cortical contributions to PF and DF are related with muscle-specific neuromechanics and whether these relationships are task-specific. This evidence may provide vital information about how PF and DF are cortically controlled after a brain lesion. Moreover, this evidence may influence rehabilitation strategies to help people post-stroke optimally gain full functional recovery. In healthy adults, MC may contribute less to the control of PF than DF, but MC's input is still necessary and depends on the nature of the task. For example, PF and DF MCE are task-specific in healthy people and similar during MVIC but different during walking (Capaday et al., 1999). Following a stroke, the motor cortical input to PF and DF changes, yet how this motor cortical control changes remains unclear. Moreover, studies have yet to examine whether motor cortical control differs between PF and DF depending on the mechanical demands of the task (e.g., a walking vs. an isolated task). Unfortunately, such potential associations have not been well investigated in people post-stroke. No study has investigated the associations between PF MCE measures and PF neuromechanics in

people post-stroke, either during walking or during MVIC. The only existing evidence is for DF during MVIC (Beaulieu et al., 2014), whereas none exists during walking.

CHAPTER THREE

RESEARCH DESIGN AND METHODS

3.1 Specific Aims and Hypotheses

The overall objectives of this project were to investigate the extent to which 1) the SOL and TA were cortically driven in people post-stroke (paretic leg) and in healthy controls (dominant leg) and 2) deficits in post stroke motor cortical control were associated with the neuromechanics of the paretic SOL and TA during walking (i.e., a functional task) and MVIC (i.e., voluntary isolated task) (Figure 3.1). Prior to any correlational analysis, we systematically investigated which MCE measure, among 8, for each muscle was the most reliable and detected the differences in variances and means between an intact CNS (healthy controls) and lesioned CNS (people post-stroke) (Aims 1 and 2 in Figure 3.1). Then we investigated the associations between the MCE and ankle neuromechanics (i.e., muscle activation, joint kinematics, and kinetics) for the paretic SOL and TA during walking and MVIC tasks in people post-stroke (Aims 3 and 4 in Figure 3.1). Eight MCE measures were quantified while the target muscle was relaxed, either SOL (Figure 3.1 A) or TA (Figure 3.1 B), on the paretic side in people post-stroke and the dominant side in healthy controls during the resting condition (rMT, MEP amplitude, MEP latency, and normalized MEP latency) and slightly contracted during the *active* condition (MEP amplitude, MEP latency, normalized MEP latency, and cSP).

The neuromechanical measures of the paretic SOL were the EMG and PI in single leg stance (SLS1 & SLS2) and second double limb support (DLS2) during walking as well as EMG and torque during MVIC (Figure 3.1 C). The neuromechanical measures of the paretic TA were the EMG and AAV during the first half of the swing (50% Swing1) during walking and EMG and torque during MVIC (Figure 3.1 D).

Specific aims for this dissertation were as follows:

Aims 1 and 2:

The overall goal of Aims 1 and 2 was to quantify eight MCE measures of SOL and TA in people post-stroke and in healthy controls. The quantification of each measure occurred in three steps. The first step was to investigate the intra-rater test-retest reliability in both groups. Only measures that were reliable in both groups were used in the next step. The second step was to examine the inter-group difference in variance for the measures that passed the first step. The third step was to examine the inter-group difference in mean for the measures that passed the second step. We employed those three steps for the following reasons. A main goal was to avoid running multiple correlations using SOL and TA MCE measures that had not been quantified to the populations of interest in our study. Furthermore, instead of examining only the reliability of each measure, we sought to identify the measure that could best distinguish damage to CNS. To determine this distinction, we investigated the inter-group differences for both variance and means. We expected low between-subject variance within a group with healthy CNS, as the integrity of the neuromotor axis is the same among the healthy participants. Conversely, within a group with damaged CNS, due to the different level of impairment of the neuromotor axis, we expected high between-subject variance because

the integrity of the neuromotor axis is not the same among stroke participants. Furthermore, the group mean of a MCE measure in healthy adults should differ from the group mean of the same measure in stroke participants. Consequently, significant inter-group differences in both variance and mean would indicate the distinction between damaged and healthy CNS. In Aims 3 and 4, therefore, we used only the MCE measures of SOL and TA that were reliable in both groups and that differed in variance and mean between groups.

The evidence for the quantification of the SOL and TA MCE is limited for both groups, especially for SOL. Although SOL MCE may differ from TA MCE, we expected that the measurement characteristics of SOL MCE would be similar to TA MCE. Therefore, the hypotheses of these two aims were based mainly on the TA MCE studies, which demonstrated good reliability for rMT, latencies, and cSP (Cacchio et al., 2009; Cacchio et al., 2011; Forster et al., 2014; Meaney, Collett, Dawes, Howells, & Izadi, 2015; Tallent et al., 2012; van Hedel et al., 2007). Furthermore, these three particular measures typically increase after a stroke (Kobayashi & Pascual-Leone, 2003).

Specific Aim 1: To determine which of the eight SOL MCE measures was reliable both in healthy controls and in people post-stroke yet differed in both variance and mean between groups (Figure 3.1 A).

When SOL was at resting state (Figure 3.2 A-I):

Hypothesis 1.1.a: SOL rMT, latency, and normalized latency of SOL would be the most reliable in both groups

Hypothesis 1.1.b: SOL rMT would be significantly higher and more variable in people post-stroke than in healthy controls.

Hypothesis 1.1.c: SOL latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.1.d: SOL normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

When SOL was at active state (Figure 3.2 A-II):

Hypothesis 1.2.a: SOL latency, normalized latency, and cSP would be the most reliable in both groups.

Hypothesis 1.2.b: SOL latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.2.c: SOL normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 1.2.d: SOL cSP would be significantly longer and more variable in people post-stroke than in healthy controls.

Specific Aim 2: To determine which of the eight TA MCE measures were reliable both in healthy controls and in people post-stroke yet differed in both variance and mean between groups (Figure 3.1 B).

When TA was at resting state (Figure 3.1 B-I):

Hypothesis 2.1.a: TA rMT, latency, and normalized latency of SOL would be the most reliable in both groups.

Hypothesis 2.1.b: TA rMT would be significantly higher and more variable in people post-stroke than in healthy controls.

Hypothesis 2.1.c: TA latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 2.1.d: TA normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

When TA was at active state (Figure 3.2 B-II):

Hypothesis 2.2.a: TA latency, normalized latency, and cSP would be the most reliable in both groups.

Hypothesis 2.2.b: TA latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 2.2.c: TA normalized latency would be significantly longer and more variable in people post-stroke than in healthy controls.

Hypothesis 2.2.d: TA cSP would be significantly longer and more variable in people post-stroke than in healthy controls.

Aims 3 and 4:

The overall goal of Aims 3 and 4 was to investigate the associations between the descending drive, quantified by MCE measures determined in the two previous aims, and the specific ankle neuromechanics of the paretic SOL and TA during walking and MVIC in people post-stroke. Instead of using clinical measures that can be influenced by multiple factors, we chose to use specific neuromechanical measures that quantify indirectly the function of each muscle during specific phases of walking and during MVIC. These neuromechanical measures included both the muscle activity and the ankle mechanics (i.e., kinematics and kinetics) of the paretic SOL and TA.

In healthy adults, Capaday et al. (1999) demonstrated that the contributions of the descending drive to SOL and TA depended on the nature of the task. Specifically, SOL MCE was less during the stance phase of walking than in plantarflexion MVIC, whereas

the TA MCE was the same during the swing phase of walking as in dorsiflexion MVIC. Since stroke detrimentally affects the integrity of the neuromotor axis in people post-stroke, the contributions of the descending drive to each muscle should be weaker than in healthy controls. However, the pattern of contributions to the paretic SOL and TA should be similar in both tasks as described in Capaday et al. (1999). Additionally, because stroke increases the MCE measures (as we hypothesized to pass all three criteria in Aims 1 and 2), and stroke decreases the neuromechanical measures used to quantify paretic SOL and TA during walking and MVIC, we expected to find negative associations between the MCE measures and the neuromechanics of the paretic SOL and TA.

Specific Aim 3: To investigate the associations between the MCE measures determined in Aim 1 and the neuromechanics of the paretic SOL during walking and MVIC in people post-stroke (Figure 3.1 C).

For the functional task (i.e., walking):

Hypothesis 3.1.a: SOL MCE would not be significantly associated with SOL EMG in stance phase (Figure 3.2 C-I).

Hypothesis 3.1.b: SOL MCE would not be significantly associated with PI (Figure 3.2 C-II).

For the isolated voluntary task (i.e., MVIC):

Hypothesis 3.2.a: SOL MCE would be significantly and negatively associated with SOL EMG (Figure 3.2 C-III).

Hypothesis 3.2.b: SOL MCE would be significantly and negatively associated with SOL isometric torque (Figure 3.2 C-IV).

Specific Aim 4: To investigate the associations between the MCE measure(s), as

determined in Aim 2, and the neuromechanics of TA during walking and MVIC in people post-stroke (Figure 3.1 D).

For the functional task (i.e., walking):

Hypothesis 4.1.a: TA MCE would be significantly and negatively associated with TA EMG in the first half of swing phase (Figure 3.2 D-I).

Hypothesis 4.1.b: TA MCE would be significantly and negatively associated with AAV (Figure 3.2 D-II).

For the isolated voluntary task (i.e., MVIC):

Hypothesis 4.2.a: TA MCE would be significantly and negatively associated with TA EMG (Figure 3.2 D-III).

Hypothesis 4.2.b: TA MCE would be significantly and negatively associated with TA isometric torque (Figure 3.2 D-IV).

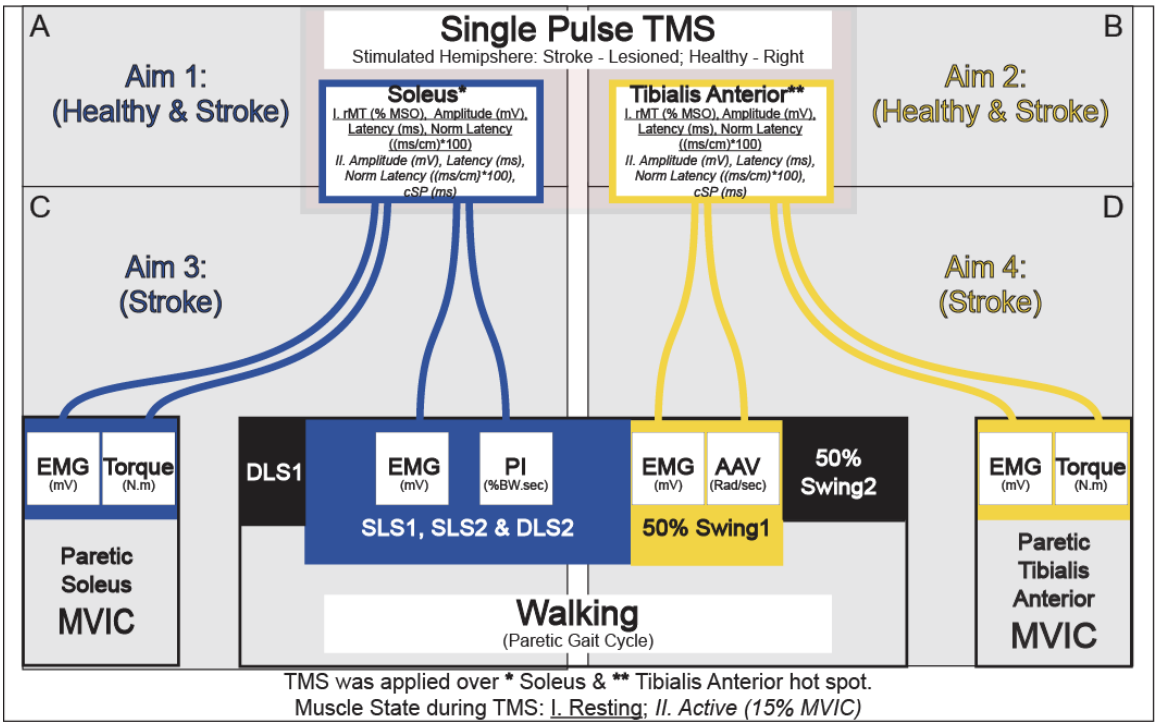

Figure 3.1: Outline of the aims

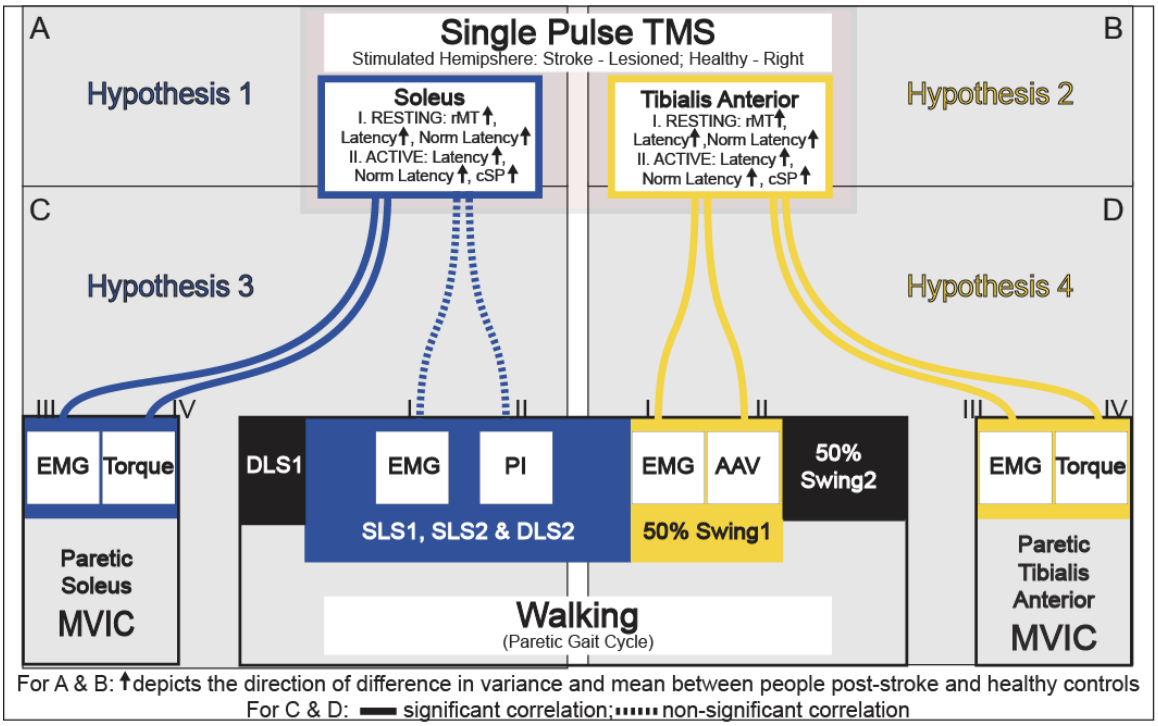

Figure 3.2: Outline of the hypotheses

3.2 Study Design

This study was cross-sectional. All experimental procedures took place in the Functional Neurostimulation Laboratory (FNL; testing MCE), Locomotion Energetics and Assessment Laboratory (LEA; testing walking neuromechanics), and the Locomotor Rehabilitation Laboratory (LRL; testing MVIC neuromechanics). All labs are located in the College of Health Professions research building (CHP-C) at the Medical University of South Carolina (MUSC), Charleston, SC.

3.3 Participants

Fifteen people with chronic stroke were recruited within a period of 6.5 months (2/11/2015 - 8/24/15). People post-stroke were included in the study if they met the following criteria: 1) 18 to 85 years of age; 2) ≥6 months post-stroke; 3) either a cortical

or subcortical lesion and either an ischemic or hemorrhagic type of stroke; 4) residual paresis in the lower extremity (FM-LE motor score <34) preservation of minimal DF and PF contraction (at least 2 of 5 on a manual muscle test); 5) able to walk 1 minute on a treadmill with minimum speed of 0.2 m/s; 6) passive ROM of 5° of plantarflexion; 7) provision of informed consent; and 8) no cognitive impairments that made it difficult to follow the instructions about experimental procedures. Individuals were excluded from the study if they 1) had a history of seizures or used medications that could lower seizure thresholds; 2) had a history of brain injury or preexisting neurological disorder; 3) had a pacemaker or intracranial metallic implants (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of, 2009); or 4) had severe arthritis or orthopedic problems that limit passive ROM.

Twenty five neurologically intact controls were recruited within a period of 3.5 months (12/1/2014 - 3/15/2015) and were free from any neurophysiological or musculoskeletal ailments.

3.4 Experimental Procedures

People post-stroke and healthy controls attended two data collection sessions (Stroke: 8 ± 2 days apart; Healthy: 7 ± 2 days apart). Before the first experimental session, a MRI session occurred either on a separate day or on the same day. That session lasted 31 and 26 minutes, respectively, for people post-stroke and for healthy controls. For the people post-stroke, the experimental procedures lasted approximately 3 and 4 hours for the first and second sessions, respectively. For the healthy controls, the experimental procedures lasted approximately 3 hours in both sessions. Figures 3.3 and

3.4 present each of the overall experimental procedures carried out in healthy controls and stroke participants, respectively.

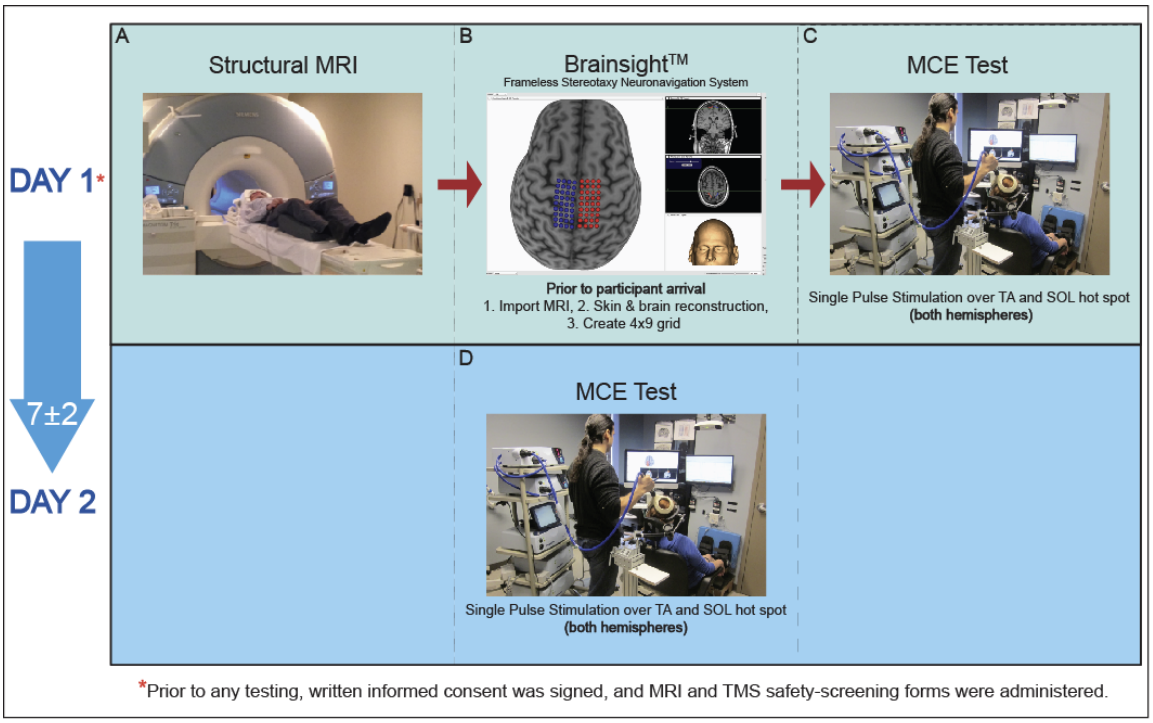

Figure 3.3: Flowchart of the experimental procedures used in healthy controls

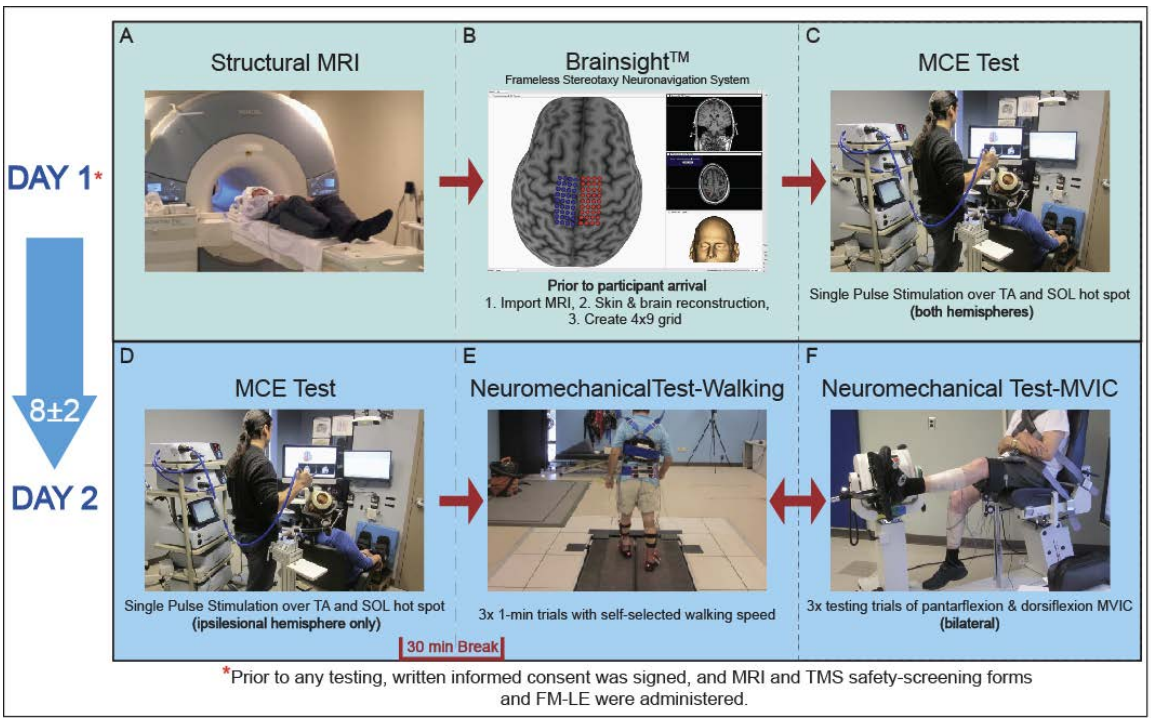

Figure 3.4: Flowchart of the experimental procedures used in people post-stroke

The first time that all participants came to the setting and prior to any testing (i.e., MRI), we informed them orally about the experimental procedures and potential risks, then asked them to read and sign written informed consents approved by the MUSC Institutional Review Board and adhering to the Declaration of Helsinki. To ensure participants' safety and qualification for MRI and TMS testing, we administered MRI (Shellock & Spinazzi, 2008) and TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2011) safety-screening questionnaires (Appendices). Then we collected anthropometric (e.g., height, body mass) and demographic (e.g., age, gender) data. For people post-stroke, we also collected information about their mobility capacity and present comorbidities, and one of three licensed physical therapists with multiple years of clinical experience administered a single clinical test (FM-LE).

The FM-LE scale was used to assess the sensorimotor function of the lower extremity in people post-stroke, and it consisted of 17 items (Fugl-Meyer, Jaasko, Leyman, Olsson, & Steglind, 1975). Each item was scored on a 0 to 2 scale; a total score of 34 points was possible (Appendices).

At the two subsequent data collection sessions, the MCE testing occurred in both groups. At the second data collection session, the walking and MVIC neuromechanics, which were measured only in people post-stroke, were always tested after MCE to avoid any interference with MCE testing (e.g., fatigue of descending pathways due to the neuromechanics testing). The order of the two neuromechanical tests was randomized; they were administered consecutively, with a short break between tests. There was a 30-minute break between the cessation of the MCE testing and beginning of the neuromechanical testing.

3.4.1 Structural MRI and TMS Frameless Stereotaxy Neuronavigation System

To ensure accurate and precise positioning of the coil throughout the MCE testing and across sessions, we used a Brainsight™ (v2.2) TMS Frameless Stereotaxy Neuronavigation System (Rogue Research Inc.; Montreal, Quebec, Canada), into which each participant's structural MRI was imported. We asked people post-stroke and healthy controls, who did not have the anatomical images of their brain taken in a previous study, to attend a MRI session. In addition to structural MRI (both groups: localizer and MPRAGE), several other MRI protocols (stroke group: flair, DKI, ep2d resting state; healthy group: flair, DKI) were carried out for secondary analyses which were part of other studies. During scanning, participants were in a supine position with a cushion placed under their knees to ensure a comfortable posture; they wore earplugs to attenuate the loud noise of the scanner and were asked to keep still (Figure 3.3 A & 3.4 A). For the structural MRI of each participant, high-resolution T-1 weighted anatomical brain images were acquired (TR=1900 ms, TE= 2.26 ms, voxel dimensions 1.0 x 1.0 x 1.0 mm, 192 slices (1 mm thickness), full brain and cerebellar coverage) using a Siemens 3T TIM trio scanner (Siemens, Erlangen, Germany) with a thirty-two-channel head coil (Siemens, Erlangen, Germany). MRI testing occurred in the Center of Biomedical Imaging located at the Medical University of South Carolina.

Shortly after the end of the MRI session, we uploaded each participant's images (i.e., DICOM files) into Brainsight and several steps were undertaken prior to the experiment (Figure 3.3 B & 3.4 B). The first step was to co-register manually each participant's MRI to two well-established brain structures, the anterior and posterior

commissures, so the individual's MRI could be mapped using a widely used stereotaxic coordinate system, the Montreal Neurological Institute (MNI) atlas. The origin of the MNI atlas was the anterior commissure (0, 0, 0); therefore, any spot in the brain had x (mediolateral), y (anteroposterior), and z (vertical) coordinates in relation to the origin. Then we reconstructed the 3D skin and curvilinear brain models. We used the 3D skin model to identify four anatomical landmarks, which we used to calculate the participant to image registration. These anatomical landmarks were the tip of the nose, bridge of the nose (i.e., nasion), and supratragic notch of the right and left ear. We used the 3D curvilinear brain model to identify manually the leg motor area on which a rectangular 15 mm (4 columns in the mediolateral axis) x 40 mm (9 rows in the anteroposterior) grid (total of 36-points) was overlaid at each hemisphere (Figure 3.5).

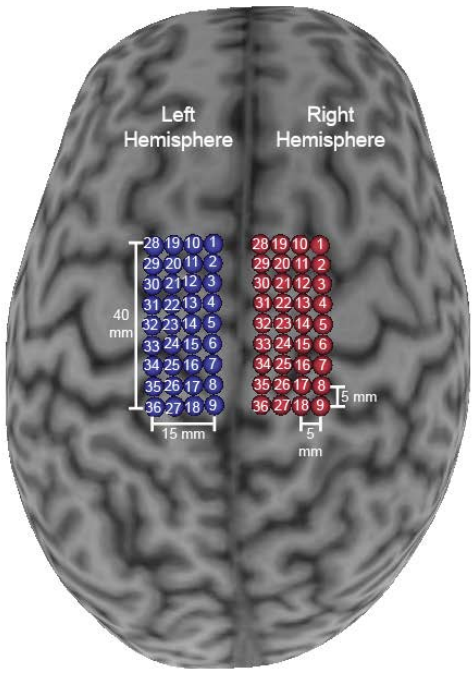

Figure 3.5: Bilateral 36-spot grid superimposed over the leg motor areas of a representative brain

The space between points was 5 mm; using 5 mm was found to determine the hot spot more accurate than longer distances (e.g., 10, 15, and 20 mm) (Brasil-Neto, McShane,

Fuhr, Hallett, & Cohen, 1992). Rather than using a scalp based target approach in which any error in orientation can alter the stimulation site, we used a cortex based approach in which error in orientation has a negligible effect on the stimulation site (Comeau, 2014). For this reason, we positioned the centered row of the grid at the center and below the surface of the leg motor cortical area where CST that innervate leg motor pools originate (Conti et al., 2014). For consistency across participants, the same person created all Brainsight files.

3.4.2 SOL and TA MCE

MCE was assessed twice in both groups, and each data collection occurred at the approximately the same time of the day (e.g., morning). In healthy controls, SOL and TA MCE were assessed bilaterally at both visits (Figure 3.3 C & D). In people post-stroke, SOL and TA MCE were assessed bilaterally at the first visit, whereas the MCE of the paretic SOL and TA were only assessed at the second visit (Figure 3.4 C & D). Other than this minor difference, all MCE procedures were the same for both groups (Figure 3.6). We strongly suggested to all participants to avoid consuming caffeine or alcohol for at least 3 hours prior to experimental procedures.|

After EMG placement and testing (Figure 3.6 Step 1; see below for further explanation), participants sat comfortably in a reclined position (~95° from horizontal) with both arms and shanks supported by armrests and limb-support pads, respectively (Figure 3.6 Step 2). To ensure consistent feet placement across participants, both feet were secured in walking boots that allowed the ankle ROM to be adjusted from 30° dorsiflexion to 45° plantarflexion in 7.5° increments. The angles at the hip, knee, and ankle were approximately 90° (flexion), 20° (flexion), and 7.5° (plantarflexion),

respectively. Both hip and knee angles were slightly adjusted in case the participant reported discomfort with the current posture. When there was a need to keep the head still during TMS application, we used a forehead rest arm that was attached on the chair and positioned opposite to the site where the coil was placed. Throughout the experiment, we instructed participants to keep still.

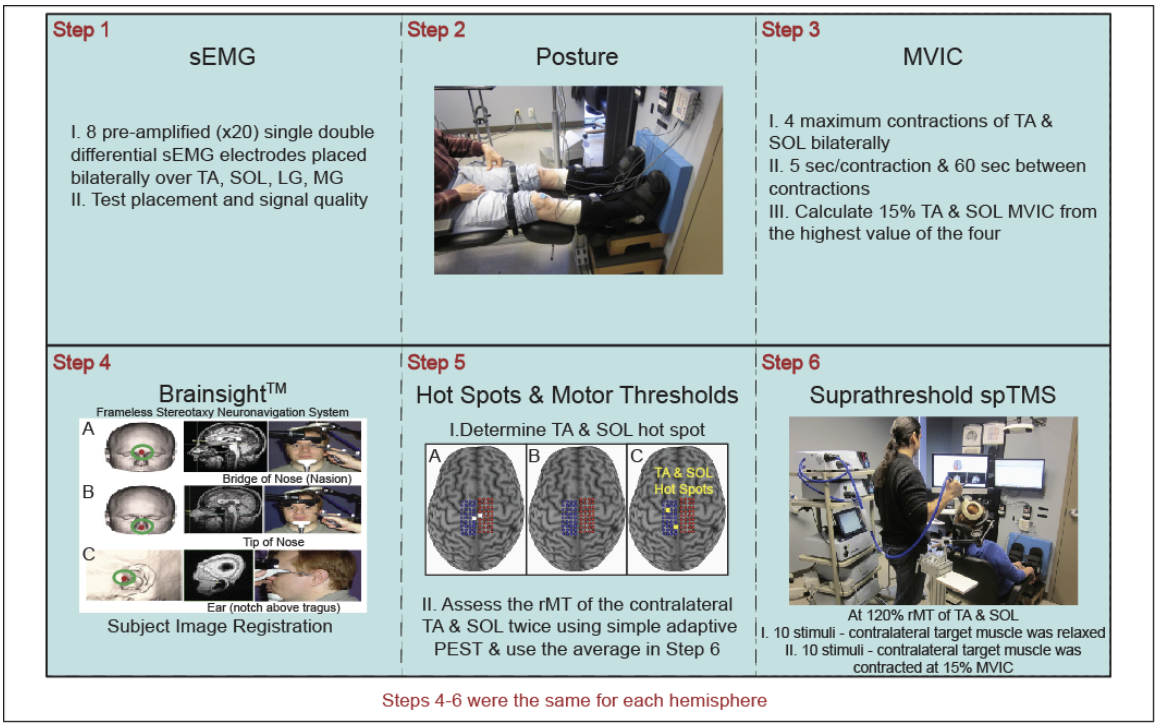

Figure 3.6: Flowchart of the experimental procedures carried out for MCE testing

After EMG placement and the participant's setup on the chair, we determined the MVIC of the SOL and TA bilaterally using EMG (Figure 3.6 Step 3). For each motion, participants were instructed to maximally contract the target muscle (e.g., SOL) 4 times (~5 seconds contractions seperated by 60 seconds of break) while they were seated at the posture described above. After each contraction, the maximum contraction, which was quantified using EMG, was calculated immediately using Spike v7.15 (Cambridge Electronic Design; Cambridge, UK) by taking the average within a 100 ms window centered around the maximum rectified and low-pass filtered (i.e., RMS amplitude of

0.165 sec) EMG. We used the same method to calculate simultaneously the joint torque and EMG during the voluntary isolated task (MVIC); see section 3.6.3 for further explanation. The highest value of the four was used to calculate the 15% MVIC, which was used during the active TMS conditions. We chose that level of MVIC because it has been commonly used in previous studies which examined TA MCE in people post-stroke (Beaulieu et al., 2014; Beaulieu, Masse-Alarie, Brouwer, & Schneider, 2015). During pilot testing, futhermore, we found that level to be achievable by people post-stroke and adequate to yield cSP during pilot testing.

Following the MVIC protocol, we prepared the neuronavigation system. First, a headband with reflective markers was placed on the participant's head. After creating a new TMS session on the participant's Brainsight file, the target grid for stimulation was selected. Our next step was to verify the proper position of the motion capture camera, Polaris Vicra System (Northen Digital Inc.; Waterloo, Ontario, Canada) by placing the headband tracker, the pointer, and the coil tracker on its capture volume space. Then we performed the participant-image registration by placing the tip of the pointer on 4 anatomical landmaks: nasion (Figure 3.6 Step 4-A), the tip of the nose (Figure 3.6 Step 4-B), and the supratragic notch of the right and left ear (Figure 3.6 Step 4-C). Once all anatomical landmarks were sampled, we verified whether registration occurred accurately by placing the tip of the pointer on random spots over the participant's skull. If the distance from the tip of the pointer to the reconstructed skin was less than 5 mm, we procceeded to TMS experiment; otherwise, participant-image registration was repeated until the desired values were obtained.

All experimental procedures involving TMS were the same among muscles and

across hemispheres. For all experiments, the same TMS user applied a single pulse stimuli on the optimal site (i.e., hot spot; see next paragraph for further details) of the target muscle using a BiStim module magnetic stimulator (The Magstim Company Limited; Whitland, UK). The stimulator was set at the standard mode; the power of the second unit was set at 0%, while ISI was greater than 0 sec (i.e., 10 sec). We used a double cone coil (part number: 9902AP; 120 mm outer and 90 mm inner diamater of each winding with an 100° angle between windings; maximal output of 1.4T; weight ~ 2 kg; average inductance 17.85 μH) that induced a posteroanterior intracranial current. The stimulated pulse was monophasic current waveform with rise and duration time of 100μs and 1ms, respectively. The TMS user controlled the coil manually, and its position in relation to the desired stimulated spot was corrected when it was necessary prior to each stimulus using the neuronavigation system. Throughout MCE testing, the ISI was controlled manually by the TMS user and ranged randomly from 5-10 s to avoid stimulus anticipation and to minimize the carry-over effects of the previous pulse to the subsequent one (Awiszus, 2003). In both conditions, we instructed participants to keep their legs still and fully relaxed between stimuli.

To ensure that the hot spot of the SOL and TA was systematically stimulated in both hemispheres and across participants, we placed and used the neuronavigated 36-spot grid over each hemisphere (Figure 3.6 Step 5-I). Prior to the hot spot grid protocol, a suprathreshold intensity was determined by applying a single stimulus over the centered spot next to the medial fissure (Figure 3.6 Step 5-A). We chose to use that single spot in that step to achieve consistency between days and across participants, and because that spot is located at the locus of the leg motor area; our decision was based on evidence

from neurophysiological studies (Alkadhi et al., 2002; Conti et al., 2014)**.** We started always at 30% MSO and gradually increased the TMS intensity by 5% increments, until the intensity that elicited MEP with peak-to-peak amplitudes was greater than 50 µV in all contralateral target muscles in 3 consecutive stimuli. Both raw waveforms with total duration 500 ms (100 and 400 ms pre- and post-TMS, respectively) and peak-to-peak amplitudes (25-125 ms post TMS) of all muscles were displayed and determined immediately after each stimulus using Signal v5.11 (Cambridge Electronic Design; Cambridge, UK). We used the intensity determined in the previous step for the hot spot hunting for two reasons. First, that intensity was most likely above SOL and TA rMT since MEPs were elicited at least in both muscles in 3 consecutive stimuli. Second, it has been suggested a slighty supratheshold intensity should be used for mapping procedures when target muscle is in the resting state (Krings et al., 1997; Saisanen et al., 2010), although in the present study a single stimulus was applied over each point of the 36-spot grid (Figure 3.6 Step 5-B) compared to 3-5 stimuli per spot used typically in mapping studies (Malcolm et al., 2006; Wassermann, McShane, Hallett, & Cohen, 1992). During hot spot hunting, which was done in both days, the stimulation order was always the same (i.e., start at the top spot in the most rightward column and move downwards) (Figure 3.5). After we completed the hot spot protocol, the amplitude values of each spot for all muscles were tranferred and sorted from high to low in a Excel spreadsheet. The hot spot of contralateral SOL and TA was defined as the spot in the grid that a single stimulus applied over that spot elicited the largest response (Rossini et al., 2015) as quantified by the peak-to-peak amplitude (µV) on the contralateral SOL and TA, respectively. Contrary to the general notion that a single spot is used to elicit MEPs in

both SOL and TA (e.g., 0-2 cm posterior and 1-2 cm lateral to the vertex when either figure-of-eight or double cone coil was used), we found that SOL and TA hot spots differed within each hemipshere in the majority of cases. Thus, each hot spot, which was the scalp position over the virtual spot at the neuronavigated grid (Figure 3.6 Step 5-C), was used to determine the rMT of each muscle and to apply 10 stimuli at 120% rMT of each muscle in two muscle state conditions. The first condition was the resting state, in which the target muscle was fully relaxed; whereas during the second condition, the active state, the target muscle was slightly contracted at 15% MVIC. For the remaining procedures the MCE testing was fixed; we always started the MCE testing on the right TA and SOL (Stroke: Day 1; Healthy: Days 1 & 2). At the second visit, when only the MCE of the paretic muscles was assessed in people post-stroke, TA was tested before SOL.

rMT is a fundamental parameter for TMS protocols, both assessment and treatment, and it can be used either as a measured outcome or to test MCE using a suprathreshold intensity to apply a specific number of stimuli (e.g., 10) in order to elicit MEPs. To determine rMT, there are few established methods (Farzan, 2014). The most widely used and recommended by the International Federation for Clinical Neurophysiology is the relative frequency method (Groppa et al., 2012), which is based on the following Rossini-Rothwell criterion (Rossini et al., 1994; Rothwell et al., 1999): the MEP response to half of N stimuli, usually 10, must be greater than 50 µV. In the present study, nevertheless, we used an adaptive threshold-hunting method over the relative frequency method because the former is more efficient (i.e., fewer stimuli are required to determine rMT) than the latter; both methods share similar precision (Silbert,

Patterson, Pevcic, Windnagel, & Thickbroom, 2013). There are two types of adaptive threshold-hunting: the simple adaptive parameter estimation by sequential testing (SA-PEST) and the maximum likelihood PEST (ML-PEST) (Awiszus & Borckardt, 2011). Between the two, we selected the SA-PEST because it determines more accurately rMT than the ML-PEST, even though the latter can determine rMT quicker (Borckardt, Nahas, Koola, & George, 2006). The first intensity used in rMT hunting was always set at 45% MSO, while the initial step size was set at 6% MSO. For example, if the first stimulus at 45% MSO induced a MEP with amplitude greater than 50 µV on the contralateral target muscle, the next intensity was set at 39% MSO. Conversely, if amplitude was less than 50 µV on the contralateral target muscle, the next intensity was set at 51% MSO. We ran twice the rMT protocol for each target muscle and then calculated the average of the two (Figure 3.6 Step 5-II). For both resting and active conditions, we used a suprathreshold intensity, 120 % of the averaged rMT. We chose that intensity because it is the most commonly used and is high enough to elicit MEP at the target muscle. We did not calculate the aMT of the target muscles because there is not well established methodology for that measure. Besides, aMT was found to be correlated with and lower than rMT (~ 82%) (Ngomo, Leonard, Moffet, & Mercier, 2012). Therefore, even when using a suprathreshold intensity of rMT, the intensity is high enough that MEP can be elicited. The resting condition was always administered prior to active condition.

During the resting condition, we asked participants to stay still and relax the target muscles bilaterally, especially the contralateral muscles, while a single pulse TMS at 120% rMT and over the hot spot of the target muscle was applied 10 times (Figure 3.6 Step 6-I). Prior to each stimulus, the muscle activity of all muscles was monitored by a

real time visual feedback displaying on a computer screen. In case any contralateral target muscles were active before or after TMS. we applied a single pulse again. The goal was to get 10 waveforms of each contralateral target muscle at rest.

During the active condition, we asked participants to contract the target muscle at 15% MVIC while a single pulse TMS at 120% rMT was applied over the hot spot of the target muscle 10 times (Figure 3.6 Step 6-II). When SOL was the target muscle, we instructed participants to push slightly down against the boot on their contralateral leg, match the moving line with the horizontal cursor, and sustain that contraction at that level for few seconds. When TA was the target muscle, we instructed participants to pull slightly up against the bootstraps on their contralateral leg, match the moving line with the horizontal cursor, and sustain that contraction at that level for few seconds. The moving line was the rectified and low-pass filtered (RMS amplitude of 0.165 sec) EMG of the target muscle, whereas the cursor was set at 15 % MVIC of the target muscle prior to the onset of the active condition. Participants were instructed to maintain contraction at least 1 sec after TMS and to relax between stimuli. As in resting condition, the muscle activity of all muscles was monitored by a real time visual feedback displaying on a computer screen.

3.4.3 SOL and TA Neuromechanics during Walking

In addition to EMG electrodes that were already attached on the participants, we positioned bilaterally 36 active LED markers on the pelvis, knee joint, shank, ankle joint, and foot using a lower extremity specific configuration. Then participants wore a safety harness (Robertson Mountaineering; Henderson, NY, USA), which was worn across the shoulders and chest and was mounted to the laboratory ceiling for protection in the event

of loss of balance. The harness off-loaded no body weight.

After setup, participants stood on the center of the treadmill for 5 sec to capture a static trial, from which a static model of each participant was reconstructed and used for the inverse dynamic analysis. During walking assessment, people post-stroke walked on a dual-belt treadmill instrumented with 2 independent 6-degree of freedom force platforms (Bertec Corporation; Columbus, OH, USA; treadmill dimensions: 206 x 139 x 39 cm; size of each belt: 175 x 30 cm; walking surface: 175 x 62 cm) under one walking speed condition, self-selected walking speed (SSWS), and grade, 0 degrees (Figure 3.4 E). During SSWS, we instructed participants to “Walk at your normal usual walking pace.” During walking assessment, a licensed physical therapist was always present to guard closely the participants as they walked on a treadmill; however, no form of manual support was provided during the actual data collection. Since participants had to walk without an assistive or orthotic device before we begun testing, they were allowed to practice treadmill walking until they felt comfortable walking without assistive and orthotic devices. During this practice time, the physical therapist offered any physical support the subject may have needed. After each participant reported comfort walking on the treadmill without any assistance, SSWS was determined by setting the treadmill speed initially at 0.2 m/s. Treadmill speed was gradually increased by 0.05 m/s increments until the participant reported that the current speed was his/her SSWS. Once SSWS was determined, participants walked for three 1-minute trials. Between trials, participants rested for at least 1 minute or until they were ready to resume. In addition, participants were allowed to sit any time they felt there was a need.

3.4.4 SOL and TA Neuromechanics during MVIC

During MVIC, people post-stroke sat comfortably with similar posture as during MCE testing on a Biodex Multi-Joint System Pro dynamometer (Biodex Medical Systems, Shirley, NY, USA) with their test leg fixed to a limb-support pad and a footplate (Figure 3.4 F). The shank was parallel to the floor, and the foot was firmly secured by placing the calcaneus in the metal heel cup mounted on the footplate, with plastic straps fastened across the toes and the dorsum of the foot. The back of the chair was set at 85° from horizontal, while the dynamometer axis of rotation was aligned with the lateral malleolus of the ankle (i.e., ankle joint axis of rotation). To ensure proximal stabilization of the participants during contractions, a waist strap, thigh strap, and two crossover shoulder straps were used.

After performing a single practice trial for each motion to ensure that participants understood the procedures, all participants performed three successive MVIC of PF and DF with the non-paretic leg. We repeated the same procedures with the paretic leg. During each contraction, we instructed participants to sit with their arms folded across their chest and to “gradually push down on” (SOL) or “gradually pull up on” (TA) the footplate “as hard” as they could and “hold it for few seconds.” Additionally, during each contraction, participants received a verbal encouragement from the experimenter to ensure maximal effort and visual feedback displayed on a screen for their torque output. Each contraction lasted approximately 5 seconds (i.e., rapid contraction and maintenance of 100% MVIC); there was a 60 seconds rest between contractions.

3.5 Data Collection

3.5.1 Muscle Activity

Activity of four muscles was collected bilaterally in all three labs using the same

sEMG electrodes and hardware, yet the software used to acquire EMG data at each lab differed. We used pre-amplified (x20) single double differential MA-411 (Motion Lab Systems; Baton Rouge, LN, USA) sEMG electrodes (body size of 38 mm x 19 mm x 8 mm, two 12 mm discs of medical grade stainless steel, a 13 x 3 mm bar of medical grade stainless steel separating the two discs, and inter-electrode distance of 17 mm). The anti-alias filter and gain were preset at 1000 Hertz (Hz) and 2000, respectively, on MA-300 Back-Pack Unit (Motion Lab Systems; Baton Rouge, LN, USA). During MCE testing, the muscle activity signal was sampled at 5000 Hz using CED Micro 1401-3 data acquisition unit (Cambridge Electronic Design; Cambridge, UK). Online data acquisition was achieved using both Spike2 v7.12 (EMG electrode placement and signal quality testing, MVIC protocol, active condition) and Signal v5.11 (rMT protocol and resting condition) software. During walking and MVIC, muscle activity signal was sampled at 2000 Hz. However, it was acquired using LabVIEW (National Instruments; Austin, TX, USA) and WinDaq v3.30 **(**DATAQ Instruments, Inc.; Akron, OH**,** USA**)** during walking and MVIC, respectively. We saved EMG data automatically after each trial as coded data files on a password-protected computer at each lab for offline analysis.

The preparation and placement of sEMG always occurred prior to MCE testing at FNL using published guidelines (Cram & Criswell, 2011; Hermens & Klug, 1999) with the participants in the standing position. First, to improve the electrode-skin contact, the areas over which the electrodes would be placed were shaved and lightly exfoliated with alcohol swaps to remove any dead skin cells and oils from the skin surface. Then, eight sEMG electrodes were attached over TA, SOL, LG, and MG bilaterally. For the TA EMG placement, participants were in upright position and instructed to lift toes upwards.

Then we placed the TA sEMG electrode at 1/3 of the line between the tip of the fibula and the tip of the medial malleolus (Cram & Criswell, 2011). For the SOL, LG, and MG placement, participants were also in upright position while raising their heels. We placed the SOL sEMG electrode at 2/3 of the line between the lateral femoral condyle to the lateral malleolus (Cram & Criswell, 2011). We placed the LG and MG sEMG electrodes at the most prominent aspect of the muscle belly 2 cm laterally and medially from the midline, respectively (Cram & Criswell, 2011). A reusable stainless steel ground reference passive electrode with a diameter of 30 mm (Natus Medical Incorporated; San Carlos, CA, USA) was placed on the patella. We applied electrolyte gel (SIGNALGEL®**,** Natus Medical Incorporated**;** San Carlos, CA, USA) on the metal surface of the ground electrode to optimize the conductive path between that surface and skin (Kamen & Gabriel, 2010). We attached all sEMG electrodes and the reference electrode on the skin using surgical tape.

After electrodes fixation and prior to experimental procedures, we first tested the electrodes placement (e.g., for clear visually detectable EMG burst) and then the quality of the signal (e.g., for baseline noise). To test the placement of the electrodes, we asked participants to either plantarflex or dorsiflex their ankle in an upright posture while the raw EMG signal of all muscles tested was displayed on a computer screen. All participants from both groups were capable of performing those tasks, yet a few people post-stroke required some assistance with standing balance while performing those ankle motions. If an electrode was misplaced, we removed and replaced it until there was a clear visually detectable EMG burst. To test the signal quality, we discharged the capacitor a few times while the TMS coil was held away from the participant seated on

the chair in resting state. The expectation was that the baseline signal at each EMG channel should be close to zero since TMS pulse was applied on air and not at the participant's head. If there was no baseline noise (e.g., 60 Hz power line hum), the peak-to-peak amplitude was less than 50 μV. If baseline noise occurred at a channel, we removed the corresponding electrode and repeated the skin preparation procedures. If the noise was still present (i.e., peak-to-peak amplitude > 50 μV), we adjusted the reference electrode's position and replaced the electrolyte gel. After we determined that the electrodes' position and signal quality were satisfactory, we secured the electrodes using underwrap. Periodically throughout the experimental procedures, we checked electrodes' position and signal quality.

3.5.2 Ankle Joint Kinematics and Kinetics during Walking

Twelve motion capture system cameras (PhaseSpace Inc.; San Leandro, CA, USA) tracked the 36 active LEDs during a static trial and walking trials, in which pelvis, shank and foot segments were defined bilaterally using a lower extremity specific configuration. Before any testing, the capture volume space surrounding the treadmill was calibrated using a calibration wand; calibration lasted less than 5 minutes. GRF was measured bilaterally by force plates, which were built-in underneath each treadmill belt. To optimize capture of steady state data on the treadmill, participants walked for 10-30 seconds prior to the 30 seconds of data collection (~ 1 min per trial). The coordinates of active LED markers, GRF, and EMG of four muscles were collected bilaterally during each trial. Coordinates data were sampled at 120 Hz, while the GRF data were sampled at 2000 Hz. Both sets of data were acquired using LabVIEW (National Instruments; Austin, TX, USA). All types of signals were saved automatically after each trial as coded data

files on a password-protected computer for offline analysis.

3.5.2 Ankle Joint Kinetics during MVIC

Peak torques and EMG of the SOL and TA were simultaneously collected during each trial. Like EMG, torque data were sampled at 2000 Hz, acquired using DI-720-USB (DATAQ Instruments, Inc.; Akron, OH**,** USA), and collected using WinDaq/Pro v3.30 (DATAQ Instruments, Inc.; Akron, OH**,** USA). Both types of signal were saved automatically after each trial as coded data files on a password-protected computer for offline analysis.

3.6 Data Analyses

All offline data analyses were carried out using a customized script written in Matlab v8.1 (Mathworks, Inc.; Natick, MA, USA).

3.6.1 SOL and TA MCE

For all measures but rMT, we calculated the value of each measure from each MEP sweep (total duration of 500 ms: 100 ms pre-TMS and 400 ms) for all muscles and then averaged these 10 values to get a single value (i.e., mean). A recent study demonstrated this approach to be more reliable than using the value calculated from the average MEP sweep in people post-stroke (Lewis et al., 2014). The EMG signal of each muscle collected during MCE testing was corrected for the gain amplification, and the units were converted from Volts to mV.

<u>Resting Condition:</u> rMT, amplitude, latency, and normalized latency were calculated to quantify SOL and TA MCE while the target muscle was relaxed. rMT was expressed as a percentage of the MSO (%) of the machine; that value was recorded

directly from the output display on the master unit of the stimulator pair. Amplitude (mV) was calculated from the raw EMG and defined as the largest difference between positive and negative peaks (i.e., peak-to-peak) of the MEP (Figure 3.7 A). Latency was calculated from the rectified EMG (Figure 3.7 B). Latency (ms) was defined as the time between TMS onset and MEP onset, which was defined as the time when a rectified EMG trace first crossed a predetermined threshold, pre-stimulus mean EMG (over a 100 ms period) plus 3 standard deviation (SD) (Cacchio et al., 2011). In addition to raw latency, we normalized latency relative to each participant's height ([latency (ms) / height (cm)] $*$ 100) because latency was found to be correlated with participant's height (Livingston, Friedlander, Gibson, & Melvin, 2013).

Active Condition: Amplitude, latency, normalized latency, and cSP were calculated to quantify SOL and TA MCE while the target muscle was slightly contracted at 15% MVIC. As in resting condition, amplitude (mV) was calculated from the raw EMG and defined as the largest difference between positive and negative peaks (i.e., peak-to-peak) of the MEP (Figure 3.7 C), and latency (ms) was calculated from the rectified EMG and defined as the time between TMS onset and MEP onset (Figure 3.7 D). Yet MEP onset in active state was calculated differently than in resting. For the calculation of the MEP onset and offset, and for EMG resumption, which was used for cSP calculation (see below), we adopted an automated approach published previously (Damron, Dearth, Hoffman, & Clark, 2008; Daskalakis et al., 2003). Additionally, we slightly modified this automated method to ensure that the MEP onset and offset were accurately calculated across muscles and participants. First, we found the time points that the rectified EMG trace crossed and predetermined threshold set to the level of pre-

stimulus mean EMG (over a 100 ms period). Then we found the peaks that were at least greater than the mean of the pre-stimulus EMG plus three SD and between those two time points. Then we searched from the first peak to 50 data points before that peak for the time that the rectified EMG trace first crossed the threshold of the mean pre-stimulus EMG; we defined that time as the MEP onset. Similarly, we searched from the last peak to 200 data points after that peak for the time that the rectified EMG trace last crossed the threshold of the mean pre-stimulus EMG; we defined that time as the MEP offset. As in resting, the normalized latency relative to each participant's height ([latency (ms) / height (cm)] ∗ 100) was calculated. Lastly, cSP (ms) was also calculated from the rectified EMG (Figure 3.7 D). In the present study, we calculated the absolute cSP (i.e., exclusion of MEP): the time between MEP offset and the resumption of baseline EMG (Saisanen, Pirinen, et al., 2008). EMG resumption was the time that the rectified EMG trace last crossed the 25% of the mean pre-stimulus EMG (Damron et al., 2008).

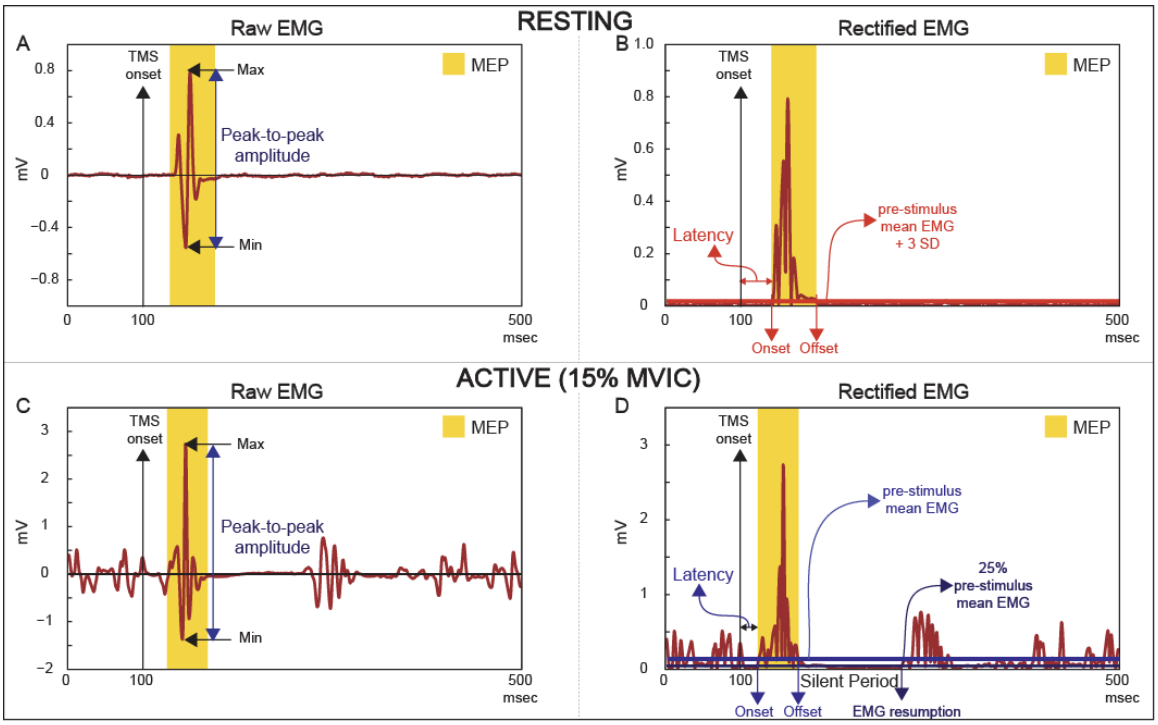

Figure 3.7: Schematic of the analysis of the MCE measures

3.6.2 SOL and TA Neuromechanics during Walking

All measures collected during gait were divided into strides (i.e., starting at foot contact of one leg and ending with the next foot contact of the same leg). Additionally, all those gait measures except GRF_{AP} were linearly interpolated to 100 points (i.e., GC was normalized to stride period and presented in %). In order to do this, specific gait events, bilateral heel strikes and toe offs, were determined using an automated method, which used GRF_V (Zeni, Richards, & Higginson, 2008). GRF_V was filtered with a low-pass 20 Hz cut-off frequency using a fourth order, zero phase lag Butterworth filter (Gottschall & Kram, 2003) and was resampled at 120 Hz. After digital filtering, any potential offsets were corrected, and body weight (N) was calculated using bilateral GRF_V, which were then normalized by the body weight and multiplied by 100. A threshold of 20 N divided by the body weight (N) multiplied by 100 was used to determine the threshold crossings in GRF_V trace. Each crossing at this preset threshold denoted bilateral heel strike and toe off (Figure 3.8).

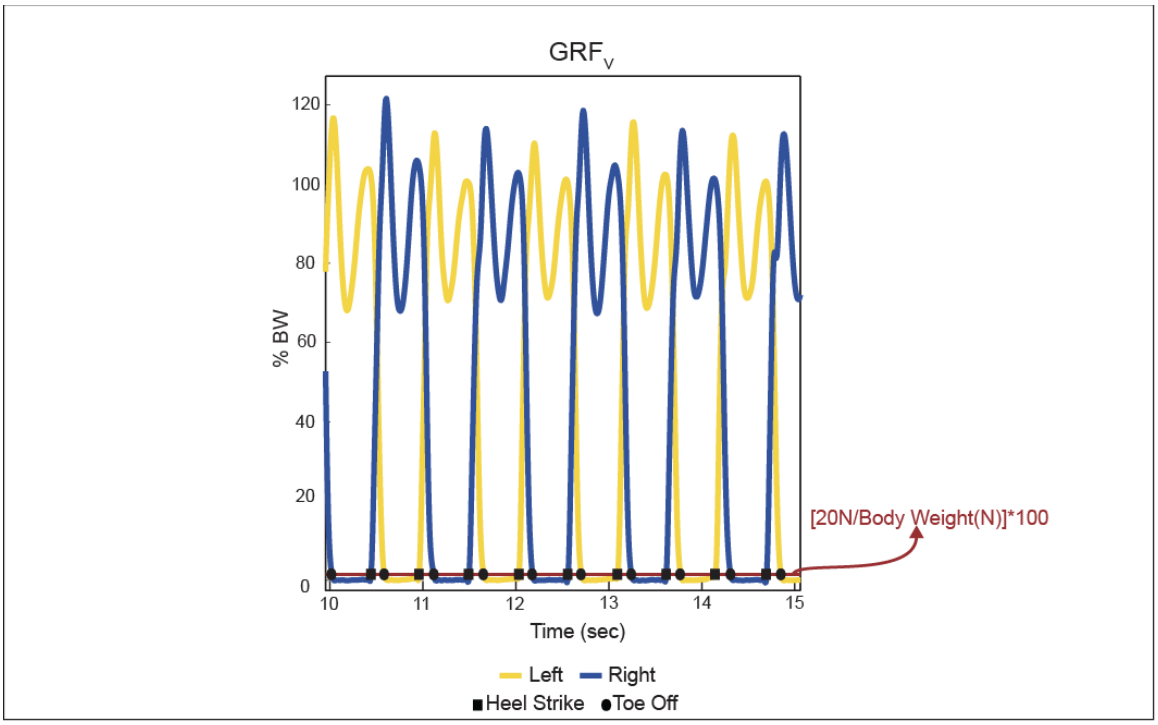

Figure 3.8: Outline of the analysis used to detect gait events

The first step for EMG analysis was to divide the EMG of each muscle by the gain (i.e., 2000) and then multiply by 1000 to convert the units from Volts to mV. Then EMG data were band-pass filtered (20-500 Hz) and band-stop filtered (156-158 Hz – to remove a 157 Hz power line hum which was detected using a spectrum analysis) using a third order zero phase lag Butterworth filter, rectified, smoothed (50 Hz filter), divided into strides, and averaged across strides. Then the average EMG of each muscle was calculated in specific gait phases based on when each muscle was typically active: single leg stance and second double limb support for SOL (Figure 3.9 A) and first half of swing for TA (Figure 3.9 B). Lastly, these EMG values (mV) were averaged across trials.

GRF_{AP} was filtered with low-pass 20 Hz cut-off frequency using a fourth order, zero phase lag Butterworth filter (Gottschall & Kram, 2003), normalized by the body weight (N), multiplied by 100, and divided into strides. In order to calculate accurately the positive area under the curve during the SLS and DLS2, we searched first for the maximum positive peak between 30 data points after the initial heel strike and next heel strike. We selected 30 data points as a mark for two reasons. First, the period of the GRF_{AP} that we were interested in occurred approximately 0.25 sec after the initial heel strike, and secondly to avoid miscalculating the fast spike of propulsion happening immediately after heel strike. After finding the positive peak, we found the times that last crossed the zero line left and right from the positive peak. Next, the area under the positive curve between those two time points was calculated (i.e., PI; % BW.sec) for each stride and averaged across strides and trials (Figure 3.9 C).

The first step for AAV analysis was to resample the coordinates of active LED markers data from 120 Hz to 100 Hz. Then AAV was calculated using the numerical

differentiation of the time-based ankle joint sagittal angle (i.e., Euler angle that was calculated using rotational matrices). All aforementioned steps were carried out using a custom written script in LabVIEW, the only data analysis steps that used a software other than Matlab. After transferring AAV data to a Matlab workspace, we divided AAV into strides. The peak AAV (rad/sec) during the first half of swing was calculated for each stride and averaged across strides and trials (Figure 3.9 D).

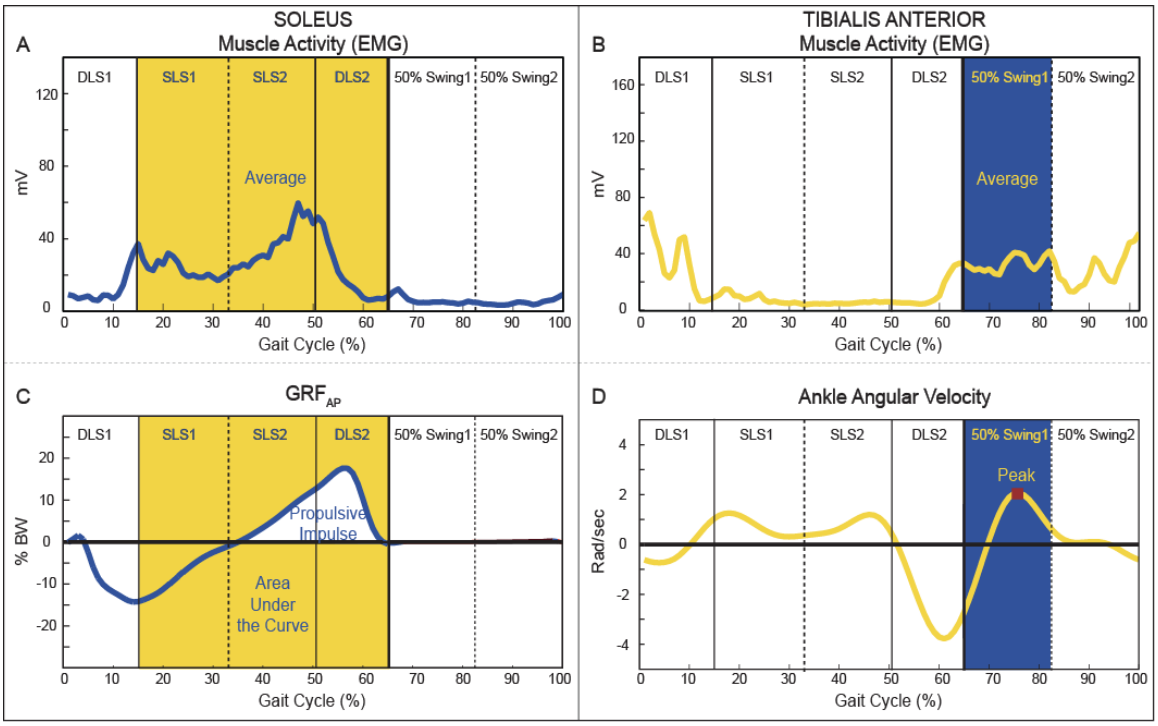

Figure 3.9: Outline of the analysis of the SOL and TA walking neuromechanics

3.6.3 SOL and TA Neuromechanics during MVIC

For both SOL and TA, the isometric torque was first low-pass filtered (10 Hz) using a third order zero lag Butterworth filter and then calculated by averaging the torque within a 100 ms window centered around the max torque of each trial (Figure 3.10 A). A 500 ms window (i.e., ± 250 ms from max) has been reported to be used for simultaneous torque and EMG analysis in healthy adults (Soylu & Arpinar-Avsar, 2010). However, in this study we used a 200 ms window (i.e., ± 100 ms from max) to avoid occasions where

the peak occurred immediately after the contraction onset, a case that was observed in pilot testing. Therefore, the 200 ms window was selected after getting consistent results in the analysis of pilot data. Then torque (N·m) was subsequently averaged across the three trials.

Similar to walking EMG analysis, the EMG of each muscle was first divided by the gain (i.e., 2000) and then multiplied by 1000 to convert the units from Volts to mV. The EMG data was band-pass (20-500 Hz) and band-stop filtered (59-61 Hz – to remove a 60 Hz power line hum which was detected using a spectrum analysis) using a third order zero phase lag Butterworth filter, rectified, smoothed (50 Hz filter), and averaged over the same time window as torque (Figure 3.10 B). Then it (mV) was subsequently averaged across the three trials.

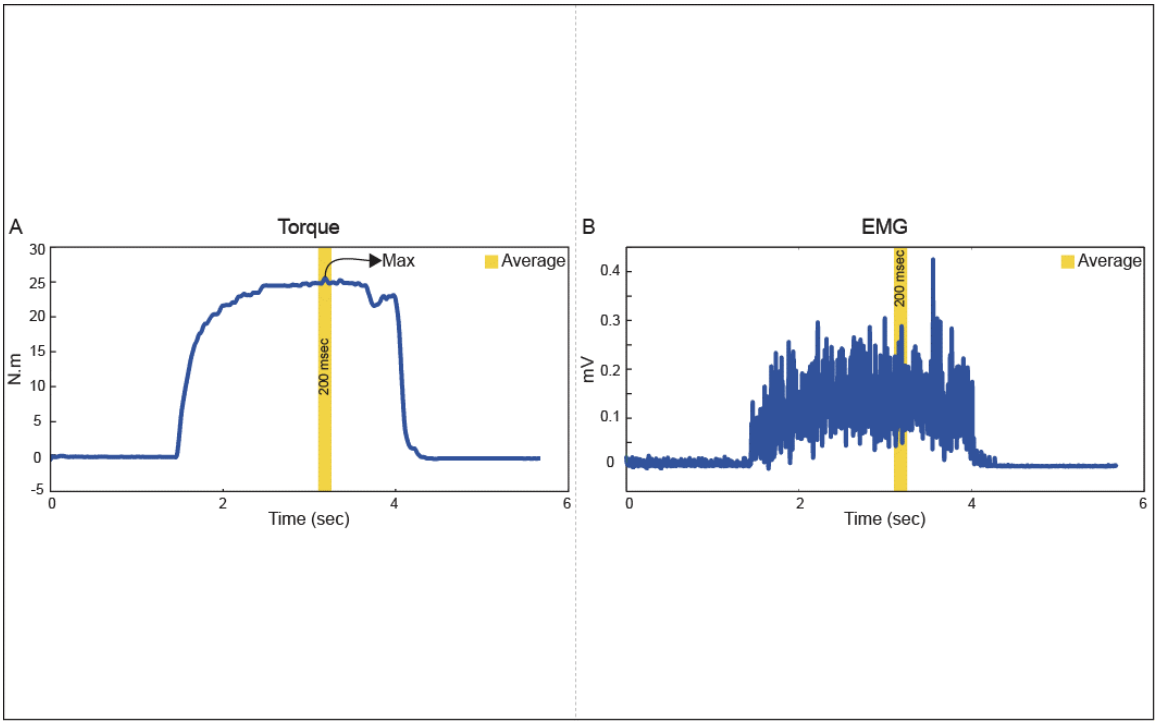

Figure 3.10: Outline of the analysis of the SOL and TA MVIC neuromechanics

3.7 Statistical Analyses

For this project, we focused our statistical analyses on the paretic SOL and TA in people post-stroke and on the right (i.e., dominant) SOL and TA in healthy controls, although we collected SOL and TA data from both legs and from LG and MG bilaterally. We will run secondary analyses for extra data collected. In addition to the mean and SD, we reported the coefficient of variation (CV), which is independent of units and is a measure of relative variation (Portney & Watkins, 2009). Therefore, three descriptive statistics (mean ± SD, CV) were reported for all data used in those analyses. The inter-group differences in age, height, weight, and test-retest period were tested. Normality of all data was tested using the Shapiro-Wilk test because it has more power to find differences than the Kolmogorov-Smirnov test (Field, 2009). We conducted statistical analyses using IBM SPSS Statistics for Windows v23 (IBM Corp, Armonk, NY, USA). Figure 3.11 presents the overall statistical analyses carried out in each aim.

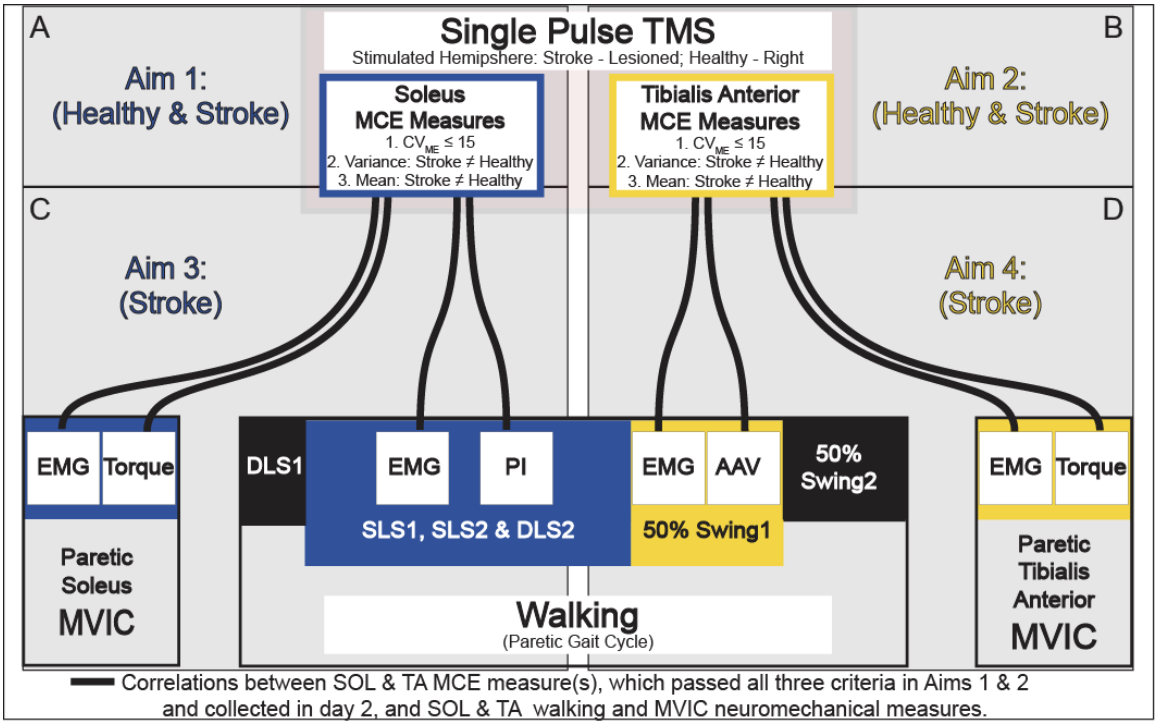

Figure 3.11: Outline of the statistical analyses.

3.7.1 Aims 1 and 2

These aims were exploratory and had two goals. The first goal was to characterize the MCE measure (s) of SOL and TA that had low methodological error (i.e., reliable) both in people post-stroke and in healthy controls. The second goal was to identify stroke-related impairments to the CNS (i.e., detect true differences between people post-stroke and healthy controls) testing the variances and means between the two groups. To achieve these goals, we ran three statistical procedures/steps that were the same for both aims. The level of significance was set at $p < 0.05$, and the test of significance was one-tailed.

First, we examined the test-retest reliability of all SOL and TA MCE measures using the coefficient of variation of method error (CV_{ME}) instead of using ICC, which is the most widely used reliability metric. ICC is the ratio of the between-subject variance to the total variance (sum of the between-subject and between-day variance), is a relative measure of reliability, has no units, and ranges from 0 to 1, with a value closer to 1 reflecting greater reliability (Weir, 2005). However, it has one main pitfall. When samples are homogeneous, ICC of the target measure is low, but low ICC does not necessarily mean the measure is unreliable. Therefore, pure reliance on ICC may lead to incorrect conclusions. For this reason, we did not use ICC as the reliability metric. Nevertheless, we calculated and reported ICC with lower and upper 95% confidence interval (CI) so we could conduct post-hoc comparisons with the present limited evidence. Briefly, we calculated ICC using the 2-way random model with absolute agreement type and average measures form. ICC values above 0.75 indicated good reliability (Portney & Watkins, 2009). We used CV_{ME} for two reasons: (1) CV_{ME} is

insensitive to the lack of variation in the samples and (2) CV_{ME} is independent of ICC (Portney & Watkins, 2009). To calculate CV_{ME}, we first calculated the method error, which is the ratio of the SD of the test-retest score differences to √2 (Portney & Watkins, 2009). Then CV_{ME} was calculated using the following equation: (2ME / (Mean of Day 1 + Mean of Day 2))*100 (Portney & Watkins, 2009). The advantage of using CV_{ME} instead of ME is that CV_{ME} is expressed as a percentage and has no units as ICC. A measure with CV_{ME} equal or less than 15 was considered a reliable measure (Stokes, 1985). For steps 2 and 3, we used measures whose CV_{ME} was lower than 15. If no measure met that requirement, we chose the measure with the lowest CV_{ME}. Based on the previous literature, we expected that at least one measure for each muscle would have low CV_{ME} (≤ 15).

The second step was to examine whether the between-subject variability differed for each group. Because the MCE measures used in these procedures would have good reliability determined in the previous step, we expected that the within-subject variability would be low in both groups. Low between-subject variability in healthy controls would result from random factors (i.e., unsystematic variation). Conversely, between-subject variability in stroke would be high because the differences in stroke characteristics had different (and larger) effects on the MCE in each stroke patient. Thus, the difference in variability between the two groups would most likely be due to the CNS lesion. Therefore, the second procedure tested whether the variance (i.e., SD^2) of a MCE measure collected in day 2 differed between people post-stroke and healthy controls using Levene's test. If the results of Levene's test were significant ($p \leq 0.05$), this finding would imply that the variance of the MCE measure differed between the two groups, and

the third procedure was carried out for that MCE measure.

The third step examined whether the means (day 2) of the two groups differed. A recent study reported that the MEP amplitude and latency of the paretic TA decreased and increased, respectively, compared to the dominant side of the healthy controls (Beaulieu et al., 2014). These changes in MCE after a stroke also reflected the lesioned CNS. We tested the normality of each distribution to ensure that we had selected the right test, either parametric or non-parametric, to examine the differences in mean between the groups. We used an independent *t*-test when the data were normally distributed; otherwise, we used the Mann–Whitney *U* test. If the group means were significantly different ($p \leq 0.05$), the MCE measure of either the paretic SOL or paretic TA was used in Aim 3 and 4, respectively.

In summary, the MCE measure(s) used in Aims 3 and 4 had to demonstrate the following: 1) good reliability, $CV_{ME} \leq 15$, in both groups, 2) significantly higher variance in people post-stroke versus in healthy controls (Levene's test), and 3) a significant difference in means between people post-stroke and healthy controls (independent *t*-test or Mann–Whitney *U* test) (Figure 3.11 A & B).

3.7.2 Aims 3 and 4

The MCE measure(s) determined by Aims 1 and 2 were used in the statistical analyses for Aims 3 and 4, respectively. Because we assessed eight MCE measures each for Aims 1 and 2, there was a possibility for more than one MCE measure to qualify for use in the correlational analyses of Aims 3 and 4. However, due to the small sample size, a maximum of two MCE measures that passed all three statistical steps in Aims 1 and 2 was used for each muscle. Therefore, the maximum total number of associations

performed for each muscle was eight; otherwise, a minimum of four associations per muscle would need to occur. Since we performed multiple associations for each aim (minimum four and maximum eight), we corrected the level of significance using a Bonferroni adjustment. In case of four associations, the level of significance was adjusted to 0.012, whereas in case of eight associations, the level of significance was adjusted to 0.006. The test of significance was two-tailed.

To test the relationships between MCE (day 2) and neuromechanics for each muscle group, we performed correlations between the MCE and walking neuromechanics and between MCE and MVIC neuromechanics (Figure 3.11 C & D). The strength and direction of each correlation was determined using correlation coefficients. The selection of the correlation coefficient, either parametric or non-parametric, was determined by testing the normality of each distribution. If both distributions were normally distributed, we used Pearson's correlation coefficient (r); otherwise, we used Spearman's correlation coefficient (r_s). Correlation coefficients less than 0.5, greater than 0.5 and less than 0.75, and greater than 0.75 indicated a poor, good, or excellent relationship, respectively (Portney & Watkins, 2009).

<u>Aim 3:</u> For this aim, we examined the relationships between paretic SOL MCE and paretic SOL neuromechanics during walking and MVIC. At least four associations were tested (Figure 3.11 C). During walking, we tested the relationships between SOL MCE measure(s) and SOL EMG, and SOL MCE measure(s) and PI. During MVIC, we tested the relationships between SOL MCE measure(s) and SOL EMG, and SOL MCE measure(s) and SOL torque.

<u>Aim 4:</u> For this aim, we examined the relationships between paretic TA MCE and

paretic TA neuromechanics during walking and MVIC. At least four associations were tested (Figure 3.11 D). During walking, we tested the relationships between TA MCE measure(s) and TA EMG, and TA MCE measure(s) and AAV. During MVIC, we tested the relationships between TA MCE measure(s) and TA EMG, and TA MCE measure(s) and TA torque.

CHAPTER FOUR

RESULTS

4.1 Group Characteristics

Healthy: Of the 25 participants, one female, and one male withdrew from the study due to TMS discomfort, one male's data could not be used due to insufficient MRI quality, and one male could not attend the second session. Therefore, all data presented here are from 21 healthy controls (8F/13M, 42 ± 11 years, 174 ± 12 cm, 75 ± 17 kg).

Stroke: Of the 15 participants, one female withdrew from the study due to TMS discomfort. Therefore, all data presented here are from 14 stroke participants (6F/8M, 62 ± 13 years, 173 ± 12 cm, 83 ± 22 kg). Lesion related characteristics (type, subtype, and location) were characterized by a stroke neurologist using each participant's MRI. Table 4.1 presents the individual and group demographical, clinical, and experimental characteristics of 14 stroke participants.

People post-stroke (N = 14) were significantly older than the healthy adults (N = 21) were (p = 0.000; Figure 4.1 A), whereas there were no significant differences between groups for height (p = 0.855; Figure 4.1 B), weight (p = 0.561; Figure 4.1 C), and test-retest period (p = 0.907; Figure 4.1 D). All participants in both groups were right-leg dominant. We determined leg dominance by simply asking participants the question: "Which leg would you use the most if you had to kick a soccer ball three times?"

#	GENDER	AGE (years)	HEIGHT (cm)	BODY MASS (kg)	STROKE TYPE	LESIONED HEMISPHERE	SUBTYPE	LESION LOCATION	PARESIS	TIME POST STROKE (months)	FM-LE	SSWS (m/sec)	TEST-RETEST (days)
1	Female	72	152	71	Ischemic	Left	Subcortical	PLIC	Right	62	27	0.60	7
2	Male	64	180	103	Ischemic	Left	Subcortical	Pontine	Right	19	25	0.50	13
3	Female	47	155	69	Ischemic	Right	Subcortical/Cortical	Temporal lobe, insular, caudate, centrum semiovale	Left	9	29	0.90	7
4	Male	47	188	127	Ischemic	Left	Subcortical	PLIC	Right	10	28	0.80	6
5	Male	79	173	64	Ischemic	Left	Subcortical	Corona radiata	Right	81	28	0.55	8
6	Male	60	168	75	Ischemic	Left	Subcortical/Cortical	Temporal lobe, parietal lobe, corona radiata	Right	13	23	0.20	9
7	Male	69	188	82	Ischemic	Left	Subcortical/Cortical	Frontal lobe, corona radiata	Right	36	26	0.35	7
8	Male	57	191	100	Ischemic	Left	Subcortical/Cortical	Frontal lobe, centrum semiovale, corona radiata	Right	62	21	0.35	6
9	Male	61	163	64	Hemor-rhagic	Left	Subcortical	Putamen	Right	21	27	0.40	11
10	Female	58	183	62	Hemor-rhagic	Left	Subcortical/Cortical	Parietal	Right	69	25	0.95	5
11	Male	71	183	120	Ischemic	Right	Subcortical/Cortical	Insula, putamen, corona radiata	Left	66	30	0.45	7
12	Female	34	168	95	Ischemic	Left	Cortical	Left frontal lobe	Right	12	19	0.40	5
13	Female	79	168	66	Ischemic	Right	Subcortical	Pontine	Left	10	26	0.35	7
14	Female	63	170	70	Ischemic	Left	Subcortical	Cerebral peduncle	Right	14	31	0.40	7
Group	Females= 6	62 ± 13 (21)	174 ± 12 (7)	83 ± 22 (26)	Ischemic= 12	Left=11	Subcortical= 7; Subcortical/Cortical= 6; Cortical= 1		Right= 11	35 ± 27 (78)	26 ± 3 (13)	0.51 ± 0.22 (43)	8 ± 2 (29)

Table 4.1: Demographical, clinical, and experimental characteristics of stroke participants

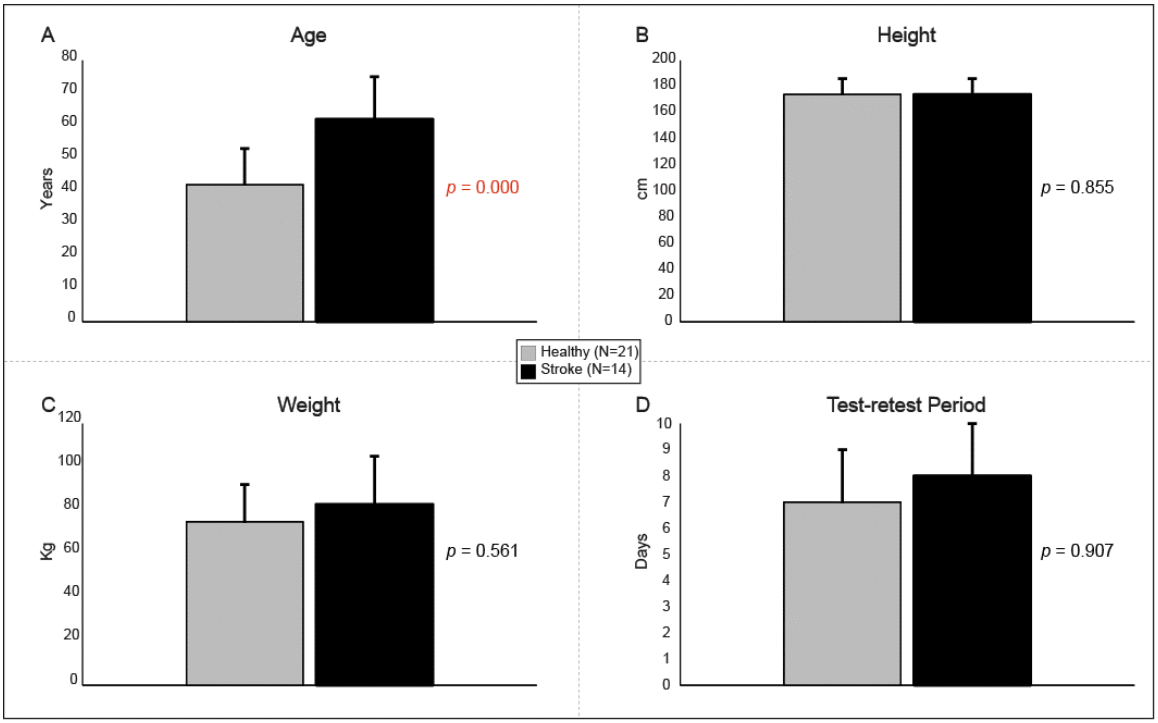

Figure 4.1: Inter-group differences in age, height, weight and test-retest period

4.2 Aim 1: Quantification of the Soleus MCE Measures in Stroke and Healthy

Table 4.2 includes the test-retest normative data of eight SOL MCE measures collected in both groups on day 1 and 2.

4.2.1 Resting Motor Threshold: Responses and Number of Stimulations

Healthy: On day 1, we could not measure the SOL rMT in two participants. In both participants, MEPs on the right SOL were inconsistently elicited using intensities greater than 85% MSO, so we stopped rMT hunting. On day 2, we could measure the SOL rMT in one of the two participants whose SOL rMT could not be determined on day 1; however, we were unable to measure the SOL rMT in the second participant. In summary, we measured the rMT of the right SOL from 19 participants on day 1 and 20 participants on day 2, and fewer than 15 stimulations were required on average to calculate rMT of the right SOL on both days (Day 1: 13 ± 5, 41; Day 2: 15 ± 6, 41).

Stroke: On day 1, we were unable to measure the SOL rMT in five participants. In four participants, we were unable to elicit a MEP on the paretic SOL even with intensities greater than 90% MSO. The fifth participant could not tolerate intensities greater than 70% MSO. On day 2, we again could not measure SOL rMT in those same four participants, but we could measure the SOL rMT of the fifth participant who could not tolerate intensities greater that 70% MSO on day 1. In summary, we measured the rMT of the paretic SOL from 9 participants on day 1 and 10 participants on day 2, and on both days fewer than 25 stimulations on average were required to calculate rMT of the paretic SOL (Day 1: 15 ± 5, 30; Day 2: 22 ± 10, 46).

4.2.2 Testing Reliability

Table 4.3 includes the test-retest reliability metrics, CV_{ME} and ICC, for eight SOL

MCE measures in both groups. For these analyses, we used data collected from 19 healthy adults and 9 stroke participants on both days.

Healthy: All measures but amplitude had CV_{ME} less than 15 during resting condition (Figure 4.2 A) while only raw and normalized latency had CV_{ME} less than 15 during the active condition (Figure 4.2 B). During the resting condition, all measures but normalized latency had ICC greater than 0.75 (Figure 4.2 C), while only amplitude and raw latency had ICC greater than 0.75 in the active condition (Figure 4.2 D).

Stroke: As in the healthy group, for all measures but amplitude, CV_{ME} was less than 15 during the resting condition (Figure 4.2 A), and only for raw and normalized latency CV_{ME} was less than 15 during the active condition (Figure 4.2 B). Of the eight measures, only rMT, amplitude, raw latency during resting (Figure 4.2 C) and cSP during active (Figure 4.2 D) had ICC greater than 0.75.

Only the following measures with CV_{ME} less than 15 in both groups were used in the next step: rMT, raw and normalized latency during resting, and raw and normalized latency during active.

4.2.3 Testing Group Variances

For the variance analyses, we used data collected with the five aforementioned measures from 20 healthy adults and 10 stroke participants on day 2.

The variances of all measures except raw latency in resting were equal between stroke participants and healthy adults (rMT: $F_{(1,28)} = 0.657$, $p = 0.425$; resting normalized latency: $F_{(1,28)} = 0.194$, $p = 0.663$; active raw latency: $F_{(1,28)} = 1.846$, $p = 0.185$; active normalized latency: $F_{(1,28)} = 1.164$, $p = 0.290$) (Figure 4.3 A, C - E). Conversely, the variance of the resting raw latency was significantly higher ($F_{(1,28)} = 6.816$, $p = 0.014$) in

stroke participants (35.9) than in healthy adults (16.6) (Figure 4.3 B). Therefore, we used only the resting raw latency to test whether the group means differed.

4.2.4 Testing Group Means

The resting raw latency in both groups was normally distributed (Stroke: $W_{(10)} = 0.854$, $p = 0.064$; Healthy: $W_{(20)} = 0.983$, $p = 0.967$); therefore, an independent *t*-test was used. The resting latency was significantly longer ($t_{(1,28)} = 2.135$, $p = 0.026$) longer in stroke participants (42.0 ± 6.0 ms) than in healthy controls (37.6 ± 4.1 ms) (Figure 4.4).

In Aim 3, therefore, only the resting raw latency was used in the correlational analyses for walking and MVIC neuromechanics of the paretic SOL.

Soleus - Resting									
Groups	Measures	Motor Threshold (% MSO)		MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)	
	Statistics	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Healthy (Right)	N	19	20	19	20	19	20	19	20
	Mean ± SD (CV)	52 ± 11 (11)	52 ± 12 (23)	0.118 ± 0.063 (53)	0.119 ± 0.058 (49)	37.5 ± 4.4 (12)	37.6 ± 4.1 (11)	22 ± 2 (9)	22 ± 2 (9)
Stroke (Paretic)	N	9	10	9	10	9	10	9	10
	Mean ± SD (CV)	51 ± 9 (18)	53 ± 9 (17)	0.138 ± 0.068 (49)	0.140 ± 0.095 (68)	42.6 ± 3.8 (9)	42.0 ± 6.0 (14)	24 ± 1 (4)	24 ± 3 (12)
Soleus - Active (15% MVIC)									
Groups	Measures	MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)		Silent Period (ms)	
	Statistics	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Healthy (Right)	N	19	20	19	20	19	20	19	20
	Mean ± SD (CV)	0.739 ± 0.637 (86)	0.832 ± 0.555 (68)	35.0 ± 4.7 (13)	33.8 ± 3.3 (10)	20 ± 2 (10)	19 ± 2 (11)	124.9 ± 44.1 (35)	157.5 ± 40.9 (26)
Stroke (Paretic)	N	9	10	9	10	9	10	9	10
	Mean ± SD (CV)	0.476 ± 0.158 (33)	0.481 ± 0.202 (42)	37.1 ± 4.2 (11)	36.6 ± 4.5 (12)	22 ± 2 (9)	21 ± 2 (10)	141.8 ± 49.0 (35)	151.1 ± 50.6 (33)

Table 4.2: Test-retest normative data, mean ± SD (CV), of eight SOL MCE measures in Healthy (right SOL) and Stroke (paretic SOL)

Soleus - Resting									
Group	Measures	Motor Threshold (% MSO)		MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)	
Healthy (N=19)	Days	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	52 ± 11 (22)	52 ± 13 (25)	0.118 ± 0.063 (53)	0.122 ± 0.059 (48)	37.5 ± 4.4 (12)	37.3 ± 4.1 (11)	22 ± 2 (9)	21 ± 2 (10)
	CV_{ME}	9		26		7		7	
	ICC $_{(95\% CI)}$	0.93 $_{(0.81 - 0.97)}$		0.86 $_{(0.64 - 0.95)}$		0.79 $_{(0.44 - 0.92)}$		0.71 $_{(0.22 - 0.89)}$	
Stroke (N=9)	Day	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	51 ± 9 (18)	52 ± 9 (17)	0.138 ± 0.068 (49)	0.142 ± 0.101 (71)	42.6 ± 3.8 (9)	42.5 ± 6.1 (14)	24 ± 1 (4)	24 ± 3 (12)
	CV_{ME}	4		31		7		7	
	ICC $_{(95\% CI)}$	0.97 $_{(0.87 - 0.99)}$		0.87 $_{(0.39 - 0.97)}$		0.8 $_{(0.01 - 0.96)}$		0.45 $_{(-2.2 - 0.88)}$	

Soleus - Active (15% MVIC)									
Group	Measures	MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)		Silent Period (ms)	
Healthy (N=19)	Days	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	0.739 ± 0.637 (86)	0.849 ± 0.565 (67)	35.0 ± 4.7 (13)	33.5 ± 3.1 (9)	20 ± 2 (10)	19 ± 1 (5)	124.9 ± 44.1 (35)	155.2 ± 40.7 (26)
	CV_{ME}	26		7		7		17	
	ICC $_{(95\% CI)}$	0.93 $_{(0.82 - 0.97)}$		0.78 $_{(0.42 - 0.92)}$		0.51 $_{(-0.19 - 0.81)}$		0.72 $_{(0.03 - 0.90)}$	
Stroke (N=9)	Day	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	0.476 ± 0.158 (33)	0.489 ± 0.213 (44)	37.1 ± 4.2 (11)	36.6 ± 4.8 (13)	22 ± 2 (9)	21 ± 3 (14)	141.8 ± 49.0 (35)	153.7 ± 52.0 (34)
	CV_{ME}	31		9		9		19	
	ICC $_{(95\% CI)}$	0.55 $_{(-1.46 - 0.90)}$		0.63 $_{(-0.89 - 92)}$		0.6 $_{(-0.95 - 0.91)}$		0.83 $_{(0.31 - 0.96)}$	

Table 4.3: Test-retest reliability, CV_{ME} and ICC $_{(95\% CI)}$, of eight SOL MCE measures in Healthy (right SOL) and Stroke (paretic SOL)

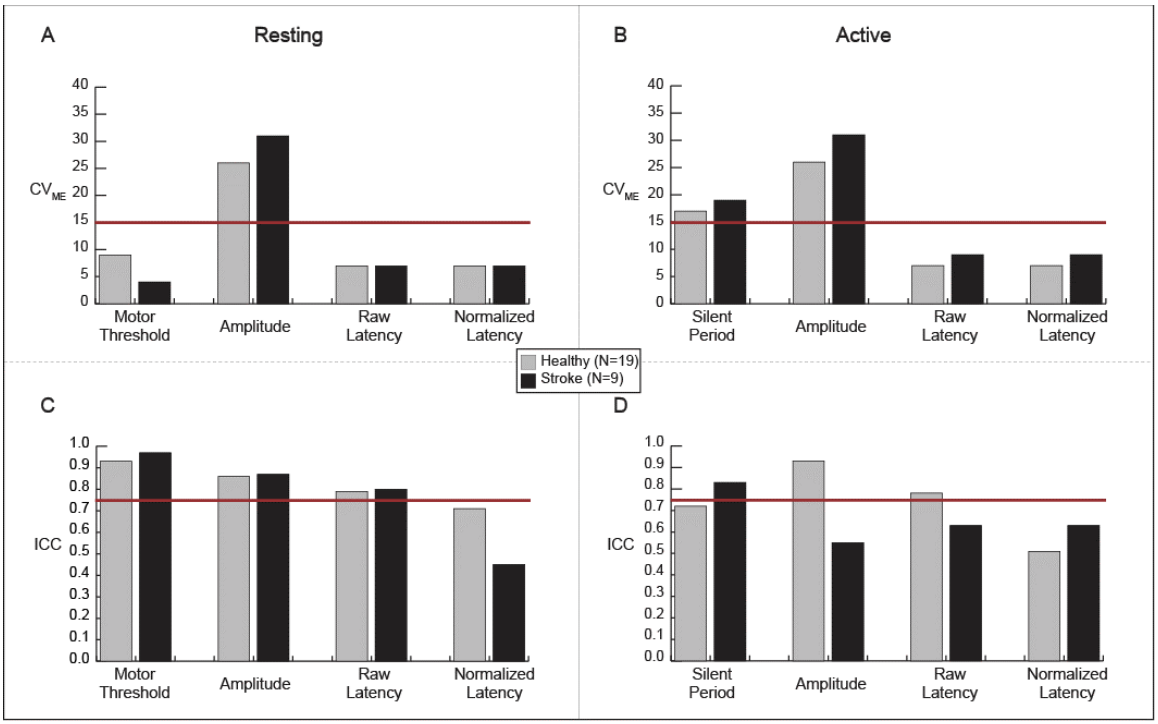

Figure 4. 2: Test-retest reliability, CV_{ME} and ICC, of eight SOL MCE measures in Healthy (right SOL) and Stroke (paretic SOL)

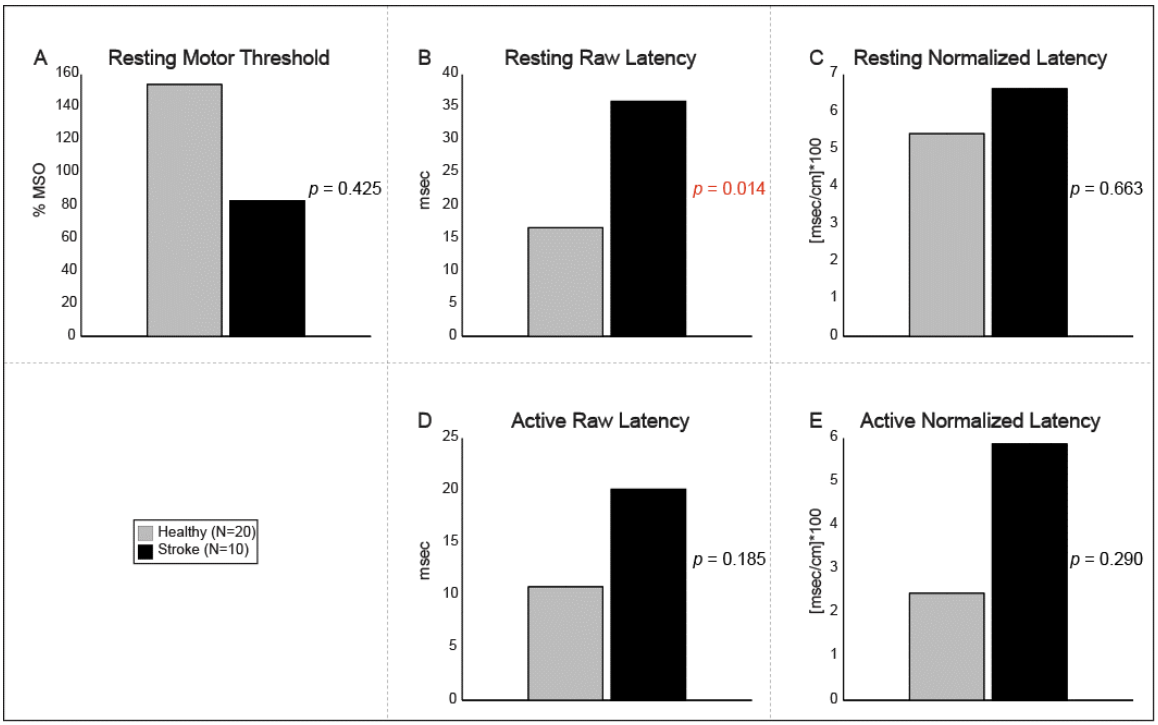

Figure 4.3: Inter-group differences in variances for five SOL MCE measures

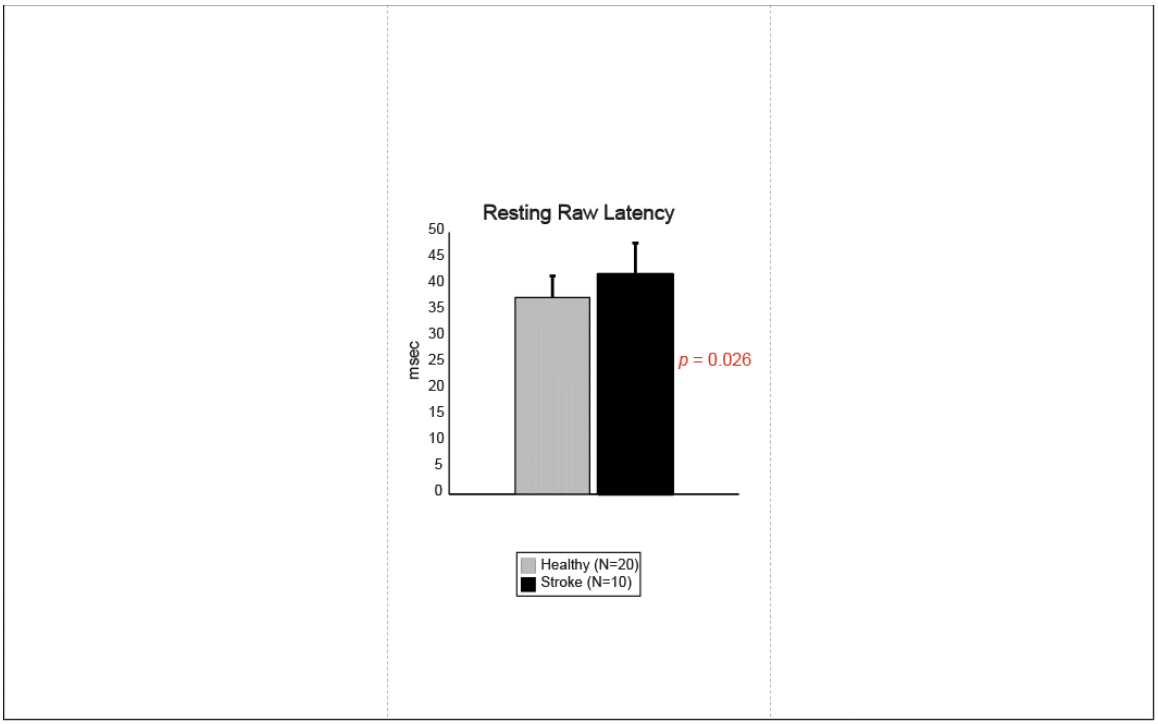

Figure 4.4: Inter-group differences in mean for the resting raw latency of SOL

4.3 Aim 2: Quantification of the Tibialis Anterior MCE Measures in Stroke and Healthy

Table 4.4 includes the test-retest normative data of eight TA MCE measures collected in both groups on day 1 and 2.

4.3.1 Resting Motor Threshold: Responses and Number of Stimulations

Healthy: On both days, we measured the rMT of the right TA in all participants (N = 21), and on average fewer than 15 stimulations were required to calculate the right TA rMT (Day 1: 14 ± 6, 40; Day 2: 13 ± 4, 33).

Stroke: On day 1, we measured the paretic TA rMT in all participants. In two participants, the paretic TA rMT was higher than 83% MSO (86 & 90% MSO), making 120% intensity greater than 100% MSO. As a result, data collected from those two participants were not included in the reliability analyses. On day 2, TA rMT was measured in all but one participant. In this one participant, MEPs on the paretic TA were

not elicited even using 100% MSO. In summary, TA rMT was measured from 14 participants on day 1 and 13 participants on day 2, while fewer than 20 stimulations were required on average to calculate paretic TA rMT on both days (Day 1: 16 ± 7, 44; Day 2: 14 ± 3, 24).

4.3.2 Testing Reliability

Table 4.5 includes the test-retest reliability metrics, CV_{ME} and ICC, for eight TA MCE measures collected in both groups. For those analyses, we used data that were collected from 21 healthy adults and 12 stroke participants on both days.

Healthy: All measures except amplitude had CV_{ME} less than 15 during the resting condition (Figure 4.5 A) while only raw and normalized latency had CV_{ME} less than 15 during the active condition (Figure 4.5 B). All measures in both conditions except active normalized latency had ICC greater than 0.75 (Figure 4.5 C & D).

Stroke: In both resting and active conditions, all measures except resting and active amplitude had CV_{ME} less than 15 (Figure 4.5 A & B), while all measures had ICC greater than 0.75 (Figure 4.5 C & D).

Only the following measures with CV_{ME} less than 15 in both groups were used in the next step: rMT, raw and normalized latency during resting, and raw and normalized latency during active.

4.3.3 Testing Group Variances

For those analyses, we used data from the five aforementioned measures collected from 21 healthy adults and 13 stroke participants on day 2.

Of five TA MCE measures, the variances of three measures differed significantly between groups. The variances between stroke participants and healthy adults were equal

for rMT ($F_{(1,32)} = 0.479$, $p = 0.494$; Figure 4.6 A) and active raw latency ($F_{(1,32)} = 1.393$, $p = 0.247$; Figure 4.6 D). The variance of resting raw latency was significantly higher ($F_{(1,32)} = 8.999$, $p = 0.005$; Figure 4.6 B) in the stroke group (55.9) than in the healthy group (13.6). Additionally, the variance of resting normalized latency was significantly higher ($F_{(1,32)} = 18.6$, $p = 0.000$; Figure 4.6 C) in the stroke group (13.2) than in the healthy group (1.7). Similarly, the variance of active normalized latency was significantly higher ($F_{(1,32)} =5.6$, $p = 0.024$; Figure 4.6 E) in the stroke group (4.2) than in the healthy group (1.4). Therefore, resting raw latency and resting and active normalized latency were used to test whether the group means differed.

4.3.4 Testing Group Means

In both groups, the distributions of the resting raw latency (Stroke: $W_{(13)} = 0.921$, $p = 0.259$; Healthy: $W_{(21)} = 0.983$, $p = 0.960$) and resting normalized latency (Stroke: $W_{(13)} = 0.887$, $p = 0.089$; Healthy: $W_{(21)} = 0.953$, $p = 0.396$) were normally distributed. The distribution of the active normalized latency was normally distributed in stroke ($W_{(13)} = 0.892$, $p = 0.103$) but not in healthy adults ($W_{(21)} = 0.883$, $p = 0.017$). Consequently, we used an independent t-test for the resting raw and normalized latency and Mann–Whitney *U* test for the active normalized latency.

The means of all three measures were different between groups. The resting raw latency was significantly ($t_{(1,32)} =3.013$, $p = 0.004$) longer in stroke participants (41.2 ± 7.5 ms) than in healthy controls (34.5 ± 3.7 ms) (Figure 4.7 A). Furthermore, the resting normalized latency was significantly ($t_{(1,32)} =3.976$, $p = 0.000$) longer in stroke (24 ± 4 ms) than in healthy controls (20 ± 1 ms) (Figure 4.7 B). Similarly, the active normalized latency was significantly ($p = 0.000$) longer in stroke (21 ± 2 ms) than in healthy controls

(19 ± 1 ms) (Figure 4.7 C).

Although three measures passed all three criteria, we could use only two measures in the correlational analyses. In Aim 4, therefore, the resting raw latency and the active normalized latency were used in the correlational analyses for walking and MVIC neuromechanics of the paretic TA.

Tibialis Anterior - Resting									
Groups	Measures	Motor Threshold (% MSO)		MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)	
	Statistics	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Healthy (Right)	N	21	21	21	21	21	21	21	21
	Mean ± SD (CV)	47 ± 11 (23)	48 ± 12 (25)	0.458 ± 0.354 (77)	0.379 ± 0.291 (77)	33.9 ± 3.1 (9)	34.5 ± 3.7 (11)	19 ± 1 (5)	20 ± 1 (5)
Stroke (Paretic)	N	14	13	12	13	12	13	12	13
	Mean ± SD (CV)	57 ± 16 (28)	56 ± 14 (25)	0.402 ± 0.262 (65)	0.343 ± 0.217 (63)	39.6 ± 6.4 (16)	41.2 ± 7.5 (18)	23 ± 3 (13)	24 ± 4 (17)

Tibialis Anterior - Active (15% MVIC)									
Groups	Measures	MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)		Silent Period (ms)	
	Statistics	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Healthy (Right)	N	21	21	21	21	21	21	21	21
	Mean ± SD (CV)	1.790 ± 0.675 (38)	1.640 ± 0.694 (42)	32.1 ± 3.0 (9)	32.6 ± 3.2 (10)	19 ± 1 (5)	19 ± 1 (5)	143.0 ± 49.1 (34)	140.1 ± 50.7 (36)
Stroke (Paretic)	N	12	13	12	13	12	13	12	13
	Mean ± SD (CV)	1.260 ± 0.555 (44)	1.263 ± 0.530 (42)	36.6 ± 5.3 (14)	36.8 ± 4.6 (12)	21 ± 2 (10)	21 ± 2 (10)	148.9 ± 44.3 (30)	148.6 ± 46.2 (31)

Table 4.4: Test-retest normative data, mean ± SD (CV), of eight TA MCE measures in Healthy (right TA) and Stroke (paretic TA)

Tibialis Anterior - Resting									
Group	Measures	Motor Threshold (% MSO)		MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)	
Healthy (N=21)	Days	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	47 ± 11 (23)	48 ± 12 (26)	0.458 ± 0.354 (77)	0.379 ± 0.291 (77)	33.9 ± 3.1 (9)	34.5 ± 3.7 (11)	19 ± 1 (5)	20 ± 1 (5)
	CV_{ME}	11		40		3		4	
	ICC $_{(95\% CI)}$	0.89 $_{(0.73 - 0.96)}$		0.84 $_{(0.61 - 0.93)}$		0.93 $_{(0.84 - 0.97)}$		0.76 $_{(0.42 - 0.90)}$	
Stroke (N=12)	Day	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	52 ± 11 (21)	54 ± 12 (22)	0.402 ± 0.262 (65)	0.338 ± 0.226 (67)	39.6 ± 6.4 (16)	40.4 ± 7.1 (18)	22.9 ± 2.7 (12)	23 ± 3 (13)
	CV_{ME}	6		31		6		6	
	ICC $_{(95\% CI)}$	0.95 $_{(0.83 - 0.99)}$		0.87 $_{(0.56 - 0.96)}$		0.93 $_{(0.75 - 0.98)}$		0.86 $_{(0.52 - 0.96)}$	

Tibialis Anterior - Active (15% MVIC)									
Group	Measures	MEP Amplitude (mV)		MEP Latency (ms)		MEP Normalized Latency ((ms/cm)*100)		Silent Period (ms)	
Healthy (N=21)	Days	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	1.790 ± 0.675 (38)	1.640 ± 0.694 (42)	32.1 ± 3.0 (9)	32.6 ± 3.2 (10)	19 ± 1 (5)	19 ± 1 (5)	143.0 ± 49.1 (34)	140.1 ± 50.7 (36)
	CV_{ME}	21		3		4		20	
	ICC $_{(95\% CI)}$	0.83 $_{(0.58 - 0.93)}$		0.93 $_{(0.84 - 0.97)}$		0.70 $_{(0.24 - 0.88)}$		0.81 $_{(0.53 - 0.92)}$	
Stroke (N=12)	Day	1	2	1	2	1	2	1	2
	Mean ± SD (CV)	1.260 ± 0.555 (44)	1.284 ± 0.548 (43)	36.6 ± 5.3 (15)	36.3 ± 4.5 (12)	21 ± 2 (10)	21 ± 2 (10)	148.9 ± 44.3 (30)	152.9 ± 45.5 (30)
	CV_{ME}	22		3		3		14	
	ICC $_{(95\% CI)}$	0.86 $_{(0.49 - 0.96)}$		0.97 $_{(0.90 - 0.99)}$		0.93 $_{(0.74 - 0.98)}$		0.88 $_{(0.58 - 0.97)}$	

Table 4.5: Test-retest reliability, CV_{ME} and ICC $_{(95\% CI)}$, of eight TA MCE measures in Healthy (right TA) and Stroke (paretic TA)

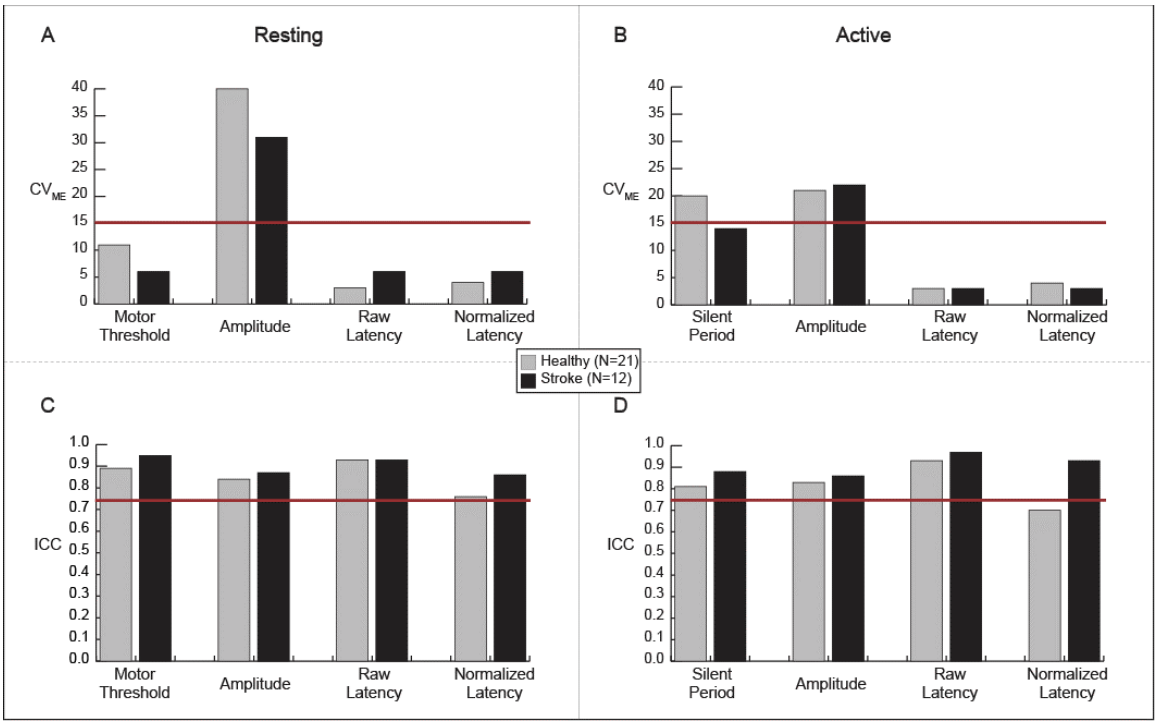

Figure 4. 5: Test-retest reliability, CV_{ME} and ICC, of eight TA MCE measures in Healthy (right TA) and Stroke (paretic TA)

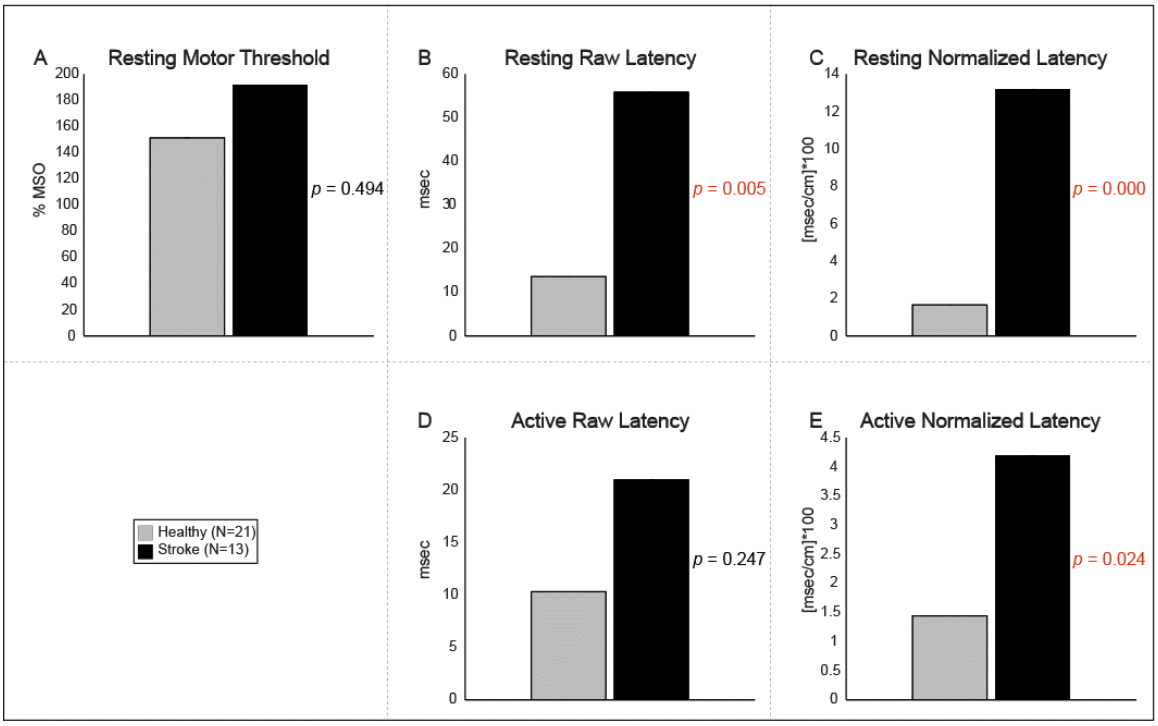

Figure 4.6: Inter-group differences in variances for five TA MCE measures

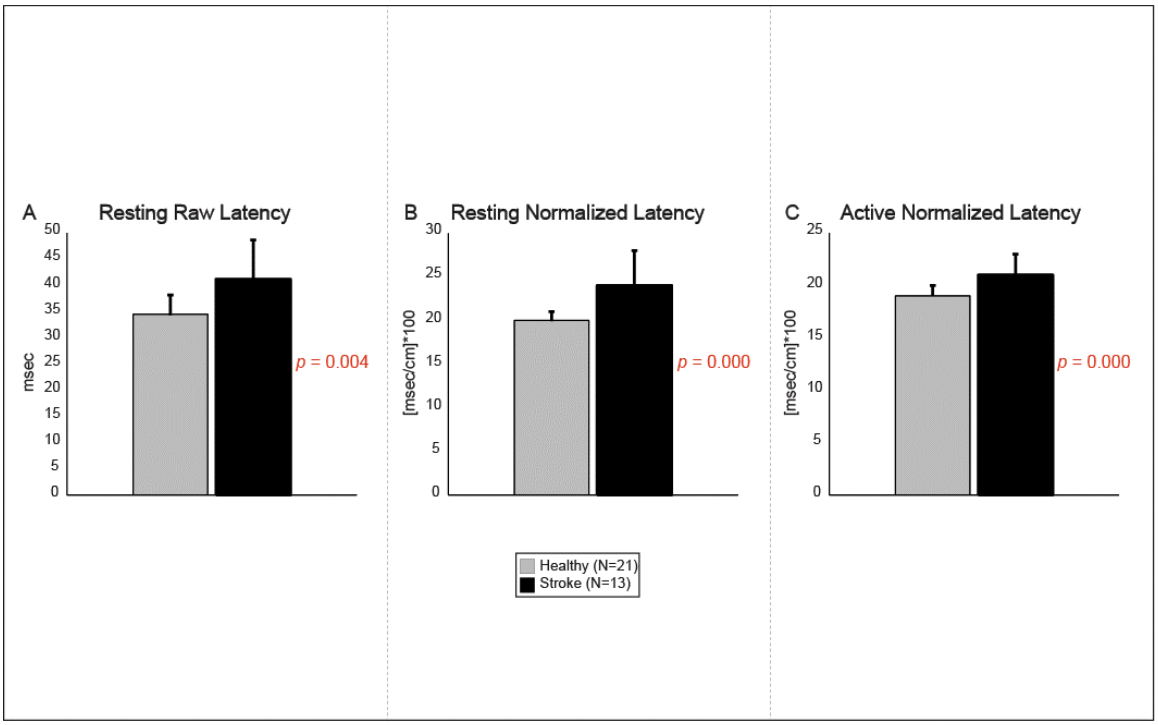

Figure 4.7: Inter-group differences in mean for three TA MCE measures

4.4 Aim 3: Associations Between MCE Measures and Neuromechanics of the Paretic Soleus

For this aim, we used data from the 10 stroke participants who had detectable MEP in the paretic SOL on day 2. Since four correlations were carried out, we considered a correlation significant only if its p was less than 0.012.

Prior to correlational analyses, we tested the normality of distributions for the paretic SOL neuromechanical measures collected during walking and MVIC. The distributions of the walking EMG ($W_{(10)} = 0.928$, $p = 0.430$), PI ($W_{(10)} = 0.954$, $p = 0.711$) and MVIC torque ($W_{(10)} = 0.955$, $p = 0.726$) were normally distributed, whereas the MVIC EMG ($W_{(10)} = 0.802$, $p = 0.015$) was not. During walking, the descriptive data of the paretic SOL EMG and PI were 0.028 ± 0.017 mV (60) and 1.36 ± 0.64 % BW.sec (47), respectively. During MVIC, the descriptive data of the paretic SOL EMG and

torque were 0.053 ± 0.058 mV (109) and 18.5 ± 9.1 N.m (49), respectively.

Walking: Resting raw latency was not significantly associated with either neuromechanical measure. Specifically, the resting raw latency of the paretic SOL had good positive and non-significant association with the paretic SOL EMG (r = 0.713, p = 0.021; Figure 4.8 A), and it had poor negative and non-significant association with the paretic SOL PI (r = -0.127, p = 0.728; Figure 4.8 B).

MVIC: Resting raw latency was not significantly associated with either neuromechanical measure. Specifically, the resting raw latency of the paretic SOL had good positive and non-significant association with the paretic SOL EMG (r_s = 0.576, p = 0.082; Figure 4.8 C), while it had poor positive and non-significant association with the paretic SOL torque (r = 0.341, p = 0.335; Figure 4.8 D).

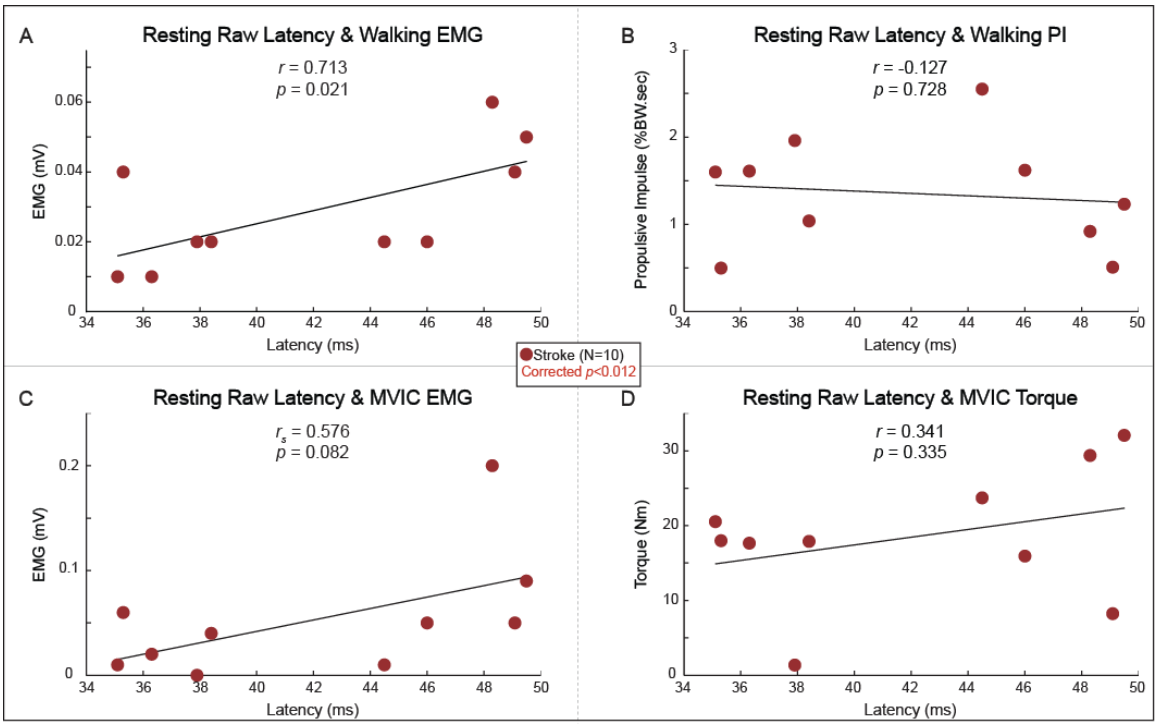

Figure 4.8: Associations between resting raw latency and neuromechanics during walking and MVIC of the paretic SOL

In summary, resting raw latency of the paretic SOL was not significantly associated with any neuromechanical measure of the paretic SOL during walking and MVIC. Despite the absence of significance in any of the four associations, the association between resting raw latency and walking EMG was positive and strong (r = 0.713), therefore as latency increased the EMG increased (Figure 4.8 A). For that specific association, the p was less than 0.05, yet higher than the corrected value of 0.012.

4.5 Aim 4: Associations Between MCE Measures and Neuromechanics of the Paretic Tibialis Anterior

For this aim, we used the data from the 13 stroke participants who had detectable MEP in the paretic TA on day 2. Because eight correlations were carried out, we considered a correlation significant only if its p was less than 0.006.

Prior to correlational analyses, we tested the normality of distributions for paretic TA neuromechanical measures collected during walking and MVIC. The distributions of the walking EMG ($W_{(13)}$ = 0.929, p = 0.335) and MVIC torque ($W_{(13)}$ = 0.935, p = 0.394) were normally distributed, but the AAV ($W_{(13)}$ = 0.853, p = 0.031) and MVIC EMG ($W_{(13)}$ = 0.836, p = 0.019) were not. During walking, the descriptive data of the paretic TA EMG and AAV were 0.071 ± 0.041 mV (58) and 0.90 ± 0.67 rad/sec (74), respectively. During MVIC, the descriptive data of the paretic TA EMG and torque were 0.137 ± 0.080 mV (58) and 11.1 ± 5.5 N.m (50), respectively.

<u>Walking</u>: Resting raw latency and active normalized latency were associated only with the angular velocity of the paretic ankle. The resting raw latency of the paretic TA had poor positive and non-significant association with the paretic TA EMG (r = 0.393, p

= 0.184; Figure 4.9 A) and excellent negative and significant association with the paretic TA AAV (r_s = -0.929, p = 0.000; Figure 4.9 B). Similarly, the active normalized latency of the paretic TA had poor positive and non-significant association with the paretic TA EMG (r = 0.393, p = 0.184; Figure 4.10 A) but good negative and significant association with the paretic TA AAV (r_s = -0.728, p = 0.005; Figure 4.10 B).

MVIC: Resting raw latency and active normalized latency were not associated with either neuromechanical measure. The resting raw latency of the paretic TA had poor negative and non-significant association with both paretic TA EMG (r_s = -0.308, p = 0.306; Figure 4.9 C) and with paretic ankle dorsiflexion torque (r = -0.128, p = 0.678; Figure 4.9 D). Similarly, the active normalized latency of the paretic TA had poor negative and non-significant association with paretic TA EMG (r_s = -0.206, p = 0.499; Figure 4.10 C) and good negative and non-significant association with paretic ankle dorsiflexion torque (r = -0.504, p = 0.079; Figure 4.10 D).

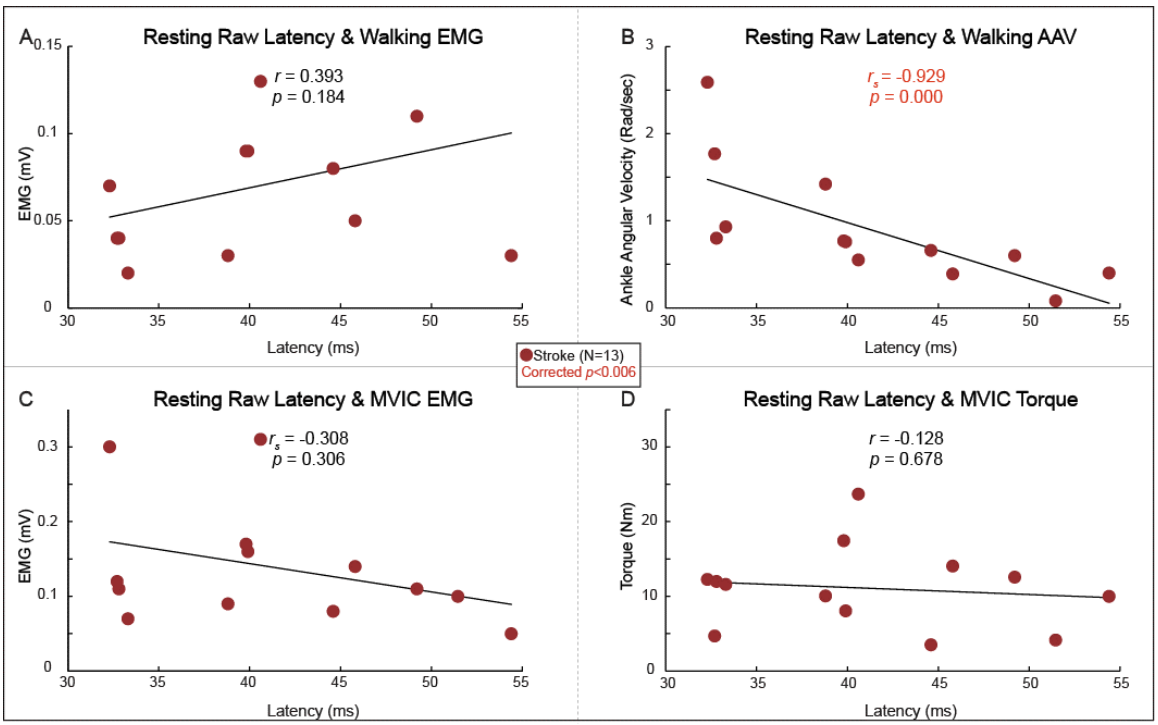

Figure 4.9: Associations between resting raw latency and neuromechanics during walking and MVIC of the paretic TA

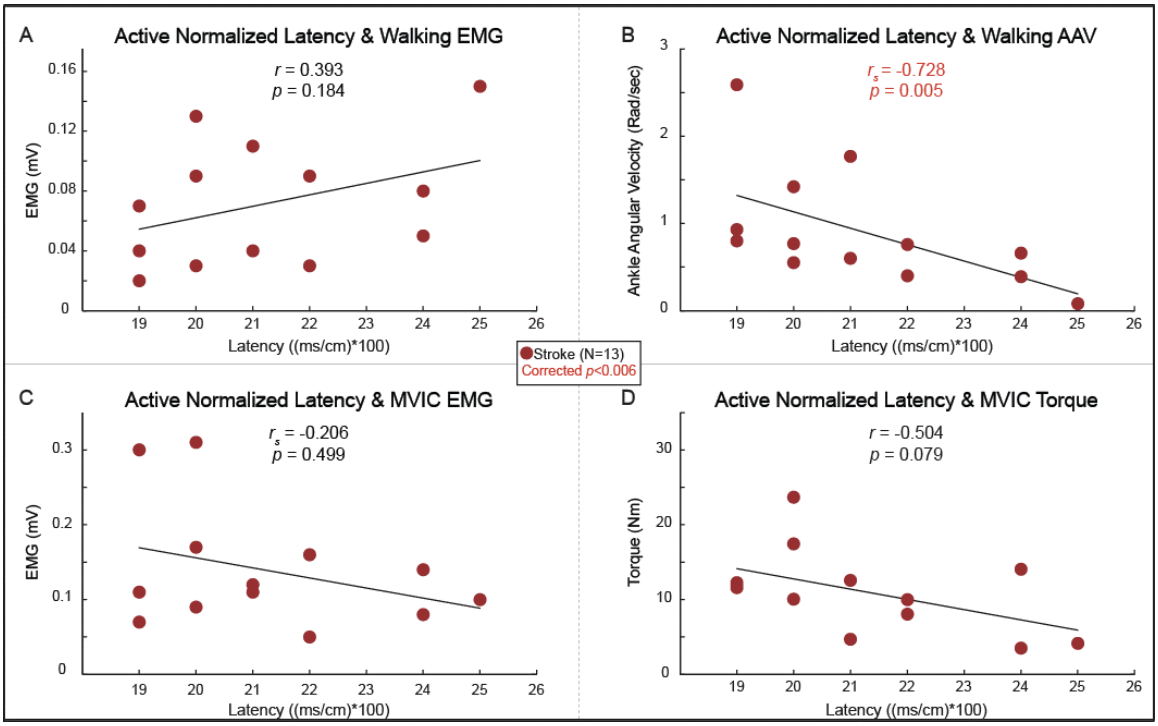

Figure 4.10: Associations between active normalized latency and neuromechanics during walking and MVIC of the paretic TA

In summary, both latencies of the paretic TA were significantly associated only with the angular velocity of the paretic ankle during walking. Notably, both associations (Figure 4.9 B & Figure 4.10 B) were strongly negative, therefore as the latency of the paretic TA increased the angular velocity of the paretic ankle during walking decreased.

CHAPTER FIVE

DISCUSSION

5.1 Overall Findings

Despite the common use of nearly all available measures to quantify SOL and TA MCE in healthy and clinical populations, evidence has been sparse regarding which MCE measure of the two muscles is reliable and, thus, preferable for detecting true differences between neurologically intact and impaired CNS. Additionally, the relationships between the descending drive and the neuromechanical measures of SOL and TA can elucidate the cortical contributions to these muscles during a motor task, yet there is limited evidence about this kind of relationship, especially in stroke. Therefore, in the present project, we first quantified the MCE of SOL and TA in healthy and stroke participants, and second, we investigated the associations between MCE and neuromechanical measures during walking and MVIC for the paretic SOL and the paretic TA in people post-stroke. For the quantification of SOL and TA MCE, we employed a relatively different methodological approach compared to previous studies with similar research goals. In addition to testing reliability, which was determined using CV_{ME} with ICC also reported, we subsequently investigated which measures differed between groups in both variance and means. We included variance in this analysis because we reasoned that a variance of a reliable MCE measure would be low in a healthy control and high in a stroke participant.

For the brain-behavior relationships, we used the MCE measures that met all three pre-defined requirements for reliability, variance, and mean, and as well as certain neuromechanical measures that quantified indirectly the biomechanical functions of the paretic SOL and TA during walking and MVIC in stroke participants.

Several findings occurred. First, not all MCE measures of SOL and TA were reliable in both groups, whereas only latencies had good reliability and differed in variance and mean between the two groups. Specifically, resting raw latency of the paretic SOL, resting raw latency of the paretic TA, and both resting and active normalized latencies of the paretic TA met all three pre-defined requirements. In the associations, therefore, one SOL and two TA MCE measures were used; thus, a total of four and eight correlations were run for the paretic SOL and TA, respectively. Second, the resting raw latency of the paretic SOL was not significantly associated with any neuromechanical measure in both walking and MVIC; however, there was a strong positive association between the resting raw latency of the paretic SOL and the EMG of the paretic SOL during the stance phase of walking. Third, both the resting raw latency and active normalized latency of the paretic TA were significantly and negatively associated only with the AAV during the swing phase of walking; the remaining associations were relatively weak and non-significant. In the following sections, we discuss in depth the meaning and importance of the findings, the clinical implications, the methodological considerations, and the future directions for each aim.

5.2 Aim 1: Quantification of the Soleus Motor Cortical Excitability in Stroke and Healthy

For this aim, we characterized eight MCE measures of the paretic SOL in stroke participants and right SOL in healthy controls. The evidence on the reliability of SOL MCE in both healthy and stroke participants is limited to a single study (Lewis et al., 2014). Therefore, our hypotheses were exploratory and based on the few prior studies that had examined TA MCE reliability in various populations (Cacchio et al., 2009; Cacchio et al., 2011; Meaney et al., 2015; Tallent et al., 2012; van Hedel et al., 2007). All those studies determined reliability using ICC; in contrast, we quantified reliability using CV_{ME}. We hypothesized that only certain measures would be reliable in both groups and their variances and means would differ between groups. Results partially supported our hypotheses. As we hypothesized, rMT, raw and normalized latency were reliable during resting in both groups whereas only raw and normalized latency were reliable during active condition in both groups. Among those five measures, only the variance and mean of the resting raw latency differed between groups. Therefore, only resting raw latency of the paretic SOL had a low measurement error and, thus, detected the differences between a neurologically intact versus a neurologically impaired CNS.

As anticipated, of the six measures that were hypothesized to be reliable, all measures were except cSP; however, the resting and active amplitude of SOL were not reliable. To the best of our knowledge, this is the first attempt to examine systematically and thoroughly the reliability of SOL MCE in healthy adults and people post-stroke while the SOL was in either resting or active state. While SOL was at complete rest, all measures but amplitude could be quantified reliably in both groups; thus, they had low measurement error. Similarly, during active, only the raw and normalized latency had good reliability in both groups. Because no prior study had investigated those SOL MCE

measures, we cannot directly compare our findings with results from previous research. To the best of our knowledge, the work by Lewis et al. (2014) is the only study that allows a relative comparison of results. In that prior study, the authors investigated the test-retest reliability of three MCE measures of SOL (aMT, MEP amplitude, and MEP area) in both healthy adults (randomly assigned side) and stroke participants (paretic side) while SOL was slightly contracted with a target force level at 10% MVIC. Although the prior research measured SOL aMT and not rMT as our study did, SOL aMT was reliable in both groups using both ICC (Healthy: 0.92; Stoke 0.82) and typical percentage error (TPE; Healthy: 5; Stroke: 8). They calculated TPE in a similar way we calculated CV_{ME}. Interestingly, our results showed that CV_{ME} was lower and ICC was higher in people post-stroke than in healthy adults while Lewis et al. (2014) found the opposite. This discrepancy might be due to certain differences in methodology (rMT versus aMT; use of neuronavigation versus no use of neuronavigation). Despite this difference, in general the previous finding about SOL aMT was in agreement with our results on SOL rMT. Findings from both studies indicate that SOL MT can be reliably measured in both healthy adults and stroke participants regardless of the state of contraction of the SOL.

In addition to rMT, our results indicated that both raw and normalized latencies were reliable in both groups while SOL was either relaxed or contracted. No other study has examined the reliability of these SOL MCE measures in either group; therefore, comparisons with previous literature are limited. Interestingly, only the ICC of the resting raw latency in both groups and the active raw latency in healthy adults indicated good reliability. However, as we discussed in section 3.7.1, the ICC may misinterpret the reliability of measure, if the group variability of that measure is low. That was the case

for the SOL latency measures whose ICC was less than 0.75. For example, the ICC of the active raw latency of the paretic SOL was 0.63, yet the group CV in both days was less than 15 (Day 1: 11; Day 2: 13), indicating low between-subject variability. That was the reason we chose using CV_{ME}, which is insensitive to variability in the samples and is independent of ICC, as the main reliability metric. Including SOL rMT, SOL latencies had a CV_{ME} much lower than 15%. Therefore, in addition to SOL rMT, the raw and normalized latencies in both conditions can be reliably measured in both healthy adults and stroke participants.

Despite the wide use of MEP amplitude to quantify SOL MCE, neither resting nor active amplitude was a reliable measure. Both measures had CV_{ME} higher than 15; hence, they had low reliability. On the other hand, only active amplitude in stroke had ICC less than 0.75. Again, if ICC was used as a reliability metric, resting amplitude in stroke and healthy and active amplitude in healthy would be considered reliable measures. However, that was the case due to high variability within each sample. Lewis et al. (2014) reported low ICC and high TPE for active amplitude of the paretic SOL but high ICC and TPE for active amplitude of SOL in healthy. Similar to our results, they found that SOL amplitude in both groups had high TPE, hence high measurement error. Combining the findings from both studies, one would postulate that SOL amplitude is not a good reliable measure to quantify SOL MCE despite the fact that the majority of studies use and report MEP amplitude as a major metric to investigate SOL MCE.

Though our hypothesis about SOL cSP was exploratory, we postulated that SOL cSP would be a reliable measure in both groups. That postulation was based on the limited evidence derived from studies which examined TA cSP in healthy (Cacchio et al.,

2009; Tallent et al., 2012; van Hedel et al., 2007). Based on CV_{ME}, SOL cSP did not have good reliability in either group whereas the ICC of SOL cSP was higher than 0.75 in stroke and lower than 0.75 in healthy. As this is the first study to investigate this measure for SOL in both healthy and stroke, we cannot make sound comparisons with previous studies. One reason that SOL cSP was not reliable in either group might be the fact that we calculated the maximum isometric SOL EMG instead of using force. Tallent et al. (2012) reported recently that TA cSP is an excellent reliable measure in healthy controls when TA was contracted at a certain level of maximum target force, either during concentric (ICC: 0.94) or eccentric (ICC: 0.96) contraction. Due to the lack of evidence on SOL cSP, we cannot ensure that this can be applied to the SOL cSP. Future studies should use similar approaches as Tallent et al. (2012) to determine whether SOL cSP can be reliably measured when it is contracted, either concentrically or eccentrically. To the best of our knowledge, our study was the first to measure SOL cSP, either in healthy or stroke.

Among the five measures with good reliability, only raw resting latency of SOL detected differences between groups. Specifically, raw resting latency of SOL had higher variance and mean in stroke than in healthy. In order to define a reliable measure, we asked whether a reliable measure could detect true differences between a neurologically intact and an impaired population. Rather than focusing only on group differences in mean, we also looked at the between group difference in variance. No group differences in variances implied that the variability of each group was due to factors other than the integrity level of CNS. As the methodology was the same across participants and between days, these factors might be due to biologically related factors (e.g., initial cortical

activation state) (Rubens & Zanto, 2012). Surprisingly, the variance of the resting normalized latency was not different between groups though the variance of the resting raw latency was. As normalized latency was the raw latency divided by the height, the fact that there was no significant difference in variance might be due to the height difference between groups. However, there was no significant difference in height between the groups. These findings indicate that although multiple SOL MCE measures were reliable in both groups, the detection of true differences between the two groups was limited only to resting raw latency. Therefore, studies that examine the SOL MCE in just healthy adults may use any of the five measures that our results demonstrated to be reliable using CV_{ME}. On the other hand, if the goal of a study is to quantify the MCE of the paretic SOL after stroke, we suggest that resting raw latency might be the only measure that should be used to ensure reliability and distinction with an intact CNS.

5.3 Aim 2: Quantification of the Tibialis Anterior MCE Measures in Stroke and Healthy

Similar to Aim 1, we characterized eight MCE measures of the paretic TA in stroke and right TA in healthy. In contrast to the limited evidence of SOL MCE reliability studies, some evidence on the reliability of certain TA MCE measures exists in healthy (Cacchio et al., 2009; Forster et al., 2014; Tallent et al., 2012; van Hedel et al., 2007), stroke (Cacchio et al., 2011), SCI (van Hedel et al., 2007), and Multiple Sclerosis (Meaney et al., 2015). Therefore, this aim's hypotheses were based on the cumulative evidence reported in those studies. As for SOL, we hypothesized that only certain TA MCE measures would be reliable in both groups and their variances and means would

differ between groups. Results partially supported the hypotheses. During resting, rMT, raw and normalized latency had good reliability in both groups whereas during active only raw and normalized latency had good reliability in both groups. Of the five measures, the variance and mean of resting raw and normalized latency and active normalized latency differed between groups. Therefore, three of these five TA MCE measures had low measurement error and detected differences between a neurologically intact and impaired CNS.

While TA was relaxed, all measures but amplitude had good reliability in both groups. Interestingly, both CV_{ME} and ICC indicated good reliability for rMT, resting raw and normalized latency. Other studies have also demonstrated good reliability for TA rMT in healthy participants (Cacchio et al., 2009; Forster et al., 2014; Tallent et al., 2012), stroke participants (Cacchio et al., 2011) and people with Multiple Sclerosis (Meaney et al., 2015). Although all of those studies relied solely on ICC to determine reliability, their results are in agreement with ours. Therefore, rMT is a reliable measure to quantify TA MCE, either in people with or without neurological impairment. No study has investigated whether resting latency of TA is reliable in either healthy or stroke populations. As for SOL latencies during resting condition, resting raw and normalized latency of TA also had good reliability, indicating that these measures, which reflect the conductivity of the signal from MC to the muscle, can also be measured consistently across days.

Although TA was slightly contracted, only raw and resting latency had good reliability in both groups. The CV_{ME} of active raw and normalized latency was very low in both groups, indicating low measurement error. On the other hand, the ICCs of the

same measures were very high except for the ICC of normalized latency in healthy, whose ICC was 0.70. This is another example that sole reliance on ICC may misinterpret the consistency of measuring a metric. No other study has reported the ICC of the active normalized latency of TA in any population; therefore, we cannot make comparisons about the reliability values of this measure. However, one study that examined the reliability of the active raw latency of TA among other measures in 16 stroke participants and 16 age-matched healthy controls did report high ICC bilaterally in both groups (Cacchio et al., 2011). Another study also reported good ICC of active raw latency in both healthy and people with SCI, especially when TA was contracted in high percentages of maximum torque effort (van Hedel et al., 2007). Although neither study calculated any reliability metric similar to CV_{ME}, their results, in conjunction with our findings, indicate that both active raw and normalized latency of TA can be reliably measured with consistent low measurement error in both healthy and stroke.

Regardless of the contraction state of TA and the integrity level of CNS, MEP amplitude had low reliability using CV_{ME} but good reliability using ICC. The CV_{ME} ranged between 31-40 in both groups for the resting amplitude whereas it was 21 in healthy and 22 in stroke for the active amplitude. Because we chose a cutoff value of 15 to distinguish a reliable measure from a non-reliable measure, amplitude might not be a consistent measure of TA MCE for either group. Conversely, the ICC for resting and active amplitude ranged from 0.82 to 0.87 in both groups. Similar to our results, other studies reported high ICC for TA amplitude. van Hedel et al. (2007) reported good reliability of TA amplitude in both healthy adults (ICC = 0.77) and people with SCI (ICC = 0.77) while TA was contracted at 40% maximum voluntary contraction quantified by

torque. Additionally, a more recent study demonstrated that the amplitude of the resting TA could be also reliably (ICC = 0.92) measured in people with Multiple Sclerosis (Meaney et al., 2015). Cacchio et al. (2011) reported similar ICCs for TA active amplitudes measured bilaterally in healthy and on the non-paretic side in stroke. However, the ICC of the TA active amplitude measured on the paretic side was 0.38, indicating poor reliability (Cacchio et al., 2011). The differences that our study and the latter study had in methodology, such as type of coil, use of neuronavigation, time between sessions, and MVIC level, may explain the disagreement between the studies about the reliability of the paretic TA active amplitude. Furthermore, the fact that cumulative evidence indicates that TA amplitude can be consistently measured due to high ICC may lead to wrong assumptions about TA MCE. As the variabilities of TA amplitudes reported here and elsewhere were high, one would expect that ICC would be high as well. As we discussed previously, this is the main drawback of using ICC to quantify reliability. As we chose CV_{ME} as the metric to determine reliability, measuring and reporting amplitude, either resting or active, to quantify TA MCE should be done with caution in both healthy and stroke participants.

The TA cSP could be reliably measured in stroke but not in healthy. For the paretic TA, CV_{ME} and ICC were 14 and 0.88, respectively. If only ICC was used to determine reliability, one could argue that the high ICC of paretic TA cSP could be due to high variability. However, because of low CV_{ME}, that might not be the case. Therefore, these findings indicate that TA cSP can be consistently measured in stroke participants. Thus far, no other study has examined the reliability of paretic TA cSP in this patient population. Conversely, two studies investigated TA cSP in healthy. The first study (van

Hedel et al., 2007) reported poor reliability for TA cSP while the second study (Cacchio et al., 2009) reported good reliability with ICC higher than 0.75. The former study calculated the absolute cSP (i.e., period between MEP offset and EMG resumption), as we did in this study while the latter calculated the relative cSP (i.e., period between MEP onset and EMG resumption). In our study, CV_{ME} and ICC of the right TA cSP was 20 and 0.79, respectively; therefore, this measure in healthy did not have good reliability based on the criterion (CV_{ME} <15) that we used in the present study. The inconsistencies of results across the three studies might be due to methodological differences. First, neither of the previous studies used a neuronavigation system, which can improve accuracy and precision of the stimulation site within and between sessions (Krings et al., 2001; Ruohonen & Karhu, 2010; Sparing et al., 2010). Second, neither of the previous studies used a double cone coil, which is considered to have an adequate stimulation depth appropriate to stimulate cortical representations of leg muscles (Deng et al., 2013, 2014; Lontis et al., 2006; Terao et al., 1994). Third, it was not clear in the methods section of the previous studies whether the TA cSP was calculated manually or automatically. In our study, we used published automated algorithms, which could assist in the consistent calculation of cSP across participants. Regardless of those differences across studies, caution should be taken when using cSP to quantify the inhibitory mechanisms of TA's neuromotor axis, especially in healthy controls.

Despite the fact that some TA MCE measures were reliable, only few of those detected differences between groups. As for SOL MCE, the variance and the mean of the resting raw latency were higher in stroke than in healthy. Additionally, the variance and the mean of the resting and active normalized latencies were also higher in stroke than in

healthy. These results indicate that TA latencies, especially after taking into account each participant's height, might be a good MCE measure that can quantify accurately the TA MCE in people post-stroke. Of course, all five measures that were found to be reliable might be used to quantify TA MCE in healthy controls. Therefore, our findings can provide some guidance concerning which measure should be used to quantify TA MCE in either healthy or stroke participants; a final decision should be thoroughly made based on the research question asked and the kind of population investigated. Researchers should bear in mind that our results are limited only to healthy and stroke participants.

5.4 Aims 1 and 2: Clinical Applications, Methodological Considerations, and Future Work

The findings of the present study may provide novel information on the measurement characteristics of certain SOL and TA MCE measures that clinical studies may utilize in the future. In recent years, non-invasive brain stimulation techniques have been employed in neurorehabilitation science, as either an assessment or intervention tool (Liew, Santarnecchi, Buch, & Cohen, 2014). However, the number of studies that have investigated the MCE of upper extremity muscles (in the thousands) is much larger than the number of studies that have investigated the MCE of the lower extremity (less than 500). Additionally, the characteristics of the upper extremity muscles MCE measures have been quantified more extensively than lower extremity MCE. Thus, despite the fact that there is no sound evidence on the measurement characteristics of the SOL and TA, studies continue to use nearly all available MCE measures to quantify SOL and TA MCE either in healthy or clinical populations. However, our findings demonstrated that of eight

measures, only the rMT and the resting and active latencies of SOL and TA can be reliably measured in both stroke and healthy participants. As those five MCE measures were reliable for both muscles and groups, one could argue that the measurement consistency of the ankle musculature MCE is independent of muscle and damage in CNS, and, as a result, the inter-muscle and inter-group differences can be investigated. Furthermore, studies that investigate the cortical plastic changes either in SOL or TA due to an intervention (e.g., behavioral, neurophysiological) should carefully choose the MCE measures to quantify these changes. According to our findings, rMT or latencies might be the most appropriate measures to use to assess these changes in neuromotor axis without the caution of high measurement error. Hence, the observed changes in SOL or TA MCE would be most likely due to the true effect of the intervention. Additionally, if a goal of a study is to assess globally the MCE of either SOL or TA, any MCE measure of the five, either rMT or latency, can be used. For example, a potential study may choose to use only the rMT to quantify SOL and TA MCE; as a result, the total number of stimuli applied over participants' brains will be small and the total assessment period will be short. Furthermore, our results provide normative data of eight MCE measures of SOL and TA in both healthy adults and stroke participants; similar studies in the future can use the present findings either for comparisons or as a guide in intervention studies. In summary, findings from the present study can be used in both scientific and clinical studies to either assess cross-sectional or longitudinal SOL and TA MCE in either healthy adults or people post-stroke.

We acknowledge a few methodological considerations that were present in these aims. One methodological consideration was the small sample size of the stroke group (N

= 14) which was similar to the sample size of the stroke groups used in Lewis et al. (2014) study (N = 13) and Cacchio et al. (2011) (N = 16). As we could not get responses on the paretic SOL and TA from all participants in both days, the sample size used to determine reliability of SOL (N = 9) and TA (N = 12) MCE measures decreased. Also, for the between group comparisons for measures collected in day 2, the sample size of the paretic SOL (N = 10) and TA (N = 13) were smaller than the sample size of the right SOL (N = 20) and TA (N = 21). Interestingly, the number of people that we could not get responses from was greater for SOL than in TA; this pattern was more apparent in the stroke group. This finding may add to the existing notion that higher stimulus is required to elicit a response to SOL than in TA (Bawa et al., 2002). Importantly, no detectable MEP does not necessarily imply absent MCE for that target muscle. If a coil with higher maximum output (e.g., figure-of-eight coil with maximum magnetic field of 2T) was used than the one we used in the present study (i.e., double cone coil with a maximum magnetic field of 1.4T), we could potentially get responses from all stroke participants.

Another methodological consideration was the significant difference in age between our groups while both Lewis et al. (2014) and Cacchio et al. (2011) recruited age-matched healthy controls. We did try to recruit age-matched healthy controls, yet it was not feasible. We do acknowledge that age has generally a significant effect on the MCE of the lower extremity muscles; in general, compared to young adults, older people have higher rMT, smaller amplitudes, and longer latencies. These changes might be due to multiple factors, such as reduction of the overall brain volume, nerve fiber degeneration, muscle atrophy, and increase of subcutaneous fat tissue (Budui, Rossi, & Zamboni, 2015; Fotenos, Snyder, Girton, Morris, & Buckner, 2005). Each factor can

affect a different component of the neuromotor axis. Reduction of brain volume results in increase of the skull to cortex distance; therefore, the coil to cortex distance increases as well. Because of these increases, higher intensities are required since as the coil to cortex distance increases, the TMS intensity increases (Danner et al., 2012; McConnell et al., 2001). Degeneration of nerve fiber may contribute to increases in latency, which reflects the conductivity of the descending pathways. Muscle atrophy and increase of the subcutaneous fat tissue may contribute to the reduced detection of the muscle activity measured by sEMG; thus, amplitudes are smaller in the elderly. Nevertheless, the summative evidence from these studies is that age does not have a significant effect in all MCE measures of leg muscles (Baudry, Collignon, & Duchateau, 2015; Rossini, Desiato, & Caramia, 1992; Saisanen, Julkunen, et al., 2008; Stevens-Lapsley, Thomas, Hedgecock, & Kluger, 2013; Tobimatsu, Sun, Fukui, & Kato, 1998). However, the MCE measure that was found to be significantly higher always in the elderly was the latency; this finding was for multiple ankle muscles: SOL (Baudry et al., 2015), TA (Eisen & Shtybel, 1990; Saisanen, Julkunen, et al., 2008), and AH (Tobimatsu et al., 1998). In the present study, we found that latencies collected in both muscle state conditions and for both muscles were significantly higher in stroke participants than in healthy. Consequently, both damage in CNS and aging might affect those inter-group differences in latencies; there was no height or weight difference between groups. However, the significant inter-group differences in variances might decouple the effect of damage in CNS and aging on latencies; therefore, latency may still distinguish a damage in CNS regardless of the factor of age. Nevertheless, as age has an effect on certain MCE measures of the lower extremities, especially latency, future studies with similar

objectives as ours should definitely recruit controls whose age is matched with the experimental or clinical group.

In addition to age, numerous factors can influence the effect of TMS over the neuromotor axis. These factors are either physiological or methodological and are present in both healthy and stroke participants (Cortes, Black-Schaffer, & Edwards, 2012; Ridding & Ziemann, 2010; Rubens & Zanto, 2012; Wassermann, 2002). The level of control is less for the physiological factors than the methodological ones. In the present study, we tried to control those physiological factors over which we had some control, such as no consumption of alcohol and caffeine at least three hours prior to experiment, level of arousal, height, weight, and gender. Similarly, we employed a methodology that was tight and consistent across participants and days. The same sEMG electrodes were used for the same muscles between days and across participants; the same person placed sEMG in all experiments using standardized protocol for the sEMG placement and testing. Furthermore, we used the simple adaptive PEST, an automated method, to determine rMT, which was tested twice for each muscle. Then we used the average of two to calculate the 120% intensity used during the resting and active conditions. Furthermore, use of the neuronavigation with each participant's structural MRI increased both the accuracy and precision of stimulating the hot spot, which was determined for each target muscle. In contrast to other studies, we detected the hot spot of each muscle in a 36-points grid (see Figure 3.5) using a suprathreshold intensity. From repeated measures of pilot experiments in our lab, we found that the hot spot of each muscle is not exactly the same, even with a 30 minute break between each hot spot hunting process. For this reason, we ran the hot spot hunting protocol on both days; most of the time, the

inter-session hot spot differed for each muscle in both hemispheres and groups. Certainly, future studies should investigate similar approaches to detect hot spot using mathematical models. Establishment of a precise method for searching for the hot spot of the target muscle may reduce a portion of the methodological error that is inherent in TMS.

One further methodological consideration was that we did not examine the reliability of RC derived measures, measures that quantify intracortical mechanisms, or measures that characterize the motor representations of SOL and TA in either group. Therefore, the application of our results is limited only to the measures tested in the present study. Furthermore, our results are limited to only the two muscles and groups that we investigated here.

Two subjects remain to elucidate the cortical control of SOL and TA: the intracortical networks and the motor representations of SOL and TA. A few studies have reported intracortical measures (i.e., SICI and SICF) of SOL and TA in both healthy and clinical populations (Beaulieu et al., 2014; Liepert, Hassa, Tuscher, & Schmidt, 2011; Liepert & Neveling, 2009; Oliveri et al., 2012; Roy, Norton, & Gorassini, 2007). However, no study examined whether these measures are reliable in either group and detect true differences between the target groups. Future studies may use similar approaches used in aims 1 and 2 to determine SOL and TA intracortical measures that have good reliability and detect truly existing differences between healthy and clinical populations. Elucidation of this subject may hold clinical significance, especially in stroke participants. It has been suggested that MCE measures, similar to the ones used in this study, might be more appropriate to quantify corticospinal integrity within the first three months after stroke whereas intracortical measures should be used after that period

(Di Pino et al., 2014). The underlying hypothesis is that the motor recovery of a certain muscle may rely on the remaining CST in the early phase after stroke while that reliance might be shifted to cortical networks in later stages of stroke (Di Pino et al., 2014). Therefore, since the majority of stroke studies recruit stroke participants in the chronic phase, it would be worth quantifying the measurement characteristics of intracortical measures of SOL and TA in both healthy and stroke participants.

Motor mapping of SOL and TA is the second subject that future work should focus on. Compared to the number of studies that examined the motor cortical area of TA (Forster et al., 2014; Niskanen et al., 2010; Thordstein et al., 2013; Vaalto et al., 2013), only one study reported the motor cortical area of SOL from a single patient with focal cortical dysplasia (Saisanen et al., 2010). A common characteristic that all these studies share is the use of the same nTMS system (Eximia, Nexstim Ltd.; Helsinki, Finland). However, this system is extremely expensive, and it is usually found in hospitals. Future work should systematically investigate and establish normative data of cortical mapping measures for SOL and TA in healthy controls using user-friendly neuronavigation systems that are more available to researchers. This will establish which motor mapping measures should be used to specifically quantify the motor representations of SOL and TA. Then, clinical studies can use these metrics to characterize plastic changes at the cortical areas of each muscle after an intervention.

5.5 Aim 3: Associations Between MCE Measures and Neuromechanics of the Paretic Soleus

For this aim, we examined the associations between one paretic SOL MCE

measure, resting raw latency, and the neuromechanics of the paretic SOL during walking and MVIC. We hypothesized that paretic SOL MCE (in this case, it was quantified using resting raw latency) would not be associated with either walking neuromechanical measure of paretic SOL whereas it would be negatively associated with both MVIC neuromechanical measures. Results partially supported our hypotheses. As we anticipated, there was no significant association between the resting raw latency and neuromechanics of the paretic SOL during walking, but, in contrast to our hypotheses, the resting raw latency of the paretic SOL was not significantly associated with either neuromechanical measure of the paretic SOL during MVIC.

During walking, resting raw latency of the paretic SOL increases were strongly associated with increases in EMG of the paretic SOL, yet the association was not statistically significant. Evidence from studies that applied TMS during walking in healthy controls supports the notion that the activity of SOL is also generated by motor cortical signals, particularly during the stance in which SOL is active ((Knikou, Hajela, & Mummidisetty, 2013; N. Petersen et al., 1998; N. T. Petersen et al., 2001). The cortical contribution to SOL in those studies was quantified calculating MEP amplitude of SOL. In contrast to those studies, Capaday et al. (1999) reported a weak linkage between the descending drive from MC to SOL and SOL EMG during stance in healthy controls; they quantified descending drive using RC derived measures. Although there were differences in methodology and results between those studies, descending drive from MC does contribute to SOL activity during the stance phase of walking; this evidence comes mainly from healthy controls. In the case of SOL, those cortical contributions might not be as strong as the contributions from spinal centers and sensory feedback, yet such

distinction is difficult to be made (J.B. Nielsen, 2002). Additionally, SOL has weak CM connections (Bawa et al., 2002) while other pathways (e.g., corticorubrospinal, corticoreticulospinal) might contribute to its activation (J. Nielsen & Petersen, 1995). Due to those particular characteristics of the SOL MCE and the fact that stroke affects multiple descending pathways, including CST, we postulated that the contributions of the descending drive to the SOL EMG during stance would be weak (i.e., non-significant association); hence other parts of the CNS might be responsible for the activation of SOL. As we hypothesized, the association was not statistical significant after correcting for multiple comparisons, but the association was strong and positive. In other words, the more impaired the descending drive to paretic SOL was, the higher the activation of the paretic SOL was during stance. A potential explanation for this unanticipated finding could be that stroke participants with a greater level of impairment in SOL MCE activate their paretic SOL at high levels just to accomplish the task during the stance phase. As stroke alters SOL MCE (i.e., resting raw latency), increase in the activation of paretic SOL might be due to the increased contributions from either sensory receptors (e.g., force receptors) or spinal networks. Whether this is true cannot be addressed by the current study. Moreover, the results from this specific association cannot be definitive because of the small sample size (N = 10) and the low p (0.021). If the size of the sample had been larger, there might have been statistically significant association. Future studies should test this assumption.

As we anticipated, resting raw latency of the paretic SOL was not significantly associated with PI. We chose PI as one of the walking neuromechanical measures of SOL because it can characterize indirectly the biomechanical function of SOL during walking,

along with LG and MG. Our results suggest that the capacity to accelerate the COM forward after stroke is not associated with the integrity of SOL MCE. After stroke, a positive association exists between paretic SOL EMG and PI in stroke participants (Turns, Neptune, & Kautz, 2007). However, PI is not exclusively dependent on the activation of SOL, along with LG and MG. Other factors, including ankle moment and power and the trailing limb, contribute to PI in both healthy adults and stroke participants (Hsiao, Knarr, Higginson, & Binder-Macleod, 2015a, 2015b; Peterson et al., 2010). Therefore, stroke participants can still generate adequate PI to accelerate forward the COM using several biomechanical patterns regardless of the level of integrity of the descending drive to the paretic SOL.

As in walking, the resting raw latency of the paretic SOL increased when the activation of the paretic SOL during MVIC increased; yet association was non-significant. Capaday et al. (1999) reported a strong linkage between the descending drive to SOL and activation of SOL during MVIC in healthy adults; therefore, we postulated that as the MEP latency of the paretic SOL would be increased due to stroke, the activation level of SOL during MVIC would be decreased. Contrary to our hypotheses, the association was non-significant and positive, but it was still strong. Results suggest that the activation of paretic SOL during MVIC may increase due to the impaired conductivity of the descending drive to the paretic SOL. A potential explanation for this unanticipated finding might be that the increase in activation of the paretic SOL during MVIC might be due to increased neuromotor output from the SOL motor pools. Paretic SOL motor pools may be activated by other descending pathways (e.g., corticorubrospinal and corticoreticulospinal) (J. Nielsen & Petersen, 1995) due to the

delayed signal from the MC to the paretic SOL via CST. Although this postulation may be true, because this association was strong but non-significant, no definitive inferences can be made.

In contrast to our hypothesis, we found no associations between resting raw latency and paretic plantarflexion torque production during MVIC. In contrast to walking in which the leg is in contact with the ground during the stance phase and factors other than SOL EMG may affect PI, during MVIC the leg is fixated to a footplate. This results in the torque production being mainly affected by the activation of the responsible muscle whose contraction is most likely generated by a descending and spinal drive and, to a lesser extent, by the feedback from sensory receptors. Nevertheless, results indicate that impaired conductivity of the descending drive to the resting paretic SOL was not associated with the paretic plantarflexion torque production during MVIC.

5.6 Aim 4: Associations Between MCE Measures and Neuromechanics of the Paretic Tibialis Anterior

For this aim, we examined the associations between two MCE measures of the paretic TA, resting raw latency and active normalized latency, and the neuromechanics of the paretic TA during walking and MVIC. We hypothesized that paretic TA MCE would be negatively and significantly associated with both walking and MVIC neuromechanical measures. Results partially supported our hypotheses. Both the resting raw latency and active normalized latency of the paretic TA were significantly, strongly, and negatively associated with only the peak AVV during the swing phase of walking. Conversely, the remaining associations were non-significant and mainly weak.

During walking, activation of paretic TA was not significantly associated with either resting raw latency or active normalized latency, both associations were weak and positive. It is generally accepted that activation of TA during walking, especially in swing phase, relies heavily on descending drives from the MC (T. H. Petersen et al., 2012). Therefore, when the descending drive to TA increases, the activation of TA also increases. Conversely, if the descending drive to TA is impaired due to a lesion at the brain level of the neuromotor axis, TA activation should be decreased as well. In the past, studies that attempted to elucidate the descending drive to TA during walking employed associations with either kinematic measures or clinical measures (Barthelemy et al., 2013; Barthelemy et al., 2015; Barthelemy et al., 2010); therefore, there is no evidence about the relationship between the descending drive to TA and the activation of TA in stroke participants. In disagreement with our hypotheses, results indicated that neither TA latencies were significantly associated with the activation of the paretic TA while the associations were positive rather negative.

During walking, the AAV decreased as both resting raw latency and active normalized latency of paretic TA increased. The significant, negative, and strong associations between latency and AAV were one of the most robust results in our study. As previously discussed, one of the TA's primary functions during walking is to clear the foot sufficiently during early and mid-swing. For this reason, previous studies examined the relationship between the descending drive to TA and toe clearance during mid-swing, in both healthy individuals and people with SCI. Similar to our results, they demonstrated a significant and negative association between the latency of the most impaired TA and toe clearance (Barthelemy et al., 2013; Barthelemy et al., 2010). Their results suggested

that delayed TA latency might indicate impaired toe clearance, which may either cause the emergence of compensatory walking patterns or increase the risk of potential falling due to foot scuffing. For reasons that have been explained in section 2.6.4, here we chose AAV to quantify kinematically the function of TA during early to mid-swing. As in the two aforementioned studies, findings from the present study suggest that delayed TA latency, either resting or active, can also indicate an impaired biomechanical pattern of the ankle during early to mid-swing.

During MVIC, activation of the paretic TA was not significantly associated with either MCE measure of the paretic TA. We expected that the more impaired the descending drive to TA was, the less the activation of the paretic TA would be during paretic dorsiflexion isometric torque. The non-significant associations for either latency might imply that impaired conductivity may not be a good indicator of TA activation during MVIC. Our findings are in agreement with Beaulieu et al. (2014). In that study, no significant associations between six MCE measures of the paretic TA, including normalized latency, and MVIC EMG were found. Findings from both studies suggest that TA MCE cannot indicate the activation of the paretic TA during MVIC.

Paretic dorsiflexion torque was not significantly associated with either latency of the paretic TA, yet both associations were negative as we predicted. As in walking, delayed latency of the paretic TA indicated less production of the paretic ankle dorsiflexion torque. However, only active normalized latency was strongly associated with the reduction of torque. This might have occurred because during active conditions motoneurons are excitable similar to MVIC whereas during resting motoneurons are silent. Contrary to our results, Beaulieu et al. (2014) found a significant association

between MCE and isometric strength of the paretic TA. Yet, that MCE measure was MEP amplitude. In their study, amplitude of the paretic TA increased as the strength of the paretic TA increased. Therefore, the cumulative evidence from both studies suggests that the MEP amplitude of the paretic TA may indicate the production of the paretic dorsiflexion torque, yet not latency.

5.7 Aims 3 and 4: Clinical Applications, Methodological Considerations, and Future Work

Findings from this study may provide insight into the understanding of the descending drive to the paretic SOL and TA, two muscles that have an important role during various motor tasks and are impaired after stroke. Furthermore, the findings may have important clinical applications. Up until now, the studies that attempted to elucidate this matter in clinical populations were limited only to associations with clinical measures and the TA (Arac et al., 1994; Barthelemy et al., 2013; Barthelemy et al., 2015; Barthelemy et al., 2010; Beaulieu et al., 2014; Hendricks et al., 2003; Piron et al., 2005; Steube, Wietholter, & Correll, 2001). First, numerous factors other than just neuromechanics may affect the outcome of the clinical measures; therefore, an existing association between the descending drive and a clinical measure may not elucidate the exact relationships between the MC and the target muscle during a motor task. Second, although TA has a crucial role during the GC, especially during the swing phase, exclusive focus on the descending drive to the TA does not clarify fully what the the cortical contributions are during the whole GC, both stance and swing phase. This issue is one of the reasons that we examined the SOL, which is mainly active during the stance

phase of gait. Therefore, investigating the relationships between the descending drive and the neuromechanics of both muscles during two motor tasks that may require neural drive in different patterns may shed light on to what extent MC in a lesioned brain contributes to the activation of the SOL and TA during a functional and isolated motor task. Limited evidence exists only in healthy individuals (Capaday et al., 1999). Here, we demonstrated statistically that only TA MCE could indicate neuromechanical patterns during walking. Our study is the first that has investigated the descending drive to the ankle antagonist muscles and the associations with specific neuromechanical measures during walking and MVIC in individuals post-stroke. Two MCE measures of the paretic TA were strongly associated with the same neuromechanical measure (AAV) only during walking. This finding implies that motor cortical contributions from a lesioned brain can strongly indicate an impaired mechanical pattern of the paretic ankle, during a specific phase of walking. Thus, these findings may have clinical implications; however, the clinical relevance of the SOL results may not be as clear. As the latency of the descending drive to the paretic SOL increased, the activation of the paretic SOL during the stance phase of walking also increased. However, it is not clear whether a reduction in latency would mitigate or exacerbate the motor function of that muscle during walking. Conversely, a delayed descending drive to the paretic TA does detrimentally influence the mechanics of the paretic ankle during the swing phase. Therefore, an intervention that can strengthen the conductivity of the descending drive to the paretic TA may improve mechanical patterns during specific motor tasks in people post-stroke.

An potential neurophysiological approach is the use of non-invasive brain stimulation techniques, such as repetitive TMS (Lefaucheur et al., 2014) and transcranial

direct current stimulation (Feng, Bowden, & Kautz, 2013); both technologies can modulate the excitability of intracortical networks. Within the last few years, a few studies investigated whether these two techniques can improve walking ability in people post-stroke. The results demonstrated that brain modulation enhanced both neuromechanical and clinical measures of walking (Chang, Kim, & Park, 2015; Chieffo et al., 2014; Kakuda et al., 2013; van Asseldonk & Boonstra, 2015; Wang et al., 2012). Although the results from these studies are promising and interesting, further investigation is required on this topic.

A few methodological considerations were present in Aims 3 and 4. The first is the relatively small sample size used for the correlational analyses of each muscle. Power analysis using results from the few studies that examined the relationships between the descending drive to TA and a mechanical measure in either stroke or SCI (Barthelemy et al., 2013; Barthelemy et al., 2015; Barthelemy et al., 2010; Beaulieu et al., 2014), demonstrated a minimum of 12 participants should be recruited to detect a statistical significance. Of the 15 recruited stroke participants, data from 14 participants were collected. Yet, the final sample size for SOL and TA was 10 and 13, respectively, due to no MEP responses detected for some stroke participants on day 2. Thus, the correlational analyses were limited to stroke participants with detectable MEPs in the paretic SOL and TA. Interestingly, the four participants with no detectable MEPs in either muscle successfully completed both walking and MVIC tasks; they activated both muscles and produced force similar to a healthy adult, especially during walking. There is some evidence that stroke participants with a discontinued CST are able to produce walking patterns (Ahn, Ahn, Kim, Hong, & Jang, 2006; Seo et al., 2014). However, as previously

stated, no detecting a MEP does not necessarily imply absent MCE for that target muscle. Multiple methodological (e.g., not strong enough coil) and physiological (e.g. low level of arousal) factors could explain the undetectable MEP responses in these four stroke participants.

A second methodological consideration is that the SOL and TA MCE were not assessed during the task that the SOL and TA neuromechanics were calculated. We do acknowledge that this discrepancy may affect the results, yet we chose this methodology for several reasons. First, we did not have access to the equipment used for TMS application during walking. To the best of our knowledge, only one group has this technology in the United States (Hajela, Mummidisetty, Smith, & Knikou, 2013; Hanna-Boutros et al., 2015; Knikou et al., 2013). Along with the application of TMS during off task, the application of TMS during a motor task may not necessarily elucidate fully the cortical contributions to that muscle during that task. Nevertheless, findings from this study, in which methodology was tightly controlled, may assist in the improvement of the methodology of studies that use TMS during walking.

Another methodological consideration specific to the walking task was the large range of SSWS (0.20 – 0.95 m/s) of the stroke participants. In general, walking speed has an effect on walking neuromechanics (Kirtley, Whittle, & Jefferson, 1985; Panizzolo et al., 2013; Peterson, Kautz, & Neptune, 2011). Although we did not run any between-subject comparisons, having participants walk at the same speed could potentially reduce the between-subject variance of neuromechanical measures. A similar methodological consideration is the potential between-subject variance within the EMG data, due to different thicknesses of subcutaneous fat tissue over the target muscles. Specifically,

subcutaneous fat thickness is negatively associated with sEMG amplitude; the greater the subcutaneous fat tissue, the smaller the sEMG amplitude (Hemingway, Biedermann, & Inglis, 1995; Nordander et al., 2003). In our study, we did not measure the subcutaneous fat tissue thickness; therefore, stroke participants with thick subcutaneous fat tissue over the target muscles might have smaller sEMG amplitudes. However, small sEMG amplitudes might not be a true indication of low muscle activation. A solution to this issue is to measure subcutaneous fat tissue thickness using a skinfold caliper, which is easy to use, inexpensive, and better tool than ultrasound for calculating fat tissue (Nordander et al., 2003). Future studies should measure the thickness of subcutaneous fat tissue and use it as a covariant within their statistical analyses. A last methodological consideration is that we did not normalize walking EMG to MVIC EMG, which is the most commonly used normalization method. Though we do acknowledge the pros and cons of EMG normalization (Cronin, Kumpulainen, Joutjarvi, Finni, & Piitulainen, 2015; Sousa & Tavares, 2012), we decided to use the raw EMG values in the correlational analyses.

Future work should extend the understanding of these relationships between the descending drive and neuromechanical measures of SOL and TA. Correlational analyses are limited to the description of the relative strength and direction between two measures. An alternative is regression analyses, which could be used for a deeper understanding of that relationship using the basis of prediction, yet to run regression analysis a larger sample size is required than for a correlational analysis. Since we found a significant and strong association between the MEP latency of the paretic TA and the angular velocity of the paretic ankle, a first step for future studies is to investigate the regression between

these two measures. Of course, a larger sample size than the one used here should be utilized. For example, if a normalized latency of the paretic TA is shown to be a strong predictor of the angular velocity of the paretic ankle, this finding implies that stroke participants who have delayed descending drive to the paretic TA will demonstrate impaired ability to move the paretic ankle during swing. If this is the case, neurorehabilitation scientists can use these findings to develop either a behavioral or a neurophysiological intervention to reverse this relationship. Furthermore, future work should further investigate whether these relationships are present in other clinical populations, such as SCI and Parkinson's.

5.8 Conclusions

The present study was a first attempt to thoroughly quantify certain MCE measures of SOL and TA in both healthy controls and stoke participants, and to elucidate the relationships between reliable MCE measures and the neuromechanics of the paretic SOL and TA during walking and MVIC. Despite the fact that nearly all MCE measures are currently used in studies to quantify the MCE of these two muscles, findings from this study showed that only a few measures can be reliably measured, and can detect differences between a neurologically intact and impaired nervous system. Furthermore, using correlational analyses, we found that certain relationships exist between the descending drive and neuromechanical measures of the paretic SOL and TA in stroke participants. Future studies may use our findings to accurately quantify the SOL and TA MCE in either healthy or stroke participants and to expand knowledge about the role of the descending drive to SOL and TA during motor tasks in neurologically impaired adults.

REFERENCES

Ackermann, H., Scholz, E., Koehler, W., & Dichgans, J. (1991). Influence of posture and voluntary background contraction upon compound muscle action potentials from anterior tibial and soleus muscle following transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol, 81*(1), 71-80.

Ahn, Y. H., Ahn, S. H., Kim, H., Hong, J. H., & Jang, S. H. (2006). Can stroke patients walk after complete lateral corticospinal tract injury of the affected hemisphere? *Neuroreport, 17*(10), 987-990.

Alkadhi, H., Crelier, G. R., Boendermaker, S. H., Golay, X., Hepp-Reymond, M. C., & Kollias, S. S. (2002). Reproducibility of primary motor cortex somatotopy under controlled conditions. *AJNR Am J Neuroradiol, 23*(9), 1524-1532.

Aminoff, M. J., Greenberg, D. A., Simon, R. P., & Greenberg, D. A. (2005). *Clinical neurology* (6th ed.). New York: Lange Medical Books/McGraw-Hill, Medical Pub. Division.

Arac, N., Sagduyu, A., Binai, S., & Ertekin, C. (1994). Prognostic value of transcranial magnetic stimulation in acute stroke. *Stroke, 25*(11), 2183-2186.

Armstrong, D. M. (1988). The supraspinal control of mammalian locomotion. *J Physiol, 405*, 1-37.

Awiszus, F. (2003). TMS and threshold hunting. *Suppl Clin Neurophysiol, 56*, 13-23.

Awiszus, F., & Borckardt, J. J. (2011). TMS Motor Threshold Assessment Tool (MTAT 1.0).

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet, 1*(8437), 1106-1107.

Barthelemy, D., Grey, M. J., Nielsen, J. B., & Bouyer, L. (2011). Involvement of the corticospinal tract in the control of human gait. *Prog Brain Res, 192*, 181-197.

Barthelemy, D., Knudsen, H., Willerslev-Olsen, M., Lundell, H., Nielsen, J. B., & Biering-Sorensen, F. (2013). Functional implications of corticospinal tract impairment on gait after spinal cord injury. *Spinal Cord, 51*(11), 852-856.

Barthelemy, D., Willerslev-Olsen, M., Lundell, H., Biering-Sørensen, F., & Nielsen, J. B. (2015). Chapter 4 - Assessment of transmission in specific descending pathways in relation to gait and balance following spinal cord injury. In S. N. Numa Dancause & R. Serge (Eds.), *Progress in Brain Research* (Vol. Volume 218, pp. 79-101): Elsevier.

Barthelemy, D., Willerslev-Olsen, M., Lundell, H., Conway, B. A., Knudsen, H., Biering-Sorensen, F., & Nielsen, J. B. (2010). Impaired transmission in the corticospinal tract and gait disability in spinal cord injured persons. *J Neurophysiol, 104*(2), 1167-1176.

Baudry, S., Collignon, S., & Duchateau, J. (2015). Influence of age and posture on spinal and corticospinal excitability. *Exp Gerontol, 69*, 62-69.

Bawa, P., Chalmers, G. R., Stewart, H., & Eisen, A. A. (2002). Responses of ankle extensor and flexor motoneurons to transcranial magnetic stimulation. *J Neurophysiol, 88*(1), 124-132.

Beaulieu, L. D., Masse-Alarie, H., Brouwer, B., & Schneider, C. (2014). Brain control of volitional ankle tasks in people with chronic stroke and in healthy individuals. *J Neurol Sci, 338*(1-2), 148-155.

Beaulieu, L. D., Masse-Alarie, H., Brouwer, B., & Schneider, C. (2015). Noninvasive neurostimulation in chronic stroke: a double-blind randomized sham-controlled testing of clinical and corticomotor effects. *Top Stroke Rehabil, 22*(1), 8-17.

Bohannon, R. W., Andrews, A. W., & Smith, M. B. (1988). Rehabilitation goals of patients with hemiplegia. *International Journal of Rehabilitation Research, 11*(2), 181-184.

Borckardt, J. J., Nahas, Z., Koola, J., & George, M. S. (2006). Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: a computer simulation evaluation of best methods. *J ect, 22*(3), 169-175.

Bowden, M. G., Balasubramanian, C. K., Neptune, R. R., & Kautz, S. A. (2006). Anterior-posterior ground reaction forces as a measure of paretic leg contribution in hemiparetic walking. *Stroke, 37*(3), 872-876.

Bowden, M. G., Embry, A. E., Perry, L. A., & Duncan, P. W. (2012). Rehabilitation of Walking After Stroke. *Curr Treat Options Neurol*.

Brasil-Neto, J. P., McShane, L. M., Fuhr, P., Hallett, M., & Cohen, L. G. (1992). Topographic mapping of the human motor cortex with magnetic stimulation: factors affecting accuracy and reproducibility. *Electroencephalogr Clin Neurophysiol, 85*(1), 9-16.

Brouwer, B., & Ashby, P. (1992). Corticospinal projections to lower limb motoneurons in man. *Exp Brain Res, 89*(3), 649-654.

Brouwer, B., & Qiao, J. (1995). Characteristics and variability of lower limb motoneuron responses to transcranial magnetic stimulation. *Electroencephalogr Clin*

Neurophysiol, 97(1), 49-54.

Budui, S. L., Rossi, A. P., & Zamboni, M. (2015). The pathogenetic bases of sarcopenia. *Clin Cases Miner Bone Metab, 12*(1), 22-26.

Cacchio, A., Cimini, N., Alosi, P., Santilli, V., & Marrelli, A. (2009). Reliability of transcranial magnetic stimulation-related measurements of tibialis anterior muscle in healthy subjects. *Clin Neurophysiol, 120*(2), 414-419.

Cacchio, A., Paoloni, M., Cimini, N., Mangone, M., Liris, G., Aloisi, P., . . . Marrelli, A. (2011). Reliability of TMS-related measures of tibialis anterior muscle in patients with chronic stroke and healthy subjects. *J Neurol Sci, 303*(1-2), 90-94.

Capaday, C., Lavoie, B. A., Barbeau, H., Schneider, C., & Bonnard, M. (1999). Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. *J Neurophysiol, 81*(1), 129-139.

Chang, M. C., Kim, D. Y., & Park, D. H. (2015). Enhancement of Cortical Excitability and Lower Limb Motor Function in Patients With Stroke by Transcranial Direct Current Stimulation. *Brain Stimul, 8*(3), 561-566.

Chen, R. (2000). Studies of human motor physiology with transcranial magnetic stimulation. *Muscle Nerve Suppl, 9*(S9), S26-32.

Chen, R., Yung, D., & Li, J. Y. (2003). Organization of ipsilateral excitatory and inhibitory pathways in the human motor cortex. *J Neurophysiol, 89*(3), 1256-1264.

Chieffo, R., De Prezzo, S., Houdayer, E., Nuara, A., Di Maggio, G., Coppi, E., . . . Leocani, L. (2014). Deep repetitive transcranial magnetic stimulation with H-coil on lower limb motor function in chronic stroke: a pilot study. *Arch Phys Med Rehabil, 95*(6), 1141-1147

Chleboun, G. S., Busic, A. B., Graham, K. K., & Stuckey, H. A. (2007). Fascicle length change of the human tibialis anterior and vastus lateralis during walking. *J Orthop Sports Phys Ther, 37*(7), 372-379.

Christensen, L. O., Andersen, J. B., Sinkjaer, T., & Nielsen, J. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. *J Physiol, 531*(Pt 2), 545-557.

Cohen, L. G., Roth, B. J., Nilsson, J., Dang, N., Panizza, M., Bandinelli, S., . . . Hallett, M. (1990). Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. *Electroencephalogr Clin Neurophysiol, 75*(4), 350-357.

Comeau, R. (2014). Neuronavigation for Transcranial Magnetic Stimulation *Transcranial Magnetic Stimulation* (pp. 31-56): Springer.

Conti, A., Raffa, G., Granata, F., Rizzo, V., Germano, A., & Tomasello, F. (2014). Navigated transcranial magnetic stimulation for "somatotopic" tractography of the corticospinal tract. *Neurosurgery, 10 Suppl 4*, 542-554; discussion 554.

Corbyn, Z. (2014). Statistics: a growing global burden. *Nature, 510*(7506), S2-3.

Cortes, M., Black-Schaffer, R. M., & Edwards, D. J. (2012). Transcranial magnetic stimulation as an investigative tool for motor dysfunction and recovery in stroke: an overview for neurorehabilitation clinicians. *Neuromodulation, 15*(4), 316-325.

Cram, J. R., & Criswell, E. (2011). *Cram's Introduction to Surface Electromyography*: Jones & Bartlett Learning.

Cronin, N. J., Avela, J., Finni, T., & Peltonen, J. (2013). Differences in contractile behaviour between the soleus and medial gastrocnemius muscles during human walking. *J Exp Biol, 216*(Pt 5), 909-914.

Cronin, N. J., Kumpulainen, S., Joutjarvi, T., Finni, T., & Piitulainen, H. (2015). Spatial variability of muscle activity during human walking: the effects of different EMG normalization approaches. *Neuroscience, 300*, 19-28.

Cuccurullo, S. J. (2004). *Physical medicine and rehabilitation board review*. New York, N.Y.: Demos.

Damron, L. A., Dearth, D. J., Hoffman, R. L., & Clark, B. C. (2008). Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *J Neurosci Methods, 173*(1), 121-128.

Danner, N., Kononen, M., Saisanen, L., Laitinen, R., Mervaala, E., & Julkunen, P. (2012). Effect of individual anatomy on resting motor threshold-computed electric field as a measure of cortical excitability. *J Neurosci Methods, 203*(2), 298-304.

Daskalakis, Z. J., Molnar, G. F., Christensen, B. K., Sailer, A., Fitzgerald, P. B., & Chen, R. (2003). An automated method to determine the transcranial magnetic stimulation-induced contralateral silent period. *Clin Neurophysiol, 114*(5), 938-944.

Deng, Z. D., Lisanby, S. H., & Peterchev, A. V. (2013). Electric field depth-focality tradeoff in transcranial magnetic stimulation: simulation comparison of 50 coil designs. *Brain Stimul, 6*(1), 1-13.

Deng, Z. D., Lisanby, S. H., & Peterchev, A. V. (2014). Coil design considerations for deep transcranial magnetic stimulation. *Clin Neurophysiol, 125*(6), 1202-1212.

Devanne, H., Lavoie, B. A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res, 114*(2), 329-338.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., . . . Di Lazzaro, V. (2014). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol, 10*(10), 597-608.

Dickstein, R. (2008). Rehabilitation of gait speed after stroke: a critical review of intervention approaches. *Neurorehabil Neural Repair, 22*(6), 649-660.

Dimitrijevic, M. R., Kofler, M., McKay, W. B., Sherwood, A. M., Van der Linden, C., & Lissens, M. A. (1992). Early and late lower limb motor evoked potentials elicited by transcranial magnetic motor cortex stimulation. *Electroencephalogr Clin Neurophysiol, 85*(6), 365-373.

Dobkin, B. H., Firestine, A., West, M., Saremi, K., & Woods, R. (2004). Ankle dorsiflexion as an fMRI paradigm to assay motor control for walking during rehabilitation. *Neuroimage, 23*(1), 370-381.

Drew, T. (1988). Motor cortical cell discharge during voluntary gait modification. *Brain Res, 457*(1), 181-187.

Drew, T. (1991). Visuomotor coordination in locomotion. *Curr Opin Neurobiol, 1*(4), 652-657.

Drew, T., Andujar, J. E., Lajoie, K., & Yakovenko, S. (2008). Cortical mechanisms involved in visuomotor coordination during precision walking. *Brain Res Rev, 57*(1), 199-211.

Drew, T., & Marigold, D. S. (2015). Taking the next step: cortical contributions to the control of locomotion. *Curr Opin Neurobiol, 33*, 25-33.

Eisen, A. A., & Shtybel, W. (1990). AAEM minimonograph #35: Clinical experience with transcranial magnetic stimulation. *Muscle Nerve, 13*(11), 995-1011.

Enoka, R. M. (2008). *Neuromechanics of human movement* (4th ed.). Champaign, IL: Human Kinetics.

Farina, D., Merletti, R., & Enoka, R. M. (2004). The extraction of neural strategies from the surface EMG. *J Appl Physiol (1985), 96*(4), 1486-1495.

Farzan, F. (2014). Single-Pulse Transcranial Magnetic Stimulation (TMS) Protocols and Outcome Measures *Transcranial Magnetic Stimulation* (pp. 69-115): Springer.

Feng, W. W., Bowden, M. G., & Kautz, S. (2013). Review of transcranial direct current stimulation in poststroke recovery. *Top Stroke Rehabil, 20*(1), 68-77.

Field, A. (2009). *Discovering Statistics using IBM SPSS Statistics*: SAGE Publications.

Forster, M. T., Limbart, M., Seifert, V., & Senft, C. (2014). Test-retest reliability of navigated transcranial magnetic stimulation of the motor cortex. *Neurosurgery, 10 Suppl 1*, 51-55; discussion 55-56.

Fotenos, A. F., Snyder, A. Z., Girton, L. E., Morris, J. C., & Buckner, R. L. (2005). Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. *Neurology, 64*(6), 1032-1039.

Fugl-Meyer, A. R., Jaasko, L., Leyman, I., Olsson, S., & Steglind, S. (1975). The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. *Scand J Rehabil Med, 7*(1), 13-31.

Fukuyama, H., Ouchi, Y., Matsuzaki, S., Nagahama, Y., Yamauchi, H., Ogawa, M., . . . Shibasaki, H. (1997). Brain functional activity during gait in normal subjects: a SPECT study. *Neurosci Lett, 228*(3), 183-186.

Geertsen, S. S., Zuur, A. T., & Nielsen, J. B. (2010). Voluntary activation of ankle muscles is accompanied by subcortical facilitation of their antagonists. *The Journal of Physiology, 588*(13), 2391-2402.

George, M. S., & Belmaker, R. H. (2007). *Transcranial magnetic stimulation in clinical psychiatry* (1st ed.). Washington, DC: American Psychiatric Pub.

Gottschall, J. S., & Kram, R. (2003). Energy cost and muscular activity required for propulsion during walking. *J Appl Physiol (1985), 94*(5), 1766-1772.

Granata, K. P., Abel, M. F., & Damiano, D. L. (2000). Joint angular velocity in spastic gait and the influence of muscle-tendon lengthening. *J Bone Joint Surg Am, 82*(2), 174-186.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., . . . Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol, 123*(5), 858-882.

Gwin, J. T., Gramann, K., Makeig, S., & Ferris, D. P. (2011). Electrocortical activity is coupled to gait cycle phase during treadmill walking. *Neuroimage, 54*(2), 1289-1296.

Hajela, N., Mummidisetty, C. K., Smith, A. C., & Knikou, M. (2013). Corticospinal reorganization after locomotor training in a person with motor incomplete

paraplegia. *Biomed Res Int, 2013*, 516427.

Hanna-Boutros, B., Sangari, S., Giboin, L. S., El Mendili, M. M., Lackmy-Vallee, A., Marchand-Pauvert, V., & Knikou, M. (2015). Corticospinal and reciprocal inhibition actions on human soleus motoneuron activity during standing and walking. *Physiol Rep, 3*(2).

Hemingway, M. A., Biedermann, H. J., & Inglis, J. (1995). Electromyographic recordings of paraspinal muscles: variations related to subcutaneous tissue thickness. *Biofeedback Self Regul, 20*(1), 39-49.

Hendricks, H. T., Pasman, J. W., van Limbeek, J., & Zwarts, M. J. (2003). Motor evoked potentials of the lower extremity in predicting motor recovery and ambulation after stroke: a cohort study. *Arch Phys Med Rehabil, 84*(9), 1373-1379.

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., Disselhorst-, & Klug, C., Hagg, G.,. (1999). *European Recommendations for Surface ElectroMyoGraphy: Results of the Seniam Project (SENIAM)* (2nd ed.). Enschede, Netherlands: Roessingh Research and Development.

Hsiao, H., Knarr, B. A., Higginson, J. S., & Binder-Macleod, S. A. (2015a). Mechanisms to increase propulsive force for individuals poststroke. *J Neuroeng Rehabil, 12*, 40.

Hsiao, H., Knarr, B. A., Higginson, J. S., & Binder-Macleod, S. A. (2015b). The relative contribution of ankle moment and trailing limb angle to propulsive force during gait. *Hum Mov Sci, 39*, 212-221.

Iglesias, C., Lourenco, G., & Marchand-Pauvert, V. (2012). Weak motor cortex contribution to the quadriceps activity during human walking. *Gait Posture, 35*(3), 360-366.

Inghilleri, M., Berardelli, A., Cruccu, G., & Manfredi, M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol, 466*, 521-534.

Jayaram, G., Stagg, C. J., Esser, P., Kischka, U., Stinear, J., & Johansen-Berg, H. (2012). Relationships between functional and structural corticospinal tract integrity and walking post stroke. *Clin Neurophysiol, 123*(12), 2422-2428.

Jorgensen, H. S., Nakayama, H., Raaschou, H. O., & Olsen, T. S. (1995). Recovery of walking function in stroke patients: the Copenhagen Stroke Study. *Arch Phys Med Rehabil, 76*(1), 27-32.

Kakuda, W., Abo, M., Watanabe, S., Momosaki, R., Hashimoto, G., Nakayama, Y., . . . Yoshida, H. (2013). High-frequency rTMS applied over bilateral leg motor areas

combined with mobility training for gait disturbance after stroke: a preliminary study. *Brain Inj, 27*(9), 1080-1086.

Kamen, G., & Gabriel, D. (2010). *Essentials of Electromyography*: Human Kinetics

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science* (4th ed.). New York: McGraw-Hill, Health Professions Division.

Kerrigan, D. C., Frates, E. P., Rogan, S., & Riley, P. O. (2000). Hip hiking and circumduction: quantitative definitions. *Am J Phys Med Rehabil, 79*(3), 247-252.

Kim, C. M., & Eng, J. J. (2004). Magnitude and pattern of 3D kinematic and kinetic gait profiles in persons with stroke: relationship to walking speed. *Gait Posture, 20*(2), 140-146.

Kirtley, C., Whittle, M. W., & Jefferson, R. J. (1985). Influence of walking speed on gait parameters. *J Biomed Eng, 7*(4), 282-288.

Knikou, M., Hajela, N., & Mummidisetty, C. K. (2013). Corticospinal excitability during walking in humans with absent and partial body weight support. *Clin Neurophysiol, 124*(12), 2431-2438.

Knutsson, E., & Richards, C. (1979). Different types of disturbed motor control in gait of hemiparetic patients. *Brain, 102*(2), 405-430.

Kobayashi, M., & Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol, 2*(3), 145-156.

Komi, P. V., Fukashiro, S., & Jarvinen, M. (1992). Biomechanical loading of Achilles tendon during normal locomotion. *Clin Sports Med, 11*(3), 521-531.

Krawetz, P., & Nance, P. (1996). Gait analysis of spinal cord injured subjects: effects of injury level and spasticity. *Arch Phys Med Rehabil, 77*(7), 635-638.

Krings, T., Buchbinder, B. R., Butler, W. E., Chiappa, K. H., Jiang, H. J., Rosen, B. R., & Cosgrove, G. R. (1997). Stereotactic transcranial magnetic stimulation: correlation with direct electrical cortical stimulation. *Neurosurgery, 41*(6), 1319-1325; discussion 1325-1316.

Krings, T., Chiappa, K. H., Foltys, H., Reinges, M. H., Cosgrove, R. G., & Thron, A. (2001). Introducing navigated transcranial magnetic stimulation as a refined brain mapping methodology. *Neurosurg Rev, 24*(4-6), 171-179.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., . . . Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *J Physiol, 471*, 501-519.

Lamontagne, A., Malouin, F., Richards, C. L., & Dumas, F. (2002). Mechanisms of disturbed motor control in ankle weakness during gait after stroke. *Gait Posture, 15*(3), 244-255.

Lavoie, B. A., Cody, F. W., & Capaday, C. (1995). Cortical control of human soleus muscle during volitional and postural activities studied using focal magnetic stimulation. *Exp Brain Res, 103*(1), 97-107.

Lefaucheur, J. P., Andre-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., . . . Garcia-Larrea, L. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin Neurophysiol, 125*(11), 2150-2206.

Lewis, G. N., Signal, N., & Taylor, D. (2014). Reliability of lower limb motor evoked potentials in stroke and healthy populations: how many responses are needed? *Clin Neurophysiol, 125*(4), 748-754.

Lieber, R. L. (2010). *Skeletal Muscle Structure, Function, and Plasticity: The Physiological Basis of Rehabilitation*: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Lieber, R. L., & Friden, J. (2000). Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve, 23*(11), 1647-1666.

Liepert, J., Hassa, T., Tuscher, O., & Schmidt, R. (2011). Motor excitability during movement imagination and movement observation in psychogenic lower limb paresis. *J Psychosom Res, 70*(1), 59-65.

Liepert, J., & Neveling, N. (2009). Motor excitability during imagination and observation of foot dorsiflexions. *J Neural Transm (Vienna), 116*(12), 1613-1619.

Liew, S. L., Santarnecchi, E., Buch, E. R., & Cohen, L. G. (2014). Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery. *Front Hum Neurosci, 8*, 378.

Livingston, S. C., Friedlander, D. L., Gibson, B. C., & Melvin, J. R. (2013). Motor evoked potential response latencies demonstrate moderate correlations with height and limb length in healthy young adults. *Neurodiagn J, 53*(1), 63-78.

Lo, Y. L., & Fook-Chong, S. (2004). A transcranial magnetic stimulation study of the ipsilateral silent period in lower limb muscles. *Neurosci Lett, 368*(3), 337-340.

Lontis, E. R., Voigt, M., & Struijk, J. J. (2006). Focality assessment in transcranial magnetic stimulation with double and cone coils. *J Clin Neurophysiol, 23*(5), 462-471.

Lotze, M., Kaethner, R. J., Erb, M., Cohen, L. G., Grodd, W., & Topka, H. (2003). Comparison of representational maps using functional magnetic resonance imaging and transcranial magnetic stimulation. *Clin Neurophysiol, 114*(2), 306-312.

Maeda, F., & Pascual-Leone, A. (2003). Transcranial magnetic stimulation: studying motor neurophysiology of psychiatric disorders. *Psychopharmacology (Berl), 168*(4), 359-376.

Malcolm, M. P., Triggs, W. J., Light, K. E., Shechtman, O., Khandekar, G., & Gonzalez Rothi, L. J. (2006). Reliability of motor cortex transcranial magnetic stimulation in four muscle representations. *Clin Neurophysiol, 117*(5), 1037-1046.

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E., & George, M. S. (2001). The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: a replication in healthy adults comparing two methods of assessing the distance to cortex. *Biol Psychiatry, 49*(5), 454-459.

Meaney, A., Collett, J., Dawes, H., Howells, K., & Izadi, H. (2015). Consistency of evoked responses to dual-stimulator, single-pulse transcranial magnetic stimulation in the lower limb of people with multiple sclerosis. *J Clin Neurosci, 22*(9), 1434-1437.

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral cortex in the intact human subject. *Nature, 285*(5762), 227.

Meyer, B. U., Britton, T. C., Kloten, H., Steinmetz, H., & Benecke, R. (1991). Coil placement in magnetic brain stimulation related to skull and brain anatomy. *Electroencephalogr Clin Neurophysiol, 81*(1), 38-46.

Mileva, K. N., Bowtell, J. L., & Kossev, A. R. (2009). Effects of low-frequency whole-body vibration on motor-evoked potentials in healthy men. *Exp Physiol, 94*(1), 103-116.

Miyai, I., Tanabe, H. C., Sase, I., Eda, H., Oda, I., Konishi, I., . . . Kubota, K. (2001). Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. *Neuroimage, 14*(5), 1186-1192.

Moosabhoy, M. A., & Gard, S. A. (2006). Methodology for determining the sensitivity of swing leg toe clearance and leg length to swing leg joint angles during gait. *Gait Posture, 24*(4), 493-501.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., . . . Stroke Statistics, S. (2015). Heart disease and stroke statistics--2015

update: a report from the American Heart Association. *Circulation, 131*(4), e29-322.

Mulroy, S., Gronley, J., Weiss, W., Newsam, C., & Perry, J. (2003). Use of cluster analysis for gait pattern classification of patients in the early and late recovery phases following stroke. *Gait Posture, 18*(1), 114-125.

Needle, A. R., Palmer, J. A., Kesar, T. M., Binder-Macleod, S. A., & Swanik, C. B. (2013). Brain Regulation of Muscle Tone and Laxity Differs in Functionally Unstable Ankles. *J Sport Rehabil, 9*, 9.

Neptune, R. R., Kautz, S. A., & Zajac, F. E. (2001). Contributions of the individual ankle plantar flexors to support, forward progression and swing initiation during walking. *J Biomech, 34*(11), 1387-1398.

Ngomo, S., Leonard, G., Moffet, H., & Mercier, C. (2012). Comparison of transcranial magnetic stimulation measures obtained at rest and under active conditions and their reliability. *J Neurosci Methods, 205*(1), 65-71.

Nielsen, J., & Petersen, N. (1995). Evidence favouring different descending pathways to soleus motoneurones activated by magnetic brain stimulation in man. *J Physiol, 486 (Pt 3)*(Pt 3), 779-788.

Nielsen, J. B. (2002). Motoneuronal drive during human walking. *Brain Res Brain Res Rev, 40*(1-3), 192-201.

Nielsen, J. B. (2003). How we walk: central control of muscle activity during human walking. *Neuroscientist, 9*(3), 195-204.

Niskanen, E., Julkunen, P., Saisanen, L., Vanninen, R., Karjalainen, P., & Kononen, M. (2010). Group-level variations in motor representation areas of thenar and anterior tibial muscles: Navigated Transcranial Magnetic Stimulation Study. *Hum Brain Mapp, 31*(8), 1272-1280.

Nordander, C., Willner, J., Hansson, G. A., Larsson, B., Unge, J., Granquist, L., & Skerfving, S. (2003). Influence of the subcutaneous fat layer, as measured by ultrasound, skinfold calipers and BMI, on the EMG amplitude. *Eur J Appl Physiol, 89*(6), 514-519.

Norkin, C. C., & White, D. J. (2003). *Measurement of Joint Motion: A Guide to Goniometry*: F a Davis Company.

Obata, H., Sekiguchi, H., Nakazawa, K., & Ohtsuki, T. (2009). Enhanced excitability of the corticospinal pathway of the ankle extensor and flexor muscles during standing in humans. *Exp Brain Res, 197*(3), 207-213.

Oliveri, M., Caltagirone, C., Loriga, R., Pompa, M. N., Versace, V., & Souchard, P. (2012). Fast increase of motor cortical inhibition following postural changes in healthy subjects. *Neurosci Lett, 530*(1), 7-11.

Olney, S. J., & Richards, C. (1996). Hemiparetic gait following stroke. Part I: Characteristics. *Gait & Posture, 4*(2), 136-148.

Panizzolo, F. A., Green, D. J., Lloyd, D. G., Maiorana, A. J., & Rubenson, J. (2013). Soleus fascicle length changes are conserved between young and old adults at their preferred walking speed. *Gait Posture, 38*(4), 764-769.

Peat, M., Dubo, H. I., Winter, D. A., Quanbury, A. O., Steinke, T., & Grahame, R. (1976). Electromyographic temporal analysis of gait: hemiplegic locomotion. *Arch Phys Med Rehabil, 57*(9), 421-425.

Petersen, N., Christensen, L. O., & Nielsen, J. (1998). The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. *J Physiol, 513 (Pt 2),* 599-610.

Petersen, N. T., Butler, J. E., Marchand-Pauvert, V., Fisher, R., Ledebt, A., Pyndt, H. S., . . . Nielsen, J. B. (2001). Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *J Physiol, 537*(Pt 2), 651-656.

Petersen, T. H., Willerslev-Olsen, M., Conway, B. A., & Nielsen, J. B. (2012). The motor cortex drives the muscles during walking in human subjects. *J Physiol, 590*(Pt 10), 2443-2452.

Peterson, C. L., Cheng, J., Kautz, S. A., & Neptune, R. R. (2010). Leg extension is an important predictor of paretic leg propulsion in hemiparetic walking. *Gait Posture, 32*(4), 451-456.

Peterson, C. L., Kautz, S. A., & Neptune, R. R. (2011). Braking and propulsive impulses increase with speed during accelerated and decelerated walking. *Gait Posture, 33*(4), 562-567.

Piron, L., Piccione, F., Tonin, P., & Dam, M. (2005). Clinical correlation between motor evoked potentials and gait recovery in poststroke patients. *Arch Phys Med Rehabil, 86*(9), 1874-1878.

Portney, L. G., & Watkins, M. P. (2009). *Foundations of Clinical Research: Applications to Practice*: Pearson/Prentice Hall.

Pouwels, S., Lalmohamed, A., Leufkens, B., de Boer, A., Cooper, C., van Staa, T., & de Vries, F. (2009). Risk of hip/femur fracture after stroke: a population-based case-control study. *Stroke, 40*(10), 3281-3285.

Press, W. H., Flannery, B. P., Teukolsky, S. A., & Vetterling, W. T. (1986). Numerical recipes, 1986. *Cambridge University Press, Cambridge, ISBN: 0, 521*(30811), 9.

Ridding, M. C., & Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol, 588*(Pt 13), 2291-2304.

Robertson, D. G. E. (2004). *Research Methods in Biomechanics*: Human Kinetics
.

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clin Neurophysiol, 122*(8), 1686.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of, T. M. S. C. G. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol, 120*(12), 2008-2039.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., . . . et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol, 91*(2), 79-92.

Rossini, P. M., Berardelli, A., Deuschl, G., Hallett, M., Maertens de Noordhout, A. M., Paulus, W., & Pauri, F. (1999). Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl, 52*, 171-185.

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., . . . Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol, 126*(6), 1071-1107.

Rossini, P. M., Desiato, M. T., & Caramia, M. D. (1992). Age-related changes of motor evoked potentials in healthy humans: non-invasive evaluation of central and peripheral motor tracts excitability and conductivity. *Brain Res, 593*(1), 14-19.

Rossini, P. M., & Rossi, S. (2007). Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. *Neurology, 68*(7), 484-488.

Rothwell, J. C., Hallett, M., Berardelli, A., Eisen, A., Rossini, P., & Paulus, W. (1999). Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl, 52*, 97-103.

Roy, F. D., Norton, J. A., & Gorassini, M. A. (2007). Role of sustained excitability of the leg motor cortex after transcranial magnetic stimulation in associative plasticity. *J Neurophysiol, 98*(2), 657-667.

Roy, F. D., Zewdie, E. T., & Gorassini, M. A. (2011). Short-interval intracortical inhibition with incomplete spinal cord injury. *Clin Neurophysiol, 122*(7), 1387-1395.

Rubens, M. T., & Zanto, T. P. (2012). Parameterization of transcranial magnetic stimulation. *J Neurophysiol, 107*(5), 1257-1259.

Ruohonen, J., & Karhu, J. (2010). Navigated transcranial magnetic stimulation. *Neurophysiol Clin, 40*(1), 7-17.

Saisanen, L., Julkunen, P., Niskanen, E., Danner, N., Hukkanen, T., Lohioja, T., . . . Kononen, M. (2008). Motor potentials evoked by navigated transcranial magnetic stimulation in healthy subjects. *J Clin Neurophysiol, 25*(6), 367-372.

Saisanen, L., Kononen, M., Julkunen, P., Maatta, S., Vanninen, R., Immonen, A., . . . Mervaala, E. (2010). Non-invasive preoperative localization of primary motor cortex in epilepsy surgery by navigated transcranial magnetic stimulation. *Epilepsy Res, 92*(2-3), 134-144.

Saisanen, L., Pirinen, E., Teitti, S., Kononen, M., Julkunen, P., Maatta, S., & Karhu, J. (2008). Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. *J Neurosci Methods, 169*(1), 231-238.

Sandbrink, F., Syed, N. A., Fujii, M. D., Dalakas, M. C., & Floeter, M. K. (2000). Motor cortex excitability in stiff-person syndrome. *Brain, 123 (Pt 11)*(Pt 11), 2231-2239.

Schubert, M., Curt, A., Colombo, G., Berger, W., & Dietz, V. (1999). Voluntary control of human gait: conditioning of magnetically evoked motor responses in a precision stepping task. *Exp Brain Res, 126*(4), 583-588.

Schubert, M., Curt, A., Jensen, L., & Dietz, V. (1997). Corticospinal input in human gait: modulation of magnetically evoked motor responses. *Exp Brain Res, 115*(2), 234-246.

Schünke, M., Schulte, E., Ross, L. M., Schumacher, U., & Lamperti, E. D. (2006). *Thieme Atlas of Anatomy: General Anatomy and Musculoskeletal System*: Thieme.

Seo, J. P., Do, K. H., Jung, G. S., Seo, S. W., Kim, K., Son, S. M., . . . Jang, S. H. (2014). The difference of gait pattern according to the state of the corticospinal tract in chronic hemiparetic stroke patients. *NeuroRehabilitation, 34*(2), 259-266.

Shellock, F. G., & Spinazzi, A. (2008). MRI safety update 2008: part 2, screening patients for MRI. *AJR Am J Roentgenol, 191*(4), 1140-1149.

Silbert, B. I., Patterson, H. I., Pevcic, D. D., Windnagel, K. A., & Thickbroom, G. W. (2013). A comparison of relative-frequency and threshold-hunting methods to determine stimulus intensity in transcranial magnetic stimulation. *Clin Neurophysiol, 124*(4), 708-712.

Soto, O., Valls-Sole, J., Shanahan, P., & Rothwell, J. (2006). Reduction of intracortical inhibition in soleus muscle during postural activity. *J Neurophysiol, 96*(4), 1711-1717.

Sousa, A. S., Silva, A., Santos, R., Sousa, F., & Tavares, J. M. (2013). Interlimb coordination during the stance phase of gait in subjects with stroke. *Arch Phys Med Rehabil, 94*(12), 2515-2522.

Sousa, A. S., & Tavares, J. M. R. (2012). Surface electromyographic amplitude normalization methods: a review. *Electromyography: New Developments, Procedures and Applications*.

Soylu, A. R., & Arpinar-Avsar, P. (2010). Detection of surface electromyography recording time interval without muscle fatigue effect for biceps brachii muscle during maximum voluntary contraction. *Journal of Electromyography and Kinesiology, 20*(4), 773-776.

Sparing, R., Hesse, M. D., & Fink, G. R. (2010). Neuronavigation for transcranial magnetic stimulation (TMS): where we are and where we are going. *Cortex, 46*(1), 118-120.

Steube, D., Wietholter, S., & Correll, C. (2001). Prognostic value of lower limb motor evoked potentials for motor impairment and disability after 8 weeks of stroke rehabilitation--a prospective investigation of 100 patients. *Electromyogr Clin Neurophysiol, 41*(8), 463-469.

Stevens-Lapsley, J. E., Thomas, A. C., Hedgecock, J. B., & Kluger, B. M. (2013). Corticospinal and intracortical excitability of the quadriceps in active older and younger healthy adults. *Arch Gerontol Geriatr, 56*(1), 279-284.

Stokes, M. (1985). Reliability and repeatability of methods for measuring muscle in physiotherapy. *Physiotherapy Theory and Practice, 1*(2), 71-76

Stokic, D. S., McKay, W. B., Scott, L., Sherwood, A. M., & Dimitrijevic, M. R. (1997). Intracortical inhibition of lower limb motor-evoked potentials after paired transcranial magnetic stimulation. *Exp Brain Res, 117*(3), 437-443.

Tallent, J., Goodall, S., Hortobagyi, T., St Clair Gibson, A., French, D. N., & Howatson, G. (2012). Repeatability of corticospinal and spinal measures during lengthening and shortening contractions in the human tibialis anterior muscle. *PLoS ONE, 7*(4), e35930.

Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. *J Clin Neurophysiol, 19*(4), 322-343.

Terao, Y., Ugawa, Y., Hanajima, R., Machii, K., Furubayashi, T., Mochizuki, H., . . . Kanazawa, I. (2000). Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. *Brain Res, 859*(1), 137-146.

Terao, Y., Ugawa, Y., Sakai, K., Uesaka, Y., Kohara, N., & Kanazawa, I. (1994). Transcranial stimulation of the leg area of the motor cortex in humans. *Acta Neurol Scand, 89*(5), 378-383.

Thordstein, M., Saar, K., Pegenius, G., & Elam, M. (2013). Individual effects of varying stimulation intensity and response criteria on area of activation for different muscles in humans. A study using navigated transcranial magnetic stimulation. *Brain Stimul, 6*(1), 49-53.

Tobimatsu, S., Sun, S. J., Fukui, R., & Kato, M. (1998). Effects of sex, height and age on motor evoked potentials with magnetic stimulation. *J Neurol, 245*(5), 256-261.

Toleikis, J. R., Sloan, T. B., & Ronai, A. K. (1991). Optimal transcranial magnetic stimulation sites for the assessment of motor function. *Electroencephalogr Clin Neurophysiol, 81*(6), 443-449.

Turns, L. J., Neptune, R. R., & Kautz, S. A. (2007). Relationships between muscle activity and anteroposterior ground reaction forces in hemiparetic walking. *Arch Phys Med Rehabil, 88*(9), 1127-1135.

Vaalto, S., Julkunen, P., Saisanen, L., Kononen, M., Maatta, S., & Karhu, J. (2013). Long-term plasticity may be manifested as reduction or expansion of cortical representations of actively used muscles in motor skill specialists. *Neuroreport, 24*(11), 596-600.

Valls-Sole, J., Alvarez, R., & Tolosa, E. S. (1994). Responses of the soleus muscle to transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol, 93*(6), 421-427.

van Asseldonk, E. H. F., & Boonstra, T. A. (2015). Transcranial Direct Current Stimulation of the Leg Motor Cortex Enhances Coordinated Motor Output During Walking With a Large Inter-Individual Variability. *Brain Stimul*.

van Hedel, H. J., Murer, C., Dietz, V., & Curt, A. (2007). The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task. *J Neurol, 254*(8), 1089-1098.

van Hedel, H. J., Wirth, B., & Curt, A. (2010). Ankle motor skill is intact in spinal cord injury, unlike stroke: implications for rehabilitation. *Neurology, 74*(16), 1271-1278.

Wang, R. Y., Tseng, H. Y., Liao, K. K., Wang, C. J., Lai, K. L., & Yang, Y. R. (2012). rTMS combined with task-oriented training to improve symmetry of interhemispheric corticomotor excitability and gait performance after stroke: a randomized trial. *Neurorehabil Neural Repair, 26*(3), 222-230.

Wasserman, E., Epstein, C. M., & Ziemann, U. (2008). *The Oxford handbook of transcranial stimulation*. Oxford ; New York: Oxford University Press.

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin Neurophysiol, 113*(7), 1165-1171.

Wassermann, E. M., McShane, L. M., Hallett, M., & Cohen, L. G. (1992). Noninvasive mapping of muscle representations in human motor cortex. *Electroencephalogr Clin Neurophysiol, 85*(1), 1-8.

Weerdesteyn, V., de Niet, M., van Duijnhoven, H. J., & Geurts, A. C. (2008). Falls in individuals with stroke. *J Rehabil Res Dev, 45*(8), 1195-1213.

Weir, J. P. (2005). Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *J Strength Cond Res, 19*(1), 231-240.

Wheaton, L. A., Villagra, F., Hanley, D. F., Macko, R. F., & Forrester, L. W. (2009). Reliability of TMS motor evoked potentials in quadriceps of subjects with chronic hemiparesis after stroke. *J Neurol Sci, 276*(1-2), 115-117.

Winter, D. A. (1983). Energy generation and absorption at the ankle and knee during fast, natural, and slow cadences. *Clin Orthop Relat Res*(175), 147-154.

Winter, D. A. (1987). *The biomechanics and motor control of human gait*. Waterloo, Ont.: University of Waterloo Press.

Winter, D. A. (1991). *The biomechanics and motor control of human gait: Normal, Elderly and Pathological.* (2nd ed.). Waterloo, Ont.: University of Waterloo Press.inter, D. A. (1992). Foot trajectory in human gait: a precise and multifactorial motor control task. *Phys Ther, 72*(1), 45-53; discussion 54-46.

Winter, D. A. (2009). *Biomechanics and motor control of human movement* (pp. xiv, 370 p.).

Wochnik-Dyjas, D., Glazowski, C., & Niewiadomska, M. (1998). Peculiarity of soleus motor potentials evoked by transcranial magnetic stimulation and electrical stimulation of tibial nerve. *Electroencephalogr Clin Neurophysiol, 109*(4), 369-375.

Yamaguchi, T., Fujiwara, T., Liu, W., & Liu, M. (2012). Effects of pedaling exercise on the intracortical inhibition of cortical leg area. *Exp Brain Res, 218*(3), 401-406.

Yang, Y. R., Tseng, C. Y., Chiou, S. Y., Liao, K. K., Cheng, S. J., Lai, K. L., & Wang, R. Y. (2013). Combination of rTMS and treadmill training modulates corticomotor inhibition and improves walking in Parkinson disease: a randomized trial. *Neurorehabil Neural Repair, 27*(1), 79-86.

Yen, C. L., Wang, R. Y., Liao, K. K., Huang, C. C., & Yang, Y. R. (2008). Gait training induced change in corticomotor excitability in patients with chronic stroke. *Neurorehabil Neural Repair, 22*(1), 22-30.

Zeni, J. A., Jr., Richards, J. G., & Higginson, J. S. (2008). Two simple methods for determining gait events during treadmill and overground walking using kinematic data. *Gait Posture, 27*(4), 710-714.

Ziemann, U., Netz, J., Szelenyi, A., & Homberg, V. (1993). Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neurosci Lett, 156*(1-2), 167-171.

APPENDICES

MRI Screening Questionnaire

TMS Screening Questionnaire

Fugl-Meyer Lower Extremity Assessment Form

Medical University of South Carolina
Center for Rehabilitation Research for Neurological Conditions
MRI Screening Questionnaire

Study:_____ Investigator:_____

Subject ID:_____ Date:_____

Subject Weight: _____ Subject Age: _____

MRI Screening Questionnaire: Directions- Ask participant the following questions and record the answers accordingly. Any additional notes may be written at the lower 'Notes' section.

	Yes	No
1. Have you had prior surgery? (If yes, state type and date of surgery in 'Notes')		
2. Have you ever had ear surgery (cochlear implant or staples)?		
3. Do you have metal clips in your head or brain from previous surgery?		
4. Do you have a pacemaker or replacement valve?		
5. Have you ever been exposed to metal being welded, drilled, or cut?		
6. Is there any possibility of metal/metal pieces in your eyes?		
7. Have you ever been treated for metal in your eyes?		
8. Have you ever been shot?		
9. Do you have any metal in your body (like shrapnel, bullets, or implants)?		
10. Do you have a permanent tattooed eyeliner, wig, or hairpiece?		
11. Do you have an infusion pump implant for taking insulin or medication?		
12. Do you have a nerve stimulator implant (TENS unit)?		
13. Do you have a false eye, especially one that is magnetic?		
14. Do you have dentures or removable dental bridges?		
15. Do you think you are claustrophobic?		
16. Do you have any trans-dermal patches (i.e. nicotine patch)?		
17. Female: Are you pregnant?		
18. Female: Do you have an IUD?		
Is there any possibility of metal, metal pieces, or metal implants in your body?	Yes	No

Notes:

_____ _____

Signature of Person Obtaining Screening **Date**

Medical University of South Carolina
Center for Rehabilitation Research for Neurological Conditions
TMS Screening Questionnaire

Study Name: _____

Pro#: _____ Subject ID: _____

Your head will be exposed to a strong magnetic pulse. To maximize safety, please answer the questions below. Please do not hesitate to ask any questions you may have regarding any of the questions.

Contraindications:

Do you have, or have you ever had, any of the following? If Yes, please explain.

Y N 1. Metallic or plastic hardware, plates, or prosthetics on your scalp?

Y N 2. Cardiac Pacemaker

Y N 3. Implanted medication pumps, intracardiac line, or central venous catheter

Y N 4. Prior diagnosis of seizure or epilepsy

Y N 5. Any electrical, mechanical, or magnetic implants

Y N 6. Any body or clothing metal above your shoulders.

Y N 6a. If yes to #6, are you willing and able to remove the item(s) for testing?

Y N 7. Any metal on your body (i.e. watch or jewelry, hair holders or pins, eye glasses, body piercings, wallet, keys)?

Y N 7a. If yes to #7, are you willing and able to remove the item(s) for testing?

Precautions:

Do you have, or have you ever had, any of the following? If Yes, please explain.

Y N 1. Previous brain neurosurgery

Y N 2. Is there any possibility you are currently pregnant?

Y N 2a. If yes to #2, what is the date of the last menstrual period?_____

Y N 3. Migraine Headaches

Y N 3a. If yes to #3, are the migraine headaches controlled?

Please list current medications: We are looking for the presence of any medicines that affect seizure threshold such as tricyclic antidepressants and neuroleptics).

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Signature of Study Personnel: _____ **Date**: _____

Medical University of South Carolina
Center for Rehabilitation Research for Neurological Conditions

Fugl-Meyer Lower Extremity Assessment Form

MOTOR FUNCTION - Lower Extremity			
TEST	ITEM	SCORE	SCORING CRITERIA
I. Reflex Activity	Achilles		0-No reflex activity can be elicited 2-Reflex activity can be elicited
	Patellar		
II. A. Flexor Synergy (in supine)	Hip flexion		0-Cannot be performed at all 1-Partial motion 2-Full motion
	Knee flexion		
	Ankle dorsiflexion		
II. B. Extensor Synergy (in sidelying)	Hip extension		0-No motion 1-Weak motion 2-Almost full strength compared to normal
	Adduction		
	Knee extension		
	Ankle plantar flexion		
III. Movement combining synergies (in sitting: knees free of chair)	A. Knee flexion beyond 90°		0-No active motion 1-From slightly extended position, knee can be flexed, but not beyond 90° 2- Knee flexion beyond 90°
	B. Ankle dorsiflexion		0-No active flexion 1-Incomplete active flexion 2-Normal dorsiflexion
IV. Movement out of synergy (in standing, hip at 0°)	A. Knee flexion		0-Knee cannot flex without hip flexion 1-Knee begins flexion without hip flexion, but does not reach to 90°, or hip flexes during motion 2-Full motion as described
	B. Ankle dorsiflexion		0-No active motion 1-Partial motion 2-Full motion
V. Normal Reflexes (sitting)	Knee flexors Patellar Achilles		0-At least 2 of the 3 phasic reflexes are markedly hyperactive 1-One reflex is markedly hyperactive, or at least 2 reflexes are lively 2-No more than one reflex is lively and none are hyperactive
VI. Coordination/speed - Supine: Heel to opposite knee (5 repetitions in rapid succession)	A. Tremor		0-Marked tremor 1-Slight tremor 2-No tremor
	B. Dysmetria		0-Pronounced or unsystematic dysmetria 1-Slight or systematic dysmetria 2- No dysmetria
	C. Speed		0-Activity is more than 6 seconds longer than unaffected side 1-(2-5) seconds longer than unaffected side 2-Less than 2 seconds difference **Total Maximum Score of the Lower Extremity = 0-34**

TOTAL SCORE OF THE LOWER EXTREMITY: **Tally 1**_____(Max = 34)

(Check addition – Tally 2 MUST EQUAL Tally 1) **Tally 2**_____(Max = 34)