Motor Cortex Excitability is Modulated by Cutaneous Electrical Stimulation of the Foot Sole

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ABSTRACT

MOTOR CORTEX EXCITABILITY IS MODULATED BY CUTANEOUS ELECTRICAL STIMULATION OF THE FOOT SOLE

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Lower limb peripheral nerve stimulation, which activates cutaneous afferents from the foot dorsum, has been shown to generate transcortical reflex responses. This suggests that the motor cortex may play a role in modulating lower limb cutaneous reflexes. Electrical stimulation of the foot sole has also previously been shown to evoke reflexes in the lower limb which are topographically organized; these reflex pathways are unknown. The current experiment investigated if a non-noxious electrical stimulus applied to distinct foot sole regions evokes excitatory changes in lower limb muscles via a transcortical pathway. Cortical and spinal excitability were assessed using transcranial magnetic stimulation and cervicomedullary junction stimulation, respectively, following electrical stimulation applied to the heel and metatarsal regions of the foot sole. Comparisons between cortically evoked responses (MEPs) and brainstem evoked responses (CMEPs) during foot sole stimulation represent the neural level of excitability. During foot sole stimulation, the CMEP amplitude increased more than the MEP amplitude. Differences between MEP and CMEP suggest a cortical loop is involved in these cutaneous reflex responses.

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Chapter 1: General Introduction and Literature Review

1.1 General Introduction

The integration of sensory afferent feedback in the generation of motor commands is essential as seen by the significant disruptions to balance, gait, and fine motor skills that occur in individuals with sensory neuropathies (Rothwell et al. 1982; Sanes 1985; Lajoie et al. 1996). Information from sensory receptors in the skin is vital for the control and precision of motor tasks. Cutaneous mechanoreceptors, particularly in the soles of the feet and in the skin around the ankle provide valuable feedback in the control of standing balance and gait. The importance of this foot sole cutaneous feedback is evident through its role in modulating descending motor commands to the lower limb (Zehr and Stein 1999). Activation of these receptors in the foot sole has also been shown to modulate reflexive muscle activity in the lower limb (Fallon 2005; Zehr et al. 2014). Numerous studies have shown an impairment in balance control when this cutaneous feedback is reduced, either naturally through ageing (Perry 2006), or experimentally through cooling (Eils et al. 2004), or anesthesia (Meyer et al. 2004). Futhermore, several interventions to augment foot sole cutaneous feedback have been developed in an attempt to mitigate balance impairments associated with sensory loss (Priplata et al. 2005; Perry et al. 2008). To develop and improve such tactile interventions, it is important to understand the neural mechanisms underlying the responses evoked by cutaneous stimulation, as well as how this stimulation affects spinal and supraspinal structures.

These are the primary aims of this literature review:

1)   Discuss the importance of sensory information derived from the skin (particularly of the foot sole).

2)   Highlight the role of afferent feedback in the generation of motor commands.

3)   Provide information on the use of transcranial magnetic stimulation to study the influence of sensory information on cortical excitability.

1.2 Skin Classification

Skin can be classified as either glabrous or hairy. Glabrous skin is the skin covering the palms of the hands and the soles of the feet. Skin found on the rest of the body (covering the limbs/trunk, excluding the face) is classified as hairy skin. Skin on the face is classified on its own due to its innervation from the fifth cranial nerve (Vallbo et al. 1995). Stimuli including vibration, deep pressure, touch, and stretch are deciphered by four distinct mechanoreceptors in glabrous and non-glabrous skin (Vallbo and Johansson 1984; Macefield 2005). There is also an additional type of receptor that exists in the non-glabrous or hairy skin: the hair follicle unit, which may contribute to a different sensory role of skin. The skin of the glabrous foot sole has been shown to dictate pressure changes at the foot sole (Kavounoudias et al. 1998) and contribute to balance control in a myriad of postural challenges (Perry et al. 2000; Meyer et al. 2004). For these reasons, the following sections will focus on literature pertaining to cutaneous receptor classification and firing properties in glabrous skin.

1.2.1 Cutaneous Mechanoreceptors in Glabrous Skin

The four types of mechanoreceptor afferents within human glabrous skin are innervated by an Aβ fiber. These receptors are categorized based on their specific mechanoreceptive endings, the rate of adaptation, as well as their ability to respond to different tactile stimuli.

These cutaneous mechanoreceptors can be identified by the morphology of their endings. There are four distinct types of receptor endings, Merkel discs, Ruffini endings, Meissner corpuscles, and Pacinian corpuscles. These cutaneous mechanoreceptors are further classified based on their adaptability to a sustained indentation, as well as the size of their receptive field. Cutaneous receptors are categorized as either slowly adapting (SA) or fast adapting (FA) based on their rate of adaptation to a constant indentation. They are further organized into type I receptors which are associated with small receptive fields, or type II receptors which have large receptive fields. The Meissner corpuscles are fast adapting receptors, which have small receptive fields (type I). The Pacinian corpuscles are also fast adapting, however they have large receptive fields, and thus are classified as type II receptors. The slowly adapting type I receptors are associated with Merkel discs, and the slowly adapting type II are associated with Ruffini endings (Macefield 2005).

1.2.2 Morphology of Cutaneous Mechanoreceptors in Glabrous Skin

These cutaneous receptors are located in different regions within the skin, and have different receptive field sizes, which contribute to the different firing responses of the afferents. Merkel discs (SAI) and Meissner corpuscles (FAI), are located within the superficial layer of the skin in the epidermis. These receptors have small oval shaped

receptive fields with discrete borders (Iggo and Muir 1969; Vallbo et al. 1979). In comparison, Ruffini endings (SAII) and Pacinian corpuscles (FAII) are located deeper within the skin in the dermal layer. These type II receptors have larger receptive fields with less clearly defined borders (Vallbo et al. 1995).

The superficial Merkel discs (or Type I slowly adapting receptors) are connected to the papillary folds of the basal layer of skin; these receptors have a semi-rigid structure and are not encapsulated. Merkel cells are a type of modified epithelial cell, they contain synaptic vesicles which store and release neuropeptide. A single primary afferent can innervate multiple Merkel disc endings (Iggo and Muir 1969). These properties contribute to the slow adaptation of these receptors to a sustained stimulus or indentation. Ruffini endings (Type II slowly adapting receptors) are located deep within the dermal layer of the skin; these receptors are encapsulated with a lamina sheath and are filled with a fluid (Chambers et al. 1972). These receptors contain collagen fibers, which connect to the surrounding tissues, allowing them to be sensitive to skin stretch. A single Ruffini corpuscle is innervated by one primary afferent.

Meissner corpuscles (Type I fast adapting receptors) are leaf-like encapsulated receptors which consist of an elongated stack of flattened epithelial cells. The primary afferent can be seen to weave between the layers of these cells (Iggo and Ogawa 1977). A single primary afferent can terminate in multiple Meissner corpuscle receptor endings.

Lastly, the Pacinian corpuscles (Type II fast adapting) are characterized as onion-like encapsulated receptors that are located deep in the dermis and in the subcutaneous tissue. They are made up of many epithelial cell layers that are filled with fluid. The primary afferent terminal extends centrally within the corpuscle, and a single afferent

innervates a single Pacinian corpuscle (Macefield 2005).

1.2.3 Firing Characteristics of Cutaneous Mechanoreceptors

Slowly adapting receptors continue to respond for the duration of the stimulus (Chambers et al. 1972). This sustained adaptation is a key component of SA receptors. In comparison, fast adapting receptors only fire when a stimulus is first presented or removed, and stop firing when the stimulation continues (Macefield 2005).

Slowly adapting receptors provide spatial information, detect intensities of stimulation, as well as touch and pressure. These receptors will have a discharge for the duration of the skin deformation. By using the technique of microneurography, researchers have been able to establish thresholds and distinct firing responses to different tactile and vibration stimulation frequencies. These experiments have found that SA afferents respond to a wide range of frequencies (0.5-400 Hz), however they are most sensitive at the lower end of the spectrum (0.5 -15 Hz) (Iggo and Ogawa 1977). Merkel discs have an irregular discharge pattern that may be a result of the innervation of multiple Merkel disc endings by a single primary afferent (Iggo and Muir 1969). SA receptors also have a high dynamic sensitivity to the onset of mechanical stimuli, in addition to providing information about sustained input. Merkel discs code for changes in pressure, texture, and are highly sensitive to edges and curvature. In comparison, Ruffini endings have a regular discharge frequency (Chambers et al. 1972). These receptors are very sensitive to skin stretch due to their connection with collagen fibers. They also have a unique characteristic in that they encode the direction of skin stretch and are not as receptive to normal forces as other cutaneous afferents (Edin et al. 1995).

Fast adapting receptors adapt rapidly to a sustained indentation. They also code for the velocity and acceleration of mechanical stimuli. Meissner's corpuscles are composed of flattened, stacked epithelial cells, which allow these receptors to be sensitive to shear forces. The FAI afferent will code for the rate (velocity) of skin indentation and distinct on-off responses. These receptors are also edge sensitive, contributing to object recognition (Vallbo et al. 1979; Macefield 2005). Meissner's corpuscles are activated by frequencies in the range of 8 to 64 Hz (Macefield 2005). Pacinian corpuscles attenuate low velocity or sustained indentations, as the force is attenuated by the dermal layers of the skin. High velocity skin deformations, however, are able to displace the viscous fluid and transmit a force through the laminar layers to the afferent terminal. These receptors code for accelerations and vibrations at high frequencies (up to 400 Hz) (Johansson et al. 1982). They are activated by blowing over the skin and are sensitive to brief mechanical events or transient vibrations (Macefield 2005).

The diverse properties of the mechanoreceptors allow the skin to code for a range of sensory events that occur in contact with its surface. Much of our understanding of the different cutaneous afferents and their characteristics has evolved from microneurography studies of these afferents in the glabrous skin of the hand (Vallbo and Johansson 1984). However, the study of these afferents in the glabrous skin of the feet is also an important area of interest as the plantar surface of the skin is a large contributor to the standing control of balance (Roll et al. 2002).

1.3 The Role of Plantar Skin in Postural Control

The plantar skin of the feet has been suggested to play a role in coding for pressure changes beneath the feet (Kavounoudias et al. 1998) and in defining support surface boundaries (Perry et al. 2000), as well as in organizing anticipatory postural adjustments (Inglis et al. 1994). The role of plantar skin in the control of standing balance has been examined in numerous studies, which have altered this feedback with either reduction (ischemia, topical or hypothermic anesthesia, diabetic neuropathy), or with augmentation (vibration, special insoles) (Magnusson et al. 1990; Priplata et al. 2003; Meyer et al. 2004; Perry et al. 2008).

Reducing cutaneous feedback from the plantar foot sole has an influence on the control of standing balance. Perry and colleagues (2000) showed (with hypothermic anesthesia) that plantar cutaneous afferents are important for maintaining postural stability during compensatory stepping responses. It has also been shown that plantar foot skin plays a significant role when balance is challenged due to the absence of vision or unipedal stance (Meyer et al. 2004).

Furthermore, increased sensory feedback from cutaneous afferents in a population with sensory decline has been shown to be used by the CNS for the control of standing balance. By applying sub-sensory stimulation to the foot soles, (Priplata et al. 2005) showed that this additional sensory information reduced postural sway during quiet stance. In addition, it has been shown that stimulating skin on the foot sole either with vibration (Kavounoudias et al. 1998) or with electrical stimulation (Zehr et al. 1997; Nakajima et al. 2006) can evoke characteristic responses associated with stance or locomotion. Previous work has induced low-level sway away from a vibrotactile

stimulus applied to localized regions of the foot sole (Kavounoudias et al. 1999). These findings suggest skin receptors can code for vibrations and pressure changes at the foot sole and the skin feedback contributes to balance control and orientation.

Electrical stimulation of the foot sole has previously been shown to evoke topographically organized reflexes in the lower limb; where heel stimulation preferentially increases the activity of plantar-flexor muscles and forefoot stimulation increases dorsi-flexor activity (Nakajima et al. 2006). This suggests that distinct foot sole regions can provide functional information pertinent to whole body orientation and muscle modulation related to balance. Altogether, studies that have altered skin feedback from the foot sole, have highlighted the skin's important role in standing balance and movement control.

1.4 Cutaneous Reflexes

Receptors from the skin provide valuable tactile feedback during standing and during walking. Mechanical or electrical stimulation of Aβ axons from skin mechanoreceptors such as Meissner and Pacinian corpuscles, Merkel discs, and Ruffini endings result in functionally relevant neural and mechanical cutaneous reflexes (Van Wezel et al. 1997; Zehr et al. 1997). These cutaneous mechanoreceptors when stimulated alter the muscle activity patterns during standing as well as during walking (Zehr et al. 1997; Kavounoudias et al. 2001; Nakajima et al. 2006; Zehr et al. 2014; Zehr 2014). Sherrington (Sherrington 1910) discovered the first knowledge of cutaneous reflexes through the study of the nociceptive flexion reflex in the hind limb of the decerebrate cat. He showed that electrical stimulation of a peripheral nerve trunk results in excitation of the ipsilateral limb alpha motoneurons to the flexor muscles and inhibition of those to the

extensors. Later, Hagbarth (Hagbarth 1960) found evidence for similar reciprocal relationships for excitatory and inhibitory influences on flexor and extensor motoneurons in man.

Non-noxious electrical stimulation of cutaneous afferents, which innervate the foot, results in complex excitatory and inhibitory reflex responses in muscles acting about the knee and ankle (Aniss et al. 1992; Gibbs et al. 1995; Zehr et al. 2001). These reflex responses are typically divided into early (<70 ms), middle (70-110 ms), and late (>110 ms) epochs. The early short latency response is mediated by a spinal pathway (Jenner and Stephens 1982).

In recent years, there has been considerable controversy about the mechanism behind the long latency cutaneous reflex responses in lower limb muscles. The common assumption is that transcortical reflex pathways do not contribute to the control of lower limb muscles, and primarily control the upper limb. However, evidence exists that suggests that long latency cutaneous reflex responses may be mediated by a transcortical pathway.

A transcortical pathway is a pathway through which somatosensory input reaches the primary motor cortex by projections from the thalamus to the primary sensory cortex. In the motor cortex, there is a caudal region (M1/c) that receives exteroceptive cutaneous input and a rostral region (M1/r), which receives proprioceptive inputs. Nielsen and colleagues (1997) investigated whether stimulation of cutaneous afferents from the foot dorsum could modulate the motor cortex output evoked using transcranial magnetic stimulation. They investigated whether the excitation observed in TA motoneurons (during tonic voluntary dorsiflexion) following sural and superficial peroneal nerve

stimulation is mediated by a transcortical reflex pathway. Stimulation of these nerves evoked a reflex response in the TA at ~70-95 ms. They observed a significant facilitation of short-latency peaks of single TA motor units evoked by transcranial magnetic stimulation (TMS). These findings suggested that the long latency reflex response in the TA evoked by cutaneous afferent stimulation in the foot is at least partly mediated by a transcortical pathway. Christensen and colleagues (1999) also investigated if a similar mechanism contributes to the facilitation observed during walking. They evoked reflex responses during different phases of walking and applied magnetic stimulation to the motor cortex at different times in relation to the cutaneous stimulation. They showed that a transcortical pathway might also contribute to long latency cutaneous reflexes during walking (Christensen et al. 1999). These studies provide support for the existence of a transcortical pathway to the lower limb muscles.

1.5 Sensory-Motor Integration

Sensory-motor integration is the relationship between the sensory system and the motor system. It is the process whereby ongoing sensory information is used to make motor plans and execute movement. This process can be affected when there are deficits in afferent input, thus altering motor output. One example of this is the deficits that occur with the skin and the afferent feedback from the skin as we age (Perry 2006). This diminished input may lead to declines in cutaneous reflex responses and contribute to increased instability and falls with age.

Somatosensory information is important and necessary for execution of known skills (Pearson 2000), the learning of new skills (Pavlides et al. 1993), as well as for fine motor control (Rabin and Gordon 2004). In clinical states, patients with reduced

somatosensory input due to peripheral de-afferentation show disrupted motor execution (Rothwell et al. 1982).

The primary somatosensory cortex (S1) is the brain region that is responsible for processing somatic input such as, touch, temperature, pain and position. The S1 is located in the post-central gyrus. It is composed of four distinct subdivisions, Brodmann areas 1, 2, 3a and 3b. Each of these cortical areas contains somatotopic maps representing the contralateral side of the body and face (Kaas et al. 1979). Functionally, each of these 4 areas differs in processing of sensory inputs. Areas 1 and 2 are associated with processing of cutaneous and deep afferent inputs, respectively (Friedman et al. 1986). Proprioceptive inputs projecting from deep tissues such as muscles and joints are received in area 3a (Iwamura et al. 1993). Cutaneous inputs are primarily processed in area 3b (Friedman et al. 2008).

Somatosensory processing begins with nerve endings originating in joint receptors, golgi tendon organs, muscle spindle fibers, and cutaneous mechanoreceptors (Riemann and Lephart 2002). These somatosensory afferents generate potentials, which are transmitted to the dorsal horn of the spinal cord to travel through the dorsal column and project to the medial lemnicus of the medulla. The second order afferent fibres then decussate and project to the ventral posterior nuclei of the thalamus. Finally the thalamocortical projections (third order afferent fibres) reach the respective areas of the primary somatosensory cortex, depending on the nature of afferent input (Mountcastle 1993).

Brodmann's area 4 is known as the primary motor cortex (M1). Its main function is to control motor movements. M1 is located in each frontal lobe, anterior to the central

sulcus in the pre-central gyrus (Jenkins et al. 2007). It is somatotopically organized, with highly innervated regions of the body such as hand and face regions holding greater representations of cortical area. Thus each region in the M1 controls voluntary action (including movement initiation and coordination) of specific muscles in the body, which that area represents (Magill 2007). Pyramidal cells in M1 form the descending tracts, which synapse and interact with spinal motoneurons, allowing for the control of movement (Canedo 1997).

The somatosensory cortex has been shown to have direct connections to the primary motor cortex, with the exception of area 3b (Jones et al. 1978). Thus it is expected that neurons within S1 can alter neural activity within M1. S1 projections to M1 can cause inhibitory post-synaptic potentials (ISPS) or excitatory post-synaptic potentials (ESPS) within M1 (Zarzecki et al. 1978; Ghosh and Porter 1988). Tetanic stimulation of S1 in cats has been shown to create long-term potentiation of neurons within M1, thus changing excitability (Sakamoto et al. 1987). Thus S1 has the capability to create short and long term changes in M1. The connectivity between S1 and M1 also has a functional importance. Cooling of area 2 in S1 led to difficulty in performing coordinated movements, which was reversed when the area was warmed again (Brinkman et al. 1985). In non-human primates, it was shown that ablation of S1 impaired the ability to learn new motor skills and the recovery of motor skills (Pavlides et al. 1993; Abela et al. 2012). This evidence indicates that the arrival of somatosensory input to S1 can mediate output from M1, as observed by changes in cortical excitability.

1.6 Cortical Excitability

The influence of somatosensory information on cortical excitability has been investigated in numerous studies. The organization of the sensorimotor cortex is dynamic thus changes in afferent input have the potential to modulate corticomotor excitability and alter cortical maps. Various modalities of afferent input (electrical, magnetic, mechanical) have been used in attempt to influence motor controlling structures. Muscle vibration applied at low amplitude has shown to produce Ia afferent input reaching both S1 and M1. Alternatively, temporary de-afferentation of the hand or forearm has shown an increase in motor evoked potential (MEP) amplitudes in muscles proximal to the site of ischemia (Brasil-Neto et al. 1992; Ziemann et al. 1998). By using transcranial magnetic stimulation, changes in corticospinal excitability (Steyvers et al. 2003), sensorimotor organization (Rosenkranz and Rothwell 2006) and in cortical motor map organization (Forner-Cordero et al. 2008) after periods of sensory modulation have been reported. Additional studies have evolved to deliver stimulation to broader areas and include afferent inflow from cutaneous afferents. Christova et al. (2011) examined the effect of continuous mechanical stimulation (25 Hz) for 20 min applied to the palmar surface of the hand. They concluded that 20 min of the mechanical stimulation induces long-lasting plastic changes in the primary motor cortex, seen by significant facilitation of the MEPs 1-hour post intervention. Repetitive stimulation of peripheral nerves has also been shown to induce reorganization of the motor cortex as well as an increase of corticospinal excitability (Hamdy et al. 1998; Ridding et al. 2000; Ridding et al. 2001; Kaelin-Lang et al. 2002). Several human studies using TMS techniques have examined how sensory afferent inputs influence cortical networks of the primary motor cortex.

Most of these types of studies have been done in the hand or arm muscles, whereas investigations involving the leg muscles have been limited. Cortical networks supplying leg muscles are facilitated with prior stimulation of ascending afferent pathways from these homonymous muscles (Deletis et al. 1992; Nielsen et al. 1997; Petersen et al. 1998). Facilitation of the MEP in ankle flexors which lasts for more than an hour can also result from repetitive electrical stimulation of the common peroneal nerve in healthy subjects (Khaslavskaia et al. 2002; Knash et al. 2003). Similarly, Roy and Gorassini (Roy and Gorassini 2008) investigated the influence of tibial nerve stimulation at the ankle on the excitability of cortical circuits in the leg area. They showed that sensory activation from the leg has a facilitatory effect on the leg primary motor cortex. Although more studies have started to investigate leg motor areas with the use of nerve or muscle stimulation, the effects of sensory stimulation of more functionally active skin areas (such as the feet) are still limited.

1.7 Research Rationale

Cutaneous afferents in the sole of the foot have been found to directly contribute to the control of posture and movement (Zehr et al. 1997; Zehr et al. 1998; Kavounoudias et al. 1998; Kavounoudias et al. 1999; Kavounoudias et al. 2001; Zehr et al. 2014). Electrical stimulation of skin on the foot sole has previously been shown to evoke topographically organized cutaneous reflexes in the lower limb; heel stimulation preferentially increases the activity of plantar flexor muscles and forefoot stimulation increases dorsiflexor activity (Nakajima et al. 2006). These cutaneous reflex responses in the lower limb muscles have been shown to occur at long latencies (70-110 ms). Interestingly, lower limb peripheral nerve stimulation, which activates cutaneous

afferents from the foot dorsum, has been shown to generate reflex responses in a similar latency range. These reflex responses evoked from the foot dorsum are purportedly supported by a transcortical pathway (Nielsen et al. 1997). This observation raises the possibility that cutaneous reflex responses evoked from the foot sole could also be, at least in part, mediated by a transcortical pathway.

1.7.1 Thesis Objective and Hypotheses

Objective: This thesis investigated whether cutaneous electrical stimulation of distinct regions of the plantar sole (heel or metatarsal) modulates excitability of the motor cortex, via a transcortical pathway. The research hypotheses were:

Primary

i.   To confirm the existence of a transcortical pathway, we must compare responses evoked subcortically to those evoked cortically (cervicomedullary motor evoked potentials; CMEPs vs MEPs).
   a. We hypothesize that MEPs and CMEPs will respond differently to changes in motoneuronal excitability.
   b. We expect that stimulation of the foot sole would facilitate motoneuronal excitability at the level of the spinal cord, represented by increases in CMEP amplitude. .

Secondary

ii.   We hypothesized that electrical stimulation of distinct regions of the foot sole would modulate lower limb motor evoked potentials (MEPs) in a location-specific manner, such that with heel stimulation we would observe an increase in MEP amplitude of the plantar flexor muscles, and a decrease with metatarsal stimulation. The opposite would be expected in the tibialis anterior muscle.

Chapter 2: General Methodology

2.1   Ethics

All experimental procedures were approved by the research ethics board at the University of Guelph, which abides by the declaration of Helsinki. All subjects completed health history questionnaires and consent prior to data collection (see Appendix).

2.2   Study Participants

Sixteen subjects (eight females; 19-29 years old) participated in *Experiment 1* of the study. Eight of the sixteen subjects returned to participate in *Experiment* 2 of the study. Subjects were free from neurological and musculoskeletal disorders. Subjects were excluded if they had history of any skin disorders, numbness, seizures, concussions, or lower limb injuries.

2.3 Experimental Set-up

2.3.1 Experiment 1 and 2

Surface electromyography (EMG) was recorded from the right soleus, medial gastrocnemius, and tibialis anterior muscles in all subjects. Skin was thoroughly cleaned with alcohol swabs prior to electrode placement. In order to obtain optimal results in the EMG response to both cutaneous reflexes and motor evoked potentials, three surface electrodes (Ag/AgCl) were placed on each muscle for a bipolar (for reflexes) and monopolar arrangement (for motor evoked potentials; MEPs and cervicomedullary motor

evoked potentials; CMEPs). A ground electrode was placed on the right lateral

malleolus. EMG signals were amplified (gain 500-1000k ;Bortek AMT-8, Calgary,

Canada) and analog signals were digitally converted at 2048Hz (Cambridge Electronic

Design, Cambridge, UK) and monitored in Spike 2 version 7.

The right plantar sole was also cleaned with alcohol prior to the placement of

electrodes for cutaneous stimulation. Two silver/silver-chloride (Ag/AgCl) stimulus

electrodes were placed on both the metatarsal (MET) and heel (HEEL) regions of the

plantar sole (Figure. 3.1**A**). The metal tabs of the electrodes were left exposed on the

lateral borders of the plantar sole regions to allow the respective stimulation leads to be

connected. Electrode gel was applied on the electrodes to reduce impedance, and lower

the voltage necessary to maintain a constant current. The electrodes were secured to the

skin using Transpore^{TM} tape.

2.3.2 Experiment 2

In addition to the above set up, in *experiment 2* subjects were set up with an

additional pair of electrodes (ConMed Clear Trace electrodes) fixed over the mastoids

for cervicomedullary junction stimulation.

2.4 Cutaneous Stimulation

Reflexes can be evoked using electrical or mechanical stimulation. In a review

article by (Zehr and Stein 1999), it was concluded that both mechanical and electrical

stimulation involve similar pathways and reflexes evoked with electrical stimulation are

qualitatively similar to natural cutaneous stimulation. Similarly, Perrier and colleagues

(2000) showed that reflexes due to electrical stimulation of the superficial peroneal nerve

are similar to those of natural skin activation of the innervation area of the superficial

peroneal nerve.

In contrast, Buford and Smith (1993) presented differences between the two modes

of stimulation for evoking reflexes in cats. They found that the kinematic responses to

electrical stimulation were smaller in size than those from the physical taps. Mechanical

stimulation may also potentially activate muscle afferents and joint receptors thus

resulting in a response of different magnitude. It can also be argued that electrical

stimulation bypasses the cutaneous mechanoreceptor endings (Caruso 1995). However,

the benefits of electrical stimulation to evoke reflexes is that they allow more control of

stimulus intensity as well as more localized control of the area and afferents being

stimulated compared to mechanical stimulation (Burke 1999).

2.4.1 Cutaneous Perceptual Threshold Testing

A Digitimer Constant Current High Voltage Stimulator (Model DS7AH) was

used to stimulate the plantar sole. Electrical stimulation consisted of a train of five

constant-current rectangular pulses (each 1.0 ms duration, inter-pulse interval 3 ms)

applied at a frequency of 2 Hz. Prior to data collection, perceptual threshold (PT) was

determined for each stimulation site. PT is defined as the stimulus intensity (current)

found to evoke detectable tactile sensation at the lowest intensity possible in the

cutaneous region of interest. Subjects stood upright while the experimenter gradually

decreased the stimulus current until the participant could barely discern the stimulus

(identified as PT) on their plantar sole. This was then repeated to ensure the correct PT

was identified. Electrical stimulation intensity was set at 2X PT to evoke a non-noxious

cutaneous sensation during each trial. It was verbally confirmed in all subjects that the stimulation intensity was not painful.

2.4.2 Cutaneous Reflexes

In order to determine an appropriate inter-stimulus interval between the train of electrical pulses to the plantar sole and the stimulation to either the cortex or brainstem, cutaneous reflexes in the right lower leg muscles were evoked. Subjects stood upright on a stable surface with their eyes open while reflexes were evoked by stimulation of the metatarsal or heel regions of the right plantar sole. In order to obtain stable and clear cutaneous reflexes, 100 stimulations were applied at each region, with a 3-minute resting period between regions.

2.5 Transcranial Magnetic Stimulation

TMS is a unique technique used to non-invasively stimulate the brain. Since its introduction in the 1980's, it has been used to investigate cortical excitability. TMS is a well-used technique that can provide information on the anatomical and functional organization of the motor system, for example, it has been used for mapping of the motor cortex (Kammer et al. 2005; Bestmann et al. 2008; Julkunen et al. 2009). TMS has also been used in the treatment of diseases such as Parkinson's (Pascual-Leone et al. 1994), Huntington's disease (Meyer et al. 1992; Lorenzano et al. 2006), and Tourette's syndrome (Ziemann et al. 1997). The ability of TMS to alter excitability has been beneficial in the treatment of these diseases. TMS can also be used to determine conduction velocities of peripheral nerves and central processing times, which is a helpful tool in the clinic.

A transcranial magnetic stimulator is composed of a capacitor and an inducer. The capacitor charges up to ~2 kV of electricity and can produce a pulse up to 5000A of energy when discharged. The inducer is a magnetic coil made up of copper wire. When a brief electrical current is passed through this hand-held stimulating coil, a magnetic field is produced perpendicular to the surface of the coil. When the coil is placed over the cortex, the magnetic field can pass through the skull and tissues unimpeded. In turn, this induces an electric current, which flows perpendicular to the magnetic field. This electric current activates underlying neurons, which are parallel to the surface of the coil (Hallett 2000). The transcranial magnetic stimulation is achieved by this intra-cortical current, which depolarizes the axon cell membranes of both cortical excitatory pyramidal cells and excitatory and inhibitory interneurons. If the depolarization exceeds a threshold level the interneurons will fire and the TMS-evoked activity can be measured with EMG. If the interneurons that are activated are located within the motor cortex, then the corticospinal tract (CS) can be activated and a motor evoked potential (MEP) will result in the skeletal muscle. This MEP is the net effect of the activation of a collection of neurons which all synapse onto the CS tract.

TMS is routinely used to investigate motor cortex excitability due to the measurable motor evoked potentials (MEPs) from peripheral muscles; however, the exact structures of excitability are debated and need to be differentiated. MEPs produced by TMS are commonly used in research and clinical evaluation, as amplitudes and latencies can indicate cortical changes (Meyer et al. 1992). The issue with MEPs, however, is that they are affected by a combination of cortical, subcortical, and spinal-cord mechanisms, which must be separated. By also stimulating at a subcortical level,

with a method known as cervicomedullary junction stimulation (discussed in a later section), we are able to classify any observed changes as originating either in the cortex or subcortex.

2.5.1 Threshold Testing

TMS was performed using a MagStim 200 stimulator and a cone coil coil to target the leg area of the motor cortex. Subjects were asked to wear a cloth head cap so that the optimal stimulus site could be marked. Subjects remained seated with their legs relaxed and knees bent ~90° while the experimenter searched for the optimal site. The vertex of the head was identified as the intersection point of the two arcs from the nasion to the inion, and the left pre-auricular point to the right pre-auricular point. Searching began 1-2 cm lateral from the vertex in the contralateral cortex. The experimenter held the coil securely in place and the coil was oriented to deliver anterior-posterior directed current in the brain. The optimal stimulus site was identified using a TMS stimulation intensity that was slightly above resting threshold. This intensity was decreased when peak-to-peak amplitude of at least 100 μV was observed in 5/10 MEPs of the SOL and MG muscles. Once the optimal site was identified, subjects were asked to stand to ensure location and intensity was suitable in an upright stance. Adjustments to location or stimulus intensity (% of maximal output of stimulator) were made if necessary to achieve the minimal peak-to-peak amplitude of the MEP.

2.5.2 Accuracy of Optimal Stimulus Site

During the experimental trials the experimenter held the TMS coil on the optimal site on the subject's head. This adds the potential of experimental error as the experimenter could shift the position of the coil from the optimal site. For this reason, custom rigid bodies were created and adhered to the TMS coil and the subject's head to record the movement of the TMS coil with respect to the head with the Optotrak 3020 motion capture system (NDI, Waterloo, ON). Each rigid body was custom made with three infrared light emitting diodes, positioned non-collinearly on a rigid piece of plastic. The head rigid body was placed on the forehead of the subject so that it could face the camera bank. The TMS coil rigid body was secured to the flat surface in the center of the figure-of-eight coil so that it could be visible by the camera bank. Movement of the rigid bodies was captured with one horizontally oriented camera bank comprised of three cameras. The camera was elevated on a custom-built stand 105 cm from the ground and 259 cm away from the subject.

The global coordinate system coincided with the International Society of Biomechanics regulations. The x-axis was aligned with the axis of progression, the y-axis was aligned with the vertical axis, and the z-axis was established as the medio-lateral axis. The local coordinate system of each rigid body was calibrated in NDI 6D Architect. The marker power was set at 60% and the tolerance level of 3D RMS error was set at 0.5 mm. Data were sampled at 100 Hz (NDI First Principles).

The calculations function of NDI First Principles was used to calculate the relative difference between the TMS rigid body and the head rigid body. Trials in which

the TMS rigid body (relative to the head rigid body) moved at least 0.5 cm away from the optimal site location were not included in the analysis

2.5.3 Timing of Afferent Stimulation Relative to Cortical/Brainstem Stimulation

In order to determine an appropriate inter-stimulus interval between the train of electrical pulses to the plantar sole and the TMS pulse to the motor cortex or the stimulation to the cervicomedullary junction (c), a simple calculation was performed. For this calculation, the time to the peak reflex response (occurring in the range of 70-110 ms) in the soleus following electrical stimulation of the right plantar sole was designated as a. This value is the estimated afferent conduction time from the plantar sole to the cortex and then to the muscle. In order to only calculate efferent conduction time, the time to peak from an average of five MEPs or CMEPs in the SOL was designated as b. The c value, which is the inter-stimulus interval between the onset of the train of electrical stimulation and the onset of the cortical (MEp) or subcortical stimulation (CMEP), is calculated by a-b. This calculation was performed for each subject and each stimulation site (heel and metatarsal), and was calculated for both *experiment 1* and *experiment 2* (Figure 3.1**B**).

2.5.4 Experiment 1: effect of foot sole stimulation on lower limb MEPs

In 16 subjects, we investigated the effect of cutaneous afferent stimulation of the foot sole on MEP responses evoked in the lower limb muscles. Data were collected while subjects remained standing with their eyes open and maintained a natural base of support on a stable surface while the experimenter held the TMS coil to the subject's head on the marked location. This position was maintained throughout the condition. Subjects were

asked to focus on a stable target straight ahead throughout the duration of the trials.

Subjects participated in two randomized conditions, plantar stimulation at either the heel

(HEEL) or metatarsal region (MET). Each condition consisted of 40 randomized trials:

20 control trials of single pulse TMS and no foot sole stimulation, and 20 stim trials of a

train of electrical cutaneous stimulation (described above). The timing of the cutaneous

stim was followed by single pulse TMS at a ISI specific to each subject (described

above). For all trials, the time between consecutive TMS stimuli was 10 ms. A 5 minute

seated resting period was imposed between conditions to avoid a confounding effect

arising from fatigue.

2.6 Cervicomedullary Junction Stimulation

An MEP, observed at the level of the muscle, is the net effect of the activation of

a collection of neurons that synapse onto the corticospinal tract. These different indirect

activation pathways produce multiple descending volleys, which arrive at the

motoneurones over a range of milliseconds. The pathway involves synapses at both the

cortical and spinal level, thus the size of the MEP depends on the excitability of both

cortical and spinal motoneurones (Taylor 2006). An ideal control would be to produce

similar descending volleys as TMS but without synaptic activation in the cortex. This

would allow changes in the amplitude of the MEP to be attributed to altered excitability

at the cortical or spinal level. With cervicomedullary stimulation we can activate the

same descending axons as TMS and thus we can test the same pathway by-passing the

cortex. Cervicomedullary stimulation is performed by electrical stimulation between

electrodes placed at the level of the mastoid processes. Stimulation of the descending

tracts at the cervicomedullary junction also evokes a short-latency excitatory response in

the muscle (CMEP) and can be used to test motoneurone excitability in awake humans. Thus, MEPs and CMEPs will respond differently to any changes in excitability, and if a change occurs in the size of an MEP during an intervention but not in the CMEP then we can conclude that the change in excitability is cortical in nature.

2.6.1 Experiment 2: effect of foot sole stimulation in lower limb CMEPs

To determine if any observed MEP changes were truly cortical in origin the effect of foot sole stimulation on evoked responses from direct activation of the corticospinal tract were also examined in 8 of the 16 subjects (in separate experimental sessions). Data were also collected while subjects remained standing with their eyes open and maintained a natural base of support. Subjects participated in the same two conditions as *Experiment 1*; HEEL stim and MET stim (randomized). Each condition consisted of 15 randomized trials: 5 of which were control trials of only brainstem stimulation, and 10 stim trials of foot sole stimulation followed by a single pulse (200 µs) brainstem stimulation at a ISI specific to the subject and protocol (described above). Effort was taken to match the size of the CMEP amplitude to the unconditioned MEP amplitude of each participant.

2.7 Analysis

2.7.1 Cutaneous Reflex Analysis

To calculate the onset of the cutaneous reflex response the amplified EMG signal was digitally rectified, smoothed (50 ms) and averaged relative to the onset of the train of electrical stimulation using Spike2 (Cambridge Electronic Designs, Cambridge, UK).

A component of the middle latency response (MLR, latency ~70-110 ms) was considered present if it rose above or fell below three standard deviations (SDs) of the mean background EMG for at least 8 ms (Nakajima et al. 2006). The cursor function in Spike was used to manually choose the peak of the reflex responses in order to calculate c.

2.7.2 Experiment Analysis

To analyze the evoked potentials, we used Signal software (version 6; Cambridge Electronic Designs, Cambridge, UK). The EMG signal from all muscles was imported into the program and smoothed (20 s pre-trigger, and 40 s sweep length). The trigger was selected based on which condition was to be analyzed (NO FOOT STIM or FOOT STIM). All of the MEP or CMEP traces within a condition were superimposed for each muscle. The cursor function in Signal was used to place cursors around the onset and at the end of the silent period of the potentials for each muscle, such that cursor *1-2* were placed on the SOL trace, *3-4* were placed on the MG trace, and *5-6* were placed around TA. By placing these cursors, only the EMG signal between the cursors (thus the potentials) was analyzed. By using the "Analysis" function of Signal, we were able to extract the RMS amplitude 50 ms before the electrical stimulation was applied, and calculate MEP/CMEP peak to peak amplitude in each muscle for both experiments.

2.8 Statistical Analysis

The size of the MEP and CMEPs were measured as the peak-to-peak amplitude of the non-rectified response. The level of background activity was measured as RMS amplitude from the rectified EMG in a 50 ms period before the stimulus and assessed using student's paired t-test to ensure there was a comparable level of EMG activity

across conditions for each muscle. Statistical analysis was performed on both the raw responses and those expressed as a percentage of difference from the NO FOOT STIM condition (Table 3.1).

Two-way repeated measures ANOVA were used to assess excitability differences in each muscle (SOL, MG, and TA) that resulted from electrical stimulation of the plantar sole during standing. The two within-subject factors (independent variables) were condition (NO FOOT STIM and FOOT STIM) and location (HEEL or MET). Peak-to-peak amplitude of each muscle was investigated in both experiments. To compare MEPs and CMEPs, responses were analyzed using a priori planned comparisons, and a two-way repeated measures ANOVA with 'site of stimulation' (cortex/MEP and brainstem/CMEP) and 'location of foot sole stimulation' (HEEL or MET) as within subject factors. For all ANOVAs, normality and sphericity of response amplitudes were evaluated using Shapiro-Wilk and Mauchley's tests, respectively. If a violation of normality occurred, the data were log transformed prior to conducting the statistical analysis. Significant effects were followed up by a priori pairwise comparisons. Significance level was established as $p < 0.05$ for all analyses. All statistical analyses were performed using SPSS version 23 (IBM Corp. Armonk NY, USA).

Chapter 3: Manuscript

3.1 Introduction

Cutaneous afferent feedback from the soles of the feet is important for the control of balance (Meyer et al. 2004). Previous work has shown that a reduction in plantar skin feedback can impair standing balance (Perry et al. 2000; Nurse and Nigg 2001; McKeon and Hertel 2007) and alter gait parameters (Eils et al. 2004). Further support for skin's role in balance comes from work that shows enhancement of cutaneous afferent feedback from the feet can improve postural control (Priplata et al. 2003; Perry et al. 2008; Galica et al. 2009). These postural responses may be mediated through cutaneous reflex loops, such as those highlighted by Fallon and colleagues (2005) where the activation of cutaneous afferents from the foot sole reflexively modulates muscle activity in the lower limb (Fallon 2005).

Cutaneous reflex responses have been demonstrated through non-noxious electrical stimulation of peripheral nerves during stance and different phases of gait (Duysens et al. 1990; Yang and Stein 1990; Aniss et al. 1992; Gibbs et al. 1995; Zehr et al. 1997; Zehr et al. 1998; Zehr et al. 2001; Haridas and Zehr 2003). These reflexes have been shown to play an important role in the modulation of postural control (Day and Cole 2002) and gait (Zehr et al. 2014), and are thus not simple withdrawal reflexes (Burke et al. 1991). The responses occurring in the short latency range (40-50 ms) are known to be of a spinal origin (Aniss et al. 1992; Kukulka 1994). In recent years there has been considerable debate over the neural mechanisms mediating the responses occurring at a longer latency specifically in muscles of the lower limb. There is some

suggestion that the long-latency reflex response may be the result of a transcortical reflex loop.

Previously, it has been shown that a transcortical pathway may contribute to the late reflex response in the tibialis anterior muscle (TA). Stimulation of the sural or superficial peroneal nerve during a seated tonic contraction produces a reflex response in the TA at a latency of approximately 70-95 ms. Nielsen and colleagues (1997) showed that the response of TA motor units to transcranial magnetic stimulation (TMS) was significantly facilitated when conditioned with the stimulation of these nerves (Nielsen et al. 1997). Overall this suggests that stimulation of cutaneous afferents from the foot dorsum can modulate motor cortex excitability.

Non-noxious cutaneous reflex responses evoked from stimulation of the three main nerve trunks innervating the dorsal and plantar skin of the foot (i.e., lateral plantar nerve, medial plantar, and superficial peroneal nerve) have been extensively studied (Aniss et al. 1992; Van Wezel et al. 1997; Nielsen et al. 1997). Additionally, the reflex responses elicited from mechanical perturbations (Eng et al. 1994; Schillings et al. 1996) and more recently, electrical (Nakajima et al. 2006) stimulation across the foot sole have been explored. Electrical stimulation of the plantar sole skin has been shown to evoke topographically organized reflexes in the lower limb muscles in sitting and standing; heel stimulation preferentially increases the activity of plantar flexor muscles and forefoot stimulation increases dorsi-flexor activity (Nakajima et al. 2006). Interestingly, these reflex responses observed in plantar and dorsi flexor muscles during standing have a similar latency range to those purportedly supported by a transcortical pathway (70-110

ms). This observation raises the possibility that these cutaneous responses evoked from the foot sole could also be, at least in part, mediated by a transcortical pathway.

To date, investigations of the cortical contribution to cutaneous reflexes in the lower limb have been restricted to sitting (with tonic contraction) (Nielsen et al. 1997) and gait conditions (Christensen et al. 1999), and have primarily focused on the TA. There has been limited investigations of a transcortical pathway from cutaneous afferents in the foot sole, and little focus on the soleus (SOL) (Roy and Gorassini 2008) and medial gastrocnemius (MG) muscle responses. The existence of long latency cutaneous reflexes in these muscles with electrical stimulation may suggest a transcortical loop could exist for these posturally active leg muscles (i.e. SOL and MG) during standing (Nakajima et al. 2006).

The purpose of the current study was to investigate if there is a transcortical loop for the foot sole cutaneous reflex responses. We used TMS to investigate whether cortical excitability of the lower limb muscles is modulated with electrical stimulation of foot sole cutaneous afferents during standing balance. Location specificity of cutaneous reflexes evoked from the foot sole has previously been demonstrated (Nakajima et al. 2006), which led to our exploration of foot region specificity on transcortical contributions.

3.2 Methods

3.2.1 Subjects

Sixteen healthy subjects (8 female) aged 19-29 years (23 ± 3 years, mean ± S.D.)

participated in this study. Subjects completed health history questionnaires and gave

written informed consent prior to data collection. Subjects were free from neurological

and musculoskeletal disorders. Subjects were excluded if they had a history of seizures,

concussions, or lower limb injuries. All experimental procedures were approved by the

research ethics board at the University of Guelph, which abides by the Declaration of

Helsinki.

3.2.2 Cutaneous Stimulation

To evoke the cutaneous reflex responses the heel and metatarsal regions of the

plantar foot sole were electrically stimulated using a constant-current stimulator (Model

DS7AH, Digitimer, Hertfordshire, United Kingdom) and two pairs of Ag/AgCl stimulus

electrodes placed on both the metatarsal and heel regions of the right plantar sole (Figure

3.1**A.**). The metal tabs of the electrodes were left exposed on the lateral borders of the

plantar sole regions to allow the stimulator leads to be connected. Electrode gel was

applied on the electrodes to reduce impedance, and lower the voltage necessary to

maintain a constant current. Electrical stimulation consisted of a train of five constant-

current rectangular pulses (each 1.0 ms duration, inter-pulse interval 3 ms) applied at a

frequency of 2 Hz (Nakajima et al. 2006).

Perceptual threshold (PT), defined as the lowest stimulus intensity (current) that evoked detectable tactile sensations, was determined for each stimulation site. PT was measured with the subjects standing while the experimenter gradually decreased the stimulus voltage until the participant could barely discern the stimulus (identified as PT) on their plantar sole. Electrical stimulation intensity was set at 2 times the perceptual threshold (2.0 x PT; 1.0-1.6 mA) during testing to evoke a non-noxious cutaneous sensation during each trial. It was verbally confirmed by all subjects that the stimulation intensity was not painful.

3.2.3 Transcranial Magnetic Stimulation

To investigate the influence of foot sole stimulation on cortical excitability, transcranial magnetic stimulation (TMS) was used. TMS was performed using two MagStim 200 stimulators connected to a Bistim module and a double cone coil (MagStim, Carmarthenshire, United Kingdom). The coil was placed over the right leg area of the motor cortex (slightly lateral to the vertex). To locate the stimulation site, subjects remained seated with their legs relaxed and knees bent ~90° while stimuli were delivered through the coil. The stimulation site was identified as the location over which motor evoked potentials (MEP) could be evoked with the lowest stimulation intensity. Once the optimal site was identified, subjects stood to verify location and establish appropriate stim intensity. The TMS stimulus intensity (% of maximal output of stimulator) was then adjusted in the standing position to produce reliable and repeatable MEPs of at least 100 μV peak-to-peak amplitude in the soleus muscle. MEPs were also evoked in MG and TA but were not monitored for amplitude. During the experimental protocol, single pulses of TMS were delivered as described below. A single pulse was

applied using simultaneous discharge of both MagStim stimulators (40-65% of maximal output of stimulators).

3.2.4 Corticospinal Tract Stimulation

The corticospinal tract was also stimulated non-invasively at the cervicomedullary junction in eight of the original sixteen subjects. Electrical stimulation of the corticospinal tract was performed using a 200 μs electrical pulse (Model D7SAH, Digitimer, Hertfordshire, United Kingdom) delivered through a pair of electrodes (Clear Trace, ConMed, Utica, New York) fixed over the mastoids with the cathode on the left. Stimulation intensity was set at a level at which a cervicomedullary motor evoked potential (CMEP) could be observed in the SOL during standing. The stimulation intensity (200-300 mA) was adjusted to produce cervicomedullary evoked potentials to match the amplitude of the previously recorded unconditioned SOL MEP amplitude of each participant.

3.2.5 Muscle Recordings

Both MEPs and CMEPs were recorded using surface electromyography (EMG). EMG was recorded from the SOL, MG, and TA muscles of the right leg using pairs of surface Ag/AgCl electrodes (Ambu Blue Sensor, Denmark, Netherlands) in a monopolar arrangement. EMG signals were amplified (gain 500-1000, band passed filtered between 10 to 1000 Hz; Bortec AMT-8 system, Bortec Biomedical Ltd, Alberta, Canada), and digitized at 2048Hz (Spike 2 version 7, Cambridge Electronic Design, Cambridge, UK).

3.2.6 Study Design

3.2.6.1. Timing of cutaneous afferent stimulation relative to cortical/brainstem stimulation

To coordinate the timing of the TMS and CMEP with the arrival of the stimulated cutaneous input, cutaneous reflex responses to foot sole electrical stimulation were evoked and the cutaneous reflex latency was calculated.

Cutaneous reflexes were evoked by stimulation of the metatarsal or heel regions of the right plantar sole while subjects stood upright on a stable surface with their eyes open. In order to obtain stable and clear cutaneous reflexes, 100 stimuli were delivered at each region (heel or metatarsal), with a 3-minute resting period between regions. The amplified EMG signal was digitally rectified, smoothed and averaged relative to the stimulus onset to calculate latency. A peak reflex response was established when the EMG rose above or fell below three standard deviations of the mean background EMG for at least 8 ms (Nakajima et al. 2006). The time of the peak reflex in the soleus muscle in response to the electrical stimulation of the plantar sole (occurring in the range of 70-110 ms) was measured. This measurement was performed for both heel & met reflex responses occurring following stimulation to both heel and metatarsal regions.

To estimate efferent conduction time from the motor cortex to the muscle, the average latency of 5 soleus MEPs were calculated during standing. For the brainstem stimulation protocol efferent conduction time from the brainstem to the muscle was calculated from the average latency of 5 CMEPs in the soleus. To estimate afferent conduction time, and determine the time at which both cutaneous and TMS input or

cervicomedullary junction would coincide in the cortex, a simple calculation was performed;

c=a-b

where the time to peak cutaneous reflex response (occurring in the range of 70-110 ms) was designated as *a*. The calculated efferent conduction time, from the MEPs or CMEPs was designated as *b.* The *c* value is the inter-stimulus interval (ISI) between the train of electrical stimulation to the plantar sole and the cortical or brainstem stimulation. This calculation enables us to align the pulses to the cortex or spinal cord to the time when the foot sole afferent information from the foot sole is presumed to be in the cortical or subcortical structure (Figure 3.1**B**).

3.2.6.2. Experiment 1: effect of foot sole stimulation on lower limb MEPs

In 16 subjects, we investigated the effect of foot sole cutaneous afferent stimulation on lower limb MEPs during quiet stance. Data were collected while subjects stood with a natural base of support and eyes open. Subjects participated in two randomized conditions, plantar stimulation at either the heel (HEEL) or metatarsal region (MET). Each condition consisted of 40 trials, block randomized: 20 control trials of single pulse TMS with no foot sole stimulation, and 20 trials with cutaneous electrical stimulation paired with TMS. The timing of the cutaneous – TMS ISI was calculated for each subject (range 48-60ms; see above). For all trials, the time between consecutive TMS pulses was 10 seconds. A 5 minute seated resting period was imposed between conditions (HEEL or MET) to avoid a confounding effect arising from fatigue.

3.2.6.3. Experiment 2: effect of foot sole stimulation on lower limb CMEPs

In eight of the 16 subjects (in separate experimental sessions) we also examined the effect of foot sole stimulation on evoked responses from direct activation of the corticospinal tract. This was to establish if any observed MEP changes were cortical in origin. Data were again collected while subjects remained standing with their eyes open and maintained a natural base of support. Subjects participated in the same two conditions as the TMS session; HEEL stim and MET stim (randomized). Each condition consisted of 15 randomized trials: 5 of which were control trials of only brainstem stimulation, and 10 stimulation trials with foot sole stimulation and single pulse brainstem stimulation. Interstimulus intervals were calculated specifically for each participant (range 57-68 ms).

3.2.7 Data Analysis and Statistics

The sizes of the MEPs and CMEPs were measured as the peak-to-peak amplitude of the non-rectified response. Evoked potentials were analyzed in Signal Software version 6 (Cambridge Electronic Design, Cambridge, UK; both MEP and CMEP). Statistical analysis was performed on the raw MEP and CMEP responses and those expressed as a percentage of difference from the NO FOOT STIM condition.

Two-way repeated measures ANOVAs were run for each muscle (SOL, MG and TA) to assess excitability differences that resulted from electrical stimulation of the plantar sole during standing. The two within-subject factors (independent variables) were condition (NO FOOT STIM or FOOT STIM) and location (HEEL or MET). Peak-to-peak amplitude for the raw MEP and CMEP responses of each muscle was investigated.

To compare MEPs and CMEPs, responses expressed as a percent difference from the NO FOOT STIM condition were analyzed using a priori planned comparisons, and a two-way repeated measures ANOVA with 'site of stimulation' (cortex/MEP vs brainstem/CMEP) and 'location of foot sole stimulation' (HEEL or MET) as within subject factors. For all ANOVAs, normality and sphericity of response amplitudes were evaluated using Shapiro-Wilk and Mauchley's tests, respectively. If a violation of normality occurred, the data were log transformed prior to conducting the statistical analyses. Significance level was set as $p < 0.05$ for all analyses. All statistical analyses were performed using SPSS version 23 (IBM Crop. Armonk NY, USA).
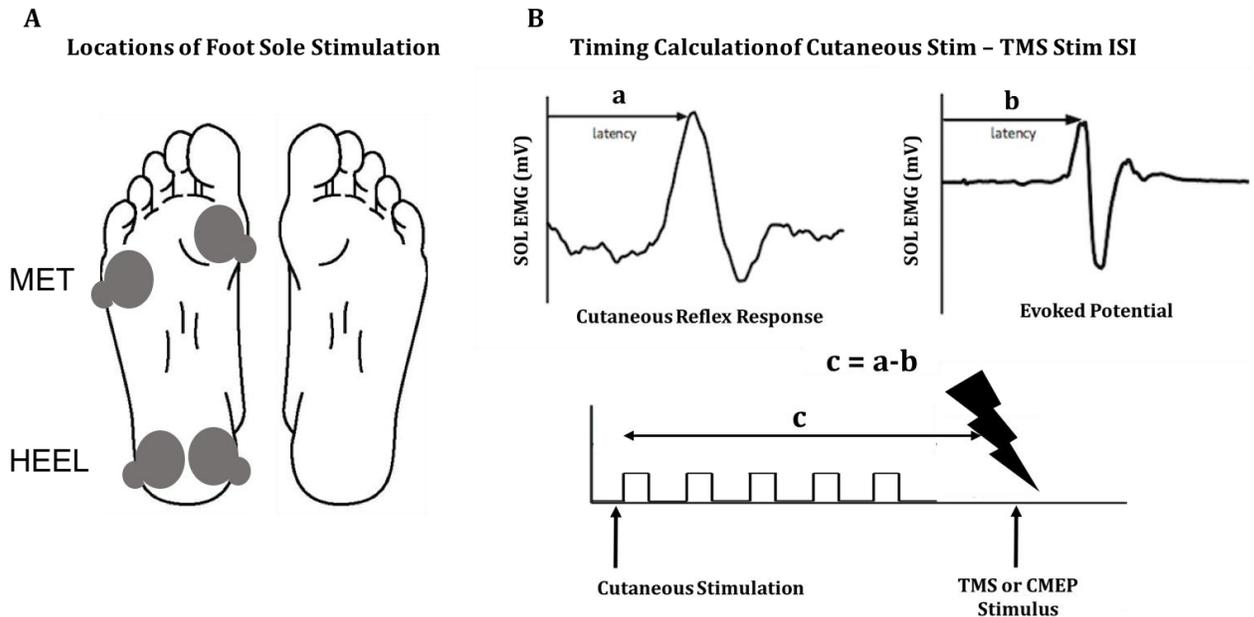
Figure 3.1: **A:** Locations of plantar sole stimulation over the metatarsal and heel regions and **B:** Timing of cutaneous stimulation relative to cortical/brainstem Stimulation. Circles represent placement of electrodes on the right foot sole for stimulation of either the metatarsal or heel region. **a** (ms) is the time to the peak reflex response (occurring between 70-110 ms) measured in the SOL following 100 electrical stimulations to either the HEEL or MET skin region on the foot sole. **b** (ms) is the time to the peak of the evoked potential in the SOL occurring with TMS or brainstem stimulation (CMEP), this is the estimated efferent conduction time. The **c** (ms) value, which is the inter-stimulus interval between the train of electrical stimulation to the plantar sole and the TMS or CMEP pulse, is calculated by **a-b**. **c**, and is the estimated afferent conduction time from the foot sole to the cortex.

3.3 Results

3.3.1 Background EMG

The RMS amplitude of background EMG activity was quantified as RMS amplitude for all muscles (SOL, MG, and TA) during the 50 ms period prior to the cutaneous stimulation onset. Background EMG was assessed to ensure there was a comparable level of EMG activity across conditions (NO FOOT STIM and FOOT STIM). There was no statistical difference in average background EMG between NO FOOT STIM and FOOT STIM conditions at both HEEL and MET locations for any of the muscles (SOL, MG, TA) in either *experiment 1* or *2* (Table 3.1).

3.3.2 Experiment 1: effect of foot sole stimulation on lower limb MEPs

Corticospinal excitability was assessed during conditions with or without electrical stimulation at two different foot sole sites on the right foot. Electrical stimulation of the foot sole modulated the size of the peak-to-peak MEPs of the plantar flexor muscles (Figure 3.2). In the averaged group data, a repeated measures ANOVA for SOL peak-to-peak amplitude showed a significant interaction for location by condition ($F_{(1,15)}$= 24.049, p<0.001). Differences in excitability were apparent between NO FOOT STIM and FOOT STIM conditions across the different foot sole locations. Post-hoc comparisons determined that SOL MEP amplitude significantly *increased* from NO FOOT STIM to FOOT STIM condition at the HEEL location (Figure 3.2*C*; NO FOOT STIM: 0.35 ± 0.055 mV, FOOT STIM: 0.48 ± 0.094 mV; $t_{(1,15)}$= 2.423, p=0.029). In contrast, MEP amplitude significantly *decreased* between NO FOOT STIM to FOOT

STIM at the MET region (Figure 3.2***D***; NO FOOT STIM: 0.29 ± 0.044 mV, FOOT STIM: 0.24 ± 0.030 mV; $t_{(1,15)}$= -2.163, p=0.0427).

A significant interaction between location and condition was also found for peak to peak MEP amplitude for the MG. Post-hoc comparisons also revealed a significant *increase* in amplitude from NO FOOT STIM to FOOT STIM at the HEEL location (Figure 3.2***C***; NO FOOT STIM: 0.11 ± 0.017 mV, FOOT STIM: 0.17± 0.029 mV; $t_{(1,15)}$= -2.253, p=0.040). In following with the SOL responses, MEP amplitude of the MG appeared to decrease from NO FOOT STIM to FOOT STIM at the MET region, although this reduction was not statistically significant (Figure 3.2***D***; NO FOOT STIM: 0.12 ± 0.023 mV, FOOT STIM: 0.11 ± 0.018 mV $t_{(1, 15)}$= 1.695, p= 0.111)

Electrical stimulation of the foot sole during standing did not significantly modify the size of the MEPs of the tibialis anterior. Although, the TA MEP amplitude showed slight decreases from NO FOOT STIM to FOOT STIM at both HEEL (0.44 ± 0.093 mV to 0.37 ± 0.11 mV) and MET (0.44 ± 0.10 mV to 0.43 ± 0.10 mV) locations (Figure 3.2***C*** and ***D***), there was no significant interaction for location by condition ($F_{(1,15)}$=0.634, p=0.438), nor were there any significant main effects for location ($F_{(1,15)}$= 0.017, p= 0.899) or condition ($F_{(1,15)}$= 2.223, p=0.157).

3.3.3 Experiment 2: effect of foot sole stimulation on lower limb CMEPs

To determine whether the excitability changes seen with foot sole stimulation occurred at the level of the cortex and/or spinal cord, we examined the effect of skin stimulation on the excitability of subcortical networks. There was no significant interaction between location and condition for SOL, however a significant increase was

found for the main effect of condition ($F_{(1,7)}$=14.223, p=0.007) for the SOL CMEP amplitude whereby there was a significant increase from NO FOOT STIM to FOOT STIM with location collapsed (Figure 3.3).

A significant interaction of location by condition was found for MG CMEP amplitude ($F_{(1,7)}$=7.567, p=0.028). Post-hoc comparisons between NO FOOT STIM and FOOT STIM conditions revealed a significant increase in CMEP at the HEEL location (Figure 3.3*C*; NO FOOT STIM: 0.10 ± 0.024 mV, FOOT STIM: 0.23 ± 0.060 mV; $t_{(1,7)}$= -3.187, p=0.015), and no significant change at the MET location (Figure 3.3***D****;* NO FOOT STIM: 0.10 ± 0.026 mV, FOOT STIM: 0.09 ± 0.019 mV; $t_{(1,7)}$= 0.702, p=0.506).

There was no significant interaction of location by condition found for TA CMEP peak-to-peak amplitude, however a significant main effect of condition ($F_{(1,7)}$= 26.023, p=0.001) was determined. CMEPs were found to increase between NO FOOT STIM and FOOT STIM conditions (0.15 mV to 0.44 mV).

3.3.4 Effect of foot sole stimulation on MEPs compared to CMEPs

To determine the level at which excitability changes occurred, we compared the amplitudes of conditioned CMEPs to the conditioned MEPs elicited over the motor cortex, for all three muscles in the eight subjects who participated in both protocols. To establish that these conditioned responses were comparable, we first used student's t tests to ensure that there were no differences between the average MEP NO FOOT STIM amplitude and the CMEP NO FOOT STIM responses for each muscle (SOL HEEL, p= 0.72; SOL MET, p= 0.53; MG HEEL, p = 0.94; MG MET, p= 0.44; TA HEEL, p= 0.11; TA MET, p= 0.16).

A two-way repeated measures ANOVA (location x site of stimulation) for the soleus revealed a significant main effect for location of foot sole stimulation (p=0.025). Heel stimulation responses (CMEP and MEP) showed a larger percent change (from the unconditioned response) compared to those of the metatarsal stimulation location (Figure 3.4*A*; HEEL: 115.34 % from NO FOOT STIM, MET: 15.16% from NO FOOT STIM; $F_{(1,7)}$= 8.087, p=0.03). A two-way repeated measures ANOVA for MG responses also revealed a significant main effect for location of foot sole stimulation. Heel stimulation responses showed a significantly larger increase in amplitude (as a percent difference from NO FOOT STIM) than those of the metatarsal location (Figure 3.4*B*; HEEL: 103.3% increase from NO FOOT STIM, MET: 2.1% decrease from NO FOOT STIM; $F_{(1,7)}$= 9.879, p= 0.02).

A significant main effect for site of stimulation was found for responses in the TA (p= 0.003). The percent increase from NO FOOT STIM in CMEP amplitude was significantly greater than responses activated at the cortex (MEP) (Figure 3.4*C*; CMEP: 185.8% increase, MEP: 13.1% increase; $F_{(1,7)}$= 19.705, p=0.003).

A priori planned comparisons between the change in response amplitude (% of NO STIM) of MEP and CMEP were also performed. For MET stimulation, the SOL CMEP showed a 45.3% increase from NO FOOT STIM, which was significantly different from the 15.0% decrease seen for the SOL MEP (Figure 3.4*A*; p<0.05). For HEEL stimulation, there was no significant difference in the change in amplitude of the conditioned MEP (as percent changes from NO FOOT STIM) compared to the conditioned CMEP (p=0.21), although the conditioned CMEP appeared to show a larger change in amplitude from NO FOOT STIM (Figure 3.4*A*; MEP: 65.3% from NO FOOT

STIM MEP, CMEP: 165.4% from NO FOOT STIM CMEP). With HEEL stimulation the conditioned MG CMEP appeared to show a larger change in response amplitude compared to the change in MG MEP (Figure 3.4*B*), although planned comparisons revealed that this difference was also non-significant (p=0.37). Foot sole stimulation at the HEEL caused a 165.3% increase in TA CMEP amplitude which was significantly different from the 6.3% decrease observed in the MEP amplitude (Figure 3.4*C*; p<0.05).

Table 3.1: Summary of statistical analysis of background EMG between NO FOOT STIM and FOOT STIM conditions across HEEL and MET foot sole locations.

	Location	Muscle		
		SOL	MG	TA
Experiment 1	HEEL	p=0.129	p=0.109	p=0.981
	MET	p=0.316	p=0.129	p=0.665
Experiment 2	HEEL	p=0.728	p=0.708	p=0.467
	MET	p=0.212	p=0.298	p=0.829

45
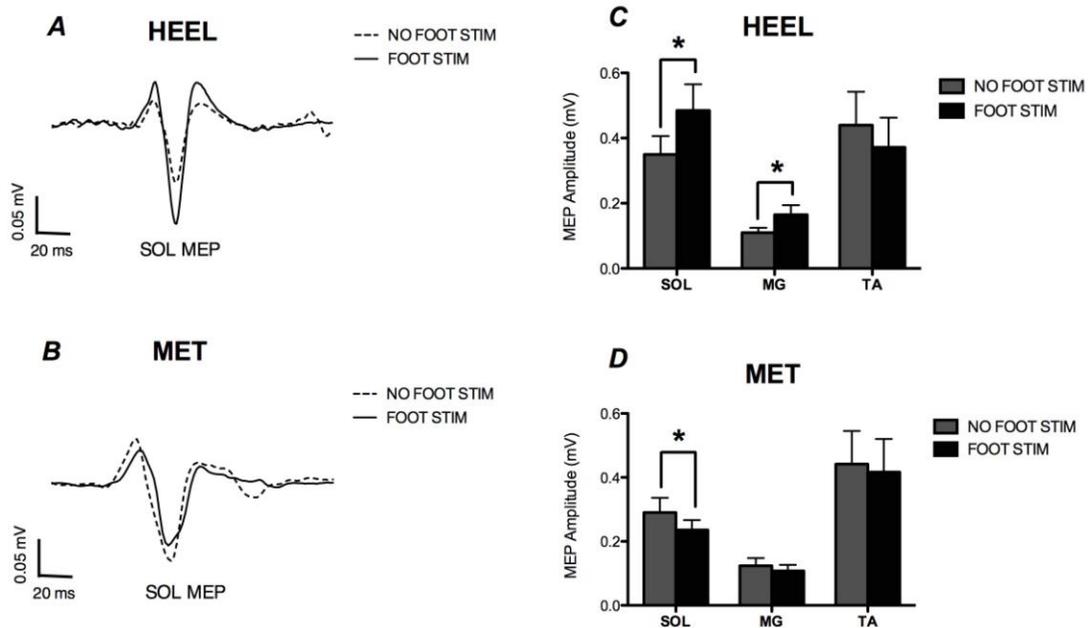
Figure 3.2: Effect of HEEL and MET stimulation on MEPs. Average SOL MEPs in a single subject with (solid line) and without (dashed line) stimulation to the *A*, HEEL of the foot sole and *B*, MET region of the foot sole. Group averaged MEP amplitudes from all 16 subjects for SOL, MG, and TA muscles for NO FOOT STIM and FOOT STIM conditions. *C*, effect of HEEL stimulation. *D*, effect of MET stimulation. Error bars represent standard error and asterisks indicate significant differences between NO FOOT STIM (grey bars) and FOOT STIM (black bars) conditions (*p<0.05).
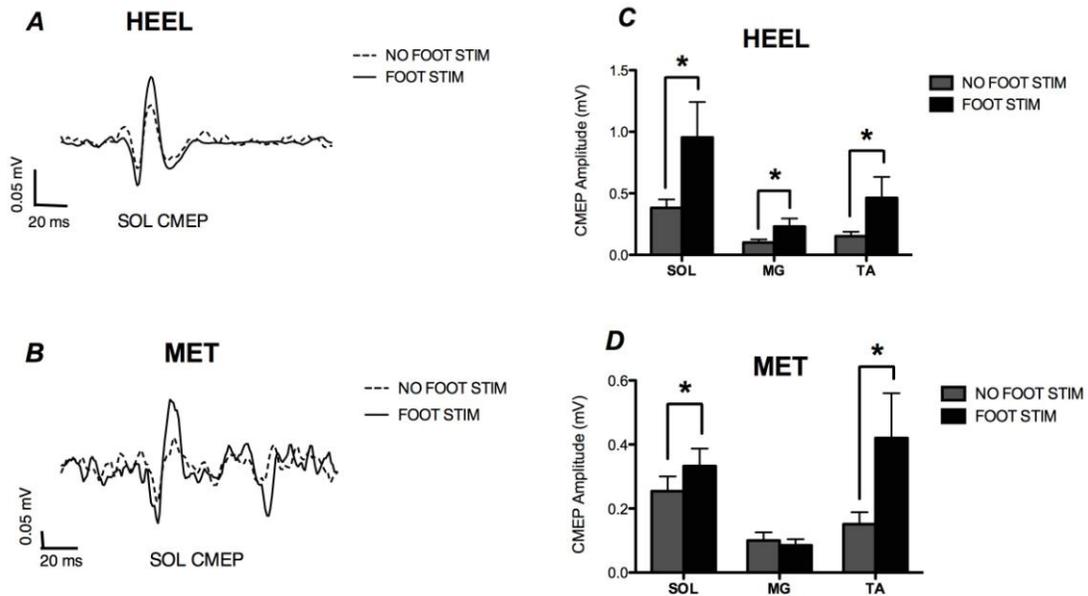
Figure 3.3: Effect of HEEL and MET stimulation on CMEPs. Average SOL CMEPs in a single subject with (solid line) and without (dashed line) stimulation to the *A,* HEEL of the foot sole and *B*, MET region of the foot sole. Group averaged CMEP amplitudes from all eight subjects for SOL, MG, and TA muscles for NO FOOT STIM and FOOT STIM conditions. *C*, effect of HEEL stimulation. *D*, effect of MET stimulation. Error bars represent standard error and asterisks indicate significant differences in CMEP amplitude between NO FOOT STIM (grey bars) and FOOT STIM (black bars) conditions (*p<0.05).
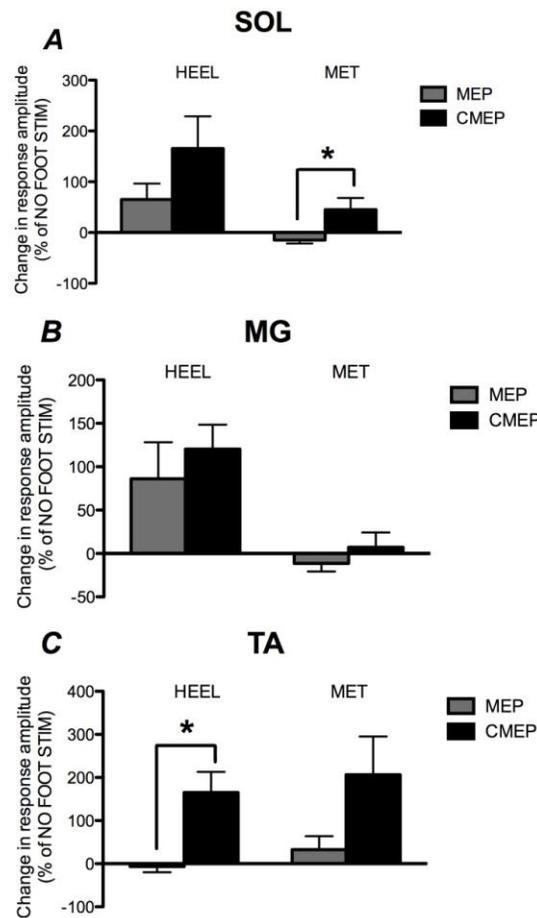
Figure 3.4: Effect of plantar sole stimulation on MEPs and CMEPs. Group data from 8 subjects showing the effect of electrical stimulation on MEPs (grey bars) and CMEPs (black bars) at both HEEL and MET stimulation locations. Responses were expressed as the FOOT STIM amplitude (MEP or CMEP), as a percent of the NO FOOT STIM condition amplitude for all three muscles, *A* SOL, *B* MG, and *C* TA. Error bars represent standard error and asterisks indicate significant differences in the size of the MEP compared to the CMEP (*p<0.05).

3.4 Discussion

In the present study we investigated whether the cutaneous evoked modulation of lower limb muscle activity observed in standing, is at least partly mediated by a transcortical pathway. We found that stimulation of the foot sole at two different functionally important regions (heel and metatarsal) modulated the excitability of the corticospinal pathway. Stimulation of the heel region of the foot sole significantly increased MEPs in the plantar flexor muscles. Conversely, stimulation to the metatarsal region saw a suppression of MEPs in the plantar flexor muscles. For the first time, these results provide evidence that there may be a cortical involvement in the modulation of cutaneous reflex responses in the SOL and MG during standing.

3.4.1 MEP modulation with cutaneous afferent stimulation of the foot sole

In humans, stimulation of cutaneous afferents from the foot sole evokes complex synaptic effects on the lower limb muscles (Delwaide et al. 1981; Aniss et al. 1992). The earliest component of these responses (40-50 ms) in the muscle are believed to be controlled at the level of the spinal cord (Aniss et al. 1992; Kukulka 1994). These responses are suggested to be evoked by fast conducting pathways, with likely no more than one interneuron involved (Aniss et al. 1992; Nielsen and Kagamihara 1993). The pathway of longer latency responses, however, has not been confirmed. Reflex responses in the lower limb muscles occurring in this long latency range (70-110 ms) can be evoked by peripheral stimulation of cutaneous afferents over distinct regions of the foot sole during standing (Nakajima et al. 2006). In fact, these reflex responses show strong location-specificity and depend on the region of foot sole stimulated. In the SOL and

MG, an excitatory response is observed following HEEL electrical stimulation, whereas an inhibitory response follows MET stimulation in the plantar flexors. A reciprocal activation to the plantar flexors is observed in the antagonist TA muscle suggesting functional modulation of these lower limb muscles (Nakajima et al. 2006). It is unknown whether these reflex responses evoked during standing are modulated through a transcortical reflex mechanism, however they occur at latencies (>70 ms) which are believed to allow time for a cutaneous volley to reach the cortex, activate the corticospinal tract and travel to the muscle (Nielsen et al. 1997). In fact, the work by Nakajima (2006) is supported by previous literature where stimulation of peripheral nerves innervating the foot (superficial peroneal, medial plantar, and tibial nerve) evokes reflex responses in the tibialis anterior at latencies of (70 - 95 ms). In this work Nielsen and colleagues (1997) argue that these reflex responses occur at latencies that support possible contributions from the motor cortex. Based on their MEP data, they are able to confirm a transcortical pathway (Nielsen et al. 1997; Roy and Gorassini 2008).

In the present study, at the level of the motor cortex we aligned the timing of the TMS pulse to correspond to the arrival of the cutaneous volley (from foot sole stimulation). The result was a significant modulation of MEP size compared to baseline MEPs measured without cutaneous stimulation. These MEPs in the plantar flexor muscles showed location specificity to plantar sole stimulation of distinct regions, similar to the location specific reflexes observed with foot sole stimulation alone. Heel cutaneous stimulation increased peak-to-peak MEP amplitude of the SOL and MG, whereas decreased peak-to-peak MEP amplitude was observed with metatarsal stimulation. The significant MEP changes provide evidence that corticospinal

excitability is modulated with cutaneous afferent stimulation, and thus these changes

may involve a transcortical mechanism.

3.4.2 MEP vs. CMEP to unravel cortical contributions

By comparing the CMEPs to the MEPs conditioned with foot sole stimulation,

we can determine the neural level (cortical or spinal) at which excitability changes occur.

A difference between the responses should indicate a contribution from the cortex to the

reflex response. The results from cervicomedullary junction stimulation showed a

facilitation of responses with plantar stimulation across both foot sole locations.

Stimulation at the HEEL location showed a facilitation of the CMEPs in the plantar

flexors that was significantly greater than the facilitation observed in the MEP responses

(Figure 3.4*A*). The smaller amplitude MEPs compared to the CMEPs suggests two

things; first that there is indeed a cortical contribution to these reflex responses, and

second, there is an overall decrease in excitation occurring at a cortical level with foot

sole stimulation. This decrease in cortical excitation is further highlighted by the

comparison of MEP and CMEP responses conditioned with metatarsal stimulation. We

show here that with stimulation to the metatarsal region of the foot sole, MEP amplitude

was significantly depressed in the SOL, whereas the CMEP response was facilitated.

This evidence suggests that the MEP depression induced by foot sole stimulation occurs

through cortical mechanisms since responses were only depressed when elicited over the

motor cortex and not at the pyramidal decussation in the brainstem, which is thought to

recruit similar descending corticospinal axons (Taylor 2006).

3.4.3 CMEP response provides evidence of differential cutaneous input to motoneuron pool

Responses evoked by stimulation at the cervicomedullary junction increased across both locations following foot sole stimulation (Fig 3.3). These results indicate that foot sole stimulation at either region has a facilitatory influence at the level of the spinal cord. This was a surprising finding as it was expected that the direction of the CMEP response would show a similar change in excitability as the reflex responses following foot sole stimulation. A cervicomedullary motor evoked potential tests the level of excitability of the motoneuron pool (Taylor 2006; McNeil et al. 2013), thus when an inhibitory reflex response (or decrease in EMG) occurs in the muscle activity, it is expected that the overall excitability of the motoneuron pool is at a lower level. In this study, a decrease in peak-to-peak amplitude of CMEP was not seen despite the decrease seen to occur in SOL and MG EMG reflex with MET stimulation. Importantly, the observed increases in CMEP amplitudes reflect a net increase in motoneuron pool excitability. Motoneuron excitability depends on the intrinsic properties of the motoneurons, as well as the excitatory and inhibitory input to the motoneurons. Because there are EMG decreases (inhibitory reflex response) in SOL/MG with MET stimulation and in TA with HEEL stimulation, it is assumed that the decrease in cortical drive is greater than the increase in afferent excitation from the skin stimulation, resulting in a net decrease in excitation to the motoneuron pool. This would leave the motoneuron pool less excitable, however, this is not reflected in the CMEP responses. A possible explanation for this is that the ongoing excitatory input from cutaneous afferents is not spread equally across the motoneuron pool. Cutaneous afferents have been shown to

have differential effects on α-motoneurons that are dependent on the type of motoneuron (high or low threshold) (Kanda et al. 1977; LaBella et al. 1989; Aniss et al. 1992). It has been demonstrated that different types of motoneurons within the same pool are subject to differential modulation by a system of cutaneous afferents (Kanda et al. 1977). Based on our results, we propose that the facilitatory input from cutaneous afferents from the foot sole increases in strength from low-threshold to higher-threshold motoneurons. Thus when an inhibitory reflex response is observed (i.e SOL with MET stim), it is because the facilitatory influence from cutaneous afferents is not as strong on low-threshold motoneurons and with the simultaneously occurring withdrawal of cortical drive, causes a net decrease in excitability. Cutaneous afferents when stimulated, however, may have a stronger facilitatory influence on larger motoneurons such that the afferent excitation is greater than the withdrawal of cortical drive, thus causing a net excitation in these higher threshold motoneurons. This excitation is not enough to cause these motoneurons to fire as we still observe an inhibitory reflex response in the SOL with MET stimulation, however they are now closer to threshold. So when a high-voltage pulse is applied to the brainstem to evoke a CMEP, these higher-threshold motoneurons will now be recruited and will generate action potentials, thus causing an increase in CMEP peak-to-peak amplitude. Cutaneous afferent stimulation has been shown to lower recruitment thresholds of higher threshold motoneurons (Kanda and Desmedt 1983). Overall, the findings from our current study suggest that cutaneous afferent feedback from the foot sole is facilitatory to the motoneurons of the lower limb, however this feedback is potentially weighted in a differential manner with a stronger influence on higher threshold motoneurons.

3.4.4 Functional Significance

Our findings provide evidence that long latency reflexes in lower leg muscles evoked by stimulation of cutaneous afferents of the foot sole are partly mediated by a transcortical pathway. We found that with foot sole stimulation during standing there is a withdrawal of cortical drive, and this withdrawal is modulated depending on the region of foot sole stimulated. In the plantar flexion muscles, MET stimulation showed a greater degree of cortical inhibition than the HEEL stimulation. This was in contrast to the TA muscle, where HEEL stimulation demonstrated a greater withdrawal of cortical input than the MET stimulation condition (Figure 3.4). These results support the idea that cortical contributions to plantar foot reflexes to lower leg muscles are organized in a location-specific manner. Specifically, a transcortical reflex loop allows for integration of sensory feedback from other sensory sources in order to fine tune or regulate the reflexive response to stimulation of different regions of the foot sole. Previously, the motor cortex has been generally accepted to play a minor role in postural control, as posture has been thought to be largely regulated by subcortical structures (Prentice and Drew 2001). Our work corroborates studies which have shown that the motor cortex may be involved when posture is compromised and requires re-stabilization (Lavoie et al. 1995; Solopova et al. 2003; Taube et al. 2006; Tokuno et al. 2009).

3.4.5 Conclusion

We have shown that a transcortical reflex pathway may be involved in the modulation of the long latency cutaneous reflex response in the lower limb. We cannot, however, exclude a contribution from other mechanisms. Based on the latency of the reflex response, it is possible that polysynaptic spinal pathways may be involved. However, changes in the response to TMS with foot sole stimulation suggest that a transcortical reflex pathway makes some contribution to the observed responses. In fact, our findings provide evidence of a cortical involvement in the location-specific organization of these reflexes elicited by stimulation to distinct regions of the skin of the foot sole. This work ultimately adds to our understanding of the many neural networks involved in postural control. Future studies are required to determine if activation of cutaneous afferents from the foot sole by natural or mechanistic stimulation (vibration), in place of electrical, causes activation of cortical networks as has been shown in the hand (Rosenkranz and Rothwell 2004; Christova et al. 2011). Additional research is essential to investigate to what extent sensory afferent stimulation can modulate cortical excitability directly, thereby providing additional insights into the neural processes associated with motor recovery following injury. Cutaneous afferent feedback from the plantar soles is important for lower limb motor function in standing and walking; dysfunction after an injury to the nervous system may result from lack of ascending afferent connections to cortical motor networks. Controlling the integration of sensory feedback may be important in rehabilitation following injury to the central nervous system.

Chapter 4: General Discussion, and Conclusions

It has been known for some time that a transcortical loop exists from cutaneous afferents in the foot to the tibialis anterior muscle (Nielsen et al. 1997; Christensen et al. 1999). However, the existence of transcortical loops to other muscles of the lower limb, such as the soleus and medial gastrocnemius has been debated, and investigations of these muscles have been limited. The experiments involving the control of the tibialis anterior muscle have mainly been completed in seated (with tonic dorsiflexion) or gait conditions, and rarely have antagonist muscles been investigated in the same study (Nielsen et al. 1997; Christensen et al. 1999; Roy and Gorassini 2008). The activity and control of muscles largely depends on the nature of the task, thus findings from studies involving the tibialis anterior can not be generalized to other lower limb muscles, especially muscles performing different actions. Thus, the objective of this thesis was to examine if a transcortical loop exists from cutaneous afferents of the foot sole to the muscles of the lower limb in a standing posture. This thesis included the investigation of transcortical loops to the soleus and medial gastrocnemius muscles in addition to the tibialis anterior. We stimulated cutaneous afferents directly from the foot sole to investigate this transcortical loop, as the plantar sole provides important functional information during standing balance. Stimulation of distinct regions of the foot sole has been shown to evoke cutaneous reflex responses which occur at latencies long enough to be mediated by a transcortical pathway. We hypothesized that stimulation of the foot sole would indeed modulate excitability of the motor cortex.

To investigate if the leg motor cortex has the potential to be involved in modulating these reflex responses, the motor cortex was stimulated using transcranial

magnetic stimulation during foot sole stimulation to either the heel or metatarsal region. Stimulation of the motor cortex causes an activation of the descending corticospinal tract to cause a response in the muscle. Thus, any changes observed at the level of the muscle could be a result of either excitability changes at the cortex, or the spinal cord, or a combination of both. To differentiate these changes between a spinal or cortical mechanism, responses in the muscle were also evoked with stimulation below the level of the motor cortex, at the brainstem. Brainstem activation allows us to bypass the synaptic activation at the level of the motor cortex, while still activating the same descending axons. The effect of foot sole stimulation on responses evoked from the brainstem and the motor cortex was examined and compared.

This thesis provides some of the first evidence to suggest that a cortical loop may exist from cutaneous afferents of the foot sole to muscles of the lower limb. Foot sole stimulation modulated both MEPs and CMEPs in the lower limb. By comparing MEP responses to the size matched CMEP responses, we found that they responded differently to changes in corticospinal excitability. In fact, MEPs showed smaller relative change in amplitudes compared to the CMEPs, this suggests two things; first that there is indeed a cortical contribution to these reflex responses, and second, there is an overall decrease in facilitation occurring at a cortical level with foot sole stimulation.

4.1 Excitability differences between foot sole stimulation locations

Corticospinal excitability was found to be modulated depending on the region of foot sole stimulated. It has been shown that stimulation to the heel and metatarsal regions evokes cutaneous reflex responses in the lower limb muscles which are specific to the location of the foot sole stimulated (Nakajima et al. 2006). These location-specific

reflexes can be found to play an important role in the clearance of an obstacle that makes contact with the plantar sole. For example, when the heel region is contacted an excitatory response is observed in the plantar flexor muscles to lift the foot away from the obstacle. In the current thesis we investigated if excitability of the motor cortex is modulated in a similar location-specific manner. We found differences in the MEP responses following stimulation to the heel region compared to the metatarsal region. Excitability differences between foot sole locations were more evident in the plantar flexor muscles, where heel stimulation increased excitability and metatarsal stimulation decreased excitability. These findings are in line with the cutaneous reflexes observed in these muscles, with an excitatory reflex response observed with heel stimulation and an inhibitory reflex response observed with metatarsal stimulation.

Interestingly, we found that these reflexes are not only modulated by a transcortical pathway, but they are functionally organized at the level of the motor cortex. We found that, at the level of the spinal cord, foot sole stimulation to either location (HEEL or MET) was able to evoke excitation, demonstrated by the increases in CMEP amplitude. CMEP responses did not display location-specific effects of foot sole stimulation. These effects were only observed in the MEP responses. This suggests that location-specific modulation occurs at supraspinal centres and that a transcortical pathway is important in the functional organization of these reflex responses.

In fact, our findings specifically reflect an inhibitory transcortical mechanism in the modulation of these reflex responses. Such that, the differences that were observed in the MEP responses between heel and metatarsal stimulation may be due to the changes in the level of cortical inhibition that occur with stimulation to the different regions of the

foot sole. For example, there may be a greater level of inhibition in the cortical networks to the plantar flexor muscles with metatarsal stimulation than heel. This cortical modulation is important in the functional role these location-specific reflexes play. The site dependence that was observed in the muscle responses evoked by stimulation of the cortex offers additional evidence that non-noxious cutaneous sensations applied to the bottom of the foot sole provide important input for balance control through the modulation of lower limb activity. The main findings of the excitability differences between foot sole sites are important to provide further detail in our understanding of the organization of cutaneous reflexes and the neural mechanisms contributing to their functional role.

4.2 Limitations

4.2.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation is a powerful tool because it allows us to manipulate brain activity non-invasively in humans. However, certain limitations exist for TMS studies, such as the inherent variability in MEPs. This may in part be due to the multiple inhibitory and excitatory converging inputs onto the corticospinal cells, and also in part due to slight coil movements, thus changing the trajectory of the magnetic pulse (Kiers et al. 1993). For these reasons, successive pulses in a single trial could yield varying sized responses. Thus, variance should be minimized as much as possible. There are many synapses involved in producing an MEP, which means an MEP represents the net response from multiple neural components in the pathway. These factors need to be

well controlled in order to attribute changes in the MEP size to the intervention, i.e foot sole stimulation.

This study involved the investigation of MEPs from three muscles during standing. A single TMS pulse typically resulted in activation of multiple adjoining muscles in concert. In order to obtain these responses, the TMS coil was held in the optimal position by an experimenter standing behind the subject. We chose this setup as the alternative would be to strap the coil on the subject's head throughout the protocol. Our chosen method allowed the weight of the coil to be off loaded from the subject's head. Although this method was beneficial for subject comfort, it did have the potential to introduce variability to the MEPs due to human error. Any movement of the coil from the identified optimal location has the potential to cause variability in the MEP responses because the stimulus would activate a different population of neurons in the motor cortex (Conforto et al. 2004). To keep a consistent location of the coil, we used Optotrak rigid bodies fixed to the coil to track if any movement of the coil occurred from the optimal site. This generated 3D kinematic data of the coil position, which were then converted and provided to the experimenter as real-time visual feedback. The experimenter was then able to use this feedback to reposition the coil if needed. Maintaining consistent coil location is crucial to obtaining reliable data. We attempted to control for this to the best of our ability by creating our own method of providing real-time feedback of coil position, however the use of neuronavigation systems in the future would be beneficial for the accuracy of the results.

Cognition and the arousal of the subject can also affect the MEP (Fischer et al. 2008). Thus, it is important to maintain stable experimental conditions such that arousal

is not affected. In order to maintain a steady arousal level, we minimized talking and interaction between the experimenter and the subject during TMS. We also made sure the experimental environment did not change throughout the protocol. Other factors that can modify the TMS response, such as aerobic exercise, genetics, and time of day, were not controlled for and might be a limitation to the interpretation of the results. It is also important for the subject to maintain a similar level of cognitive attention throughout the protocol as merely imagining the activation of a muscle or imagining the task can cause enhancement of MEPs (Strafella and Paus 2000; Patuzzo et al. 2003; Fourkas et al. 2006). Although we asked subjects to focus their attention to the wall in front of them, it is difficult to control what they are thinking. Thus it is possible that their cognitive state could have affected the results. In future studies, one way to prevent any drastic cognitive fluctuations is to give the subjects a cognitive task to focus on during the protocol, such as counting the number of times a light flashes. This would help maintain a consistent cognitive state throughout the trials.

4.2.2 Timing of responses

The basic idea of this type of study is to use TMS as a probe of the excitability of the corticospinal pathways at a certain time in relation to the activation of the skin afferents. The rationale of this is that the magnetic stimulus activates the corticospinal neurones at a site where the size of the descending volley evoked by the TMS stimulus might be influenced by the change in excitability of the corticospinal neurons (due to the cutaneous afferents). We hypothesized that cutaneous stimulation would modulate excitability at a cortical level, thus we stimulated the motor cortex when we expected the cutaneous volley to have arrived at the cortical level. To achieve estimates of the afferent

and efferent conduction times we recorded the cutaneous reflexes evoked by foot sole stimulation and the motor potentials evoked by transcranial magnetic stimulation of the corticospinal tract. Based on the hypothesis that these cutaneous reflexes are transcortical, we were able to subtract the efferent conduction time (MEP latency) from the reflex latency to establish the minimum time the cutaneous volley required to reach the cortex. These calculations were based on a study from (Nielsen et al. 1997) who performed similar calculations to establish the conduction time required for a transcortical reflex. These authors however, were able to determine the exact afferent conduction time for the cutaneous volley to reach the cerebral cortex by recording somatosensory evoked potentials (SEP). Recording cerebral potentials is a more reliable method to determine an appropriate interval between cutaneous stimulation and TMS. Our calculation of the ISI between foot sole stimulation and TMS is based on the fact that these cutaneous reflexes in the muscles occur at latencies which have sufficient time to be modulated either through multi-synaptic connections in the spinal cord or through a cortical loop. Therefore, if we assume the cutaneous volley has the potential to be modulated through the cortex we can align the TMS pulse with that timing. Based on this assumption our calculation of the afferent conduction time is acceptable. However, to truly validate this assumption the recording of SEPs may be warranted. The interpretation of the results of this study may be greatly impacted if the cortical stimulus is applied at an incorrect interval following the cutaneous stimulation. Previous studies have shown that MEP responses in lower limb muscles are both suppressed and increased by a preceding sensory nerve stimulus depending on the conditioning-test interval that was used (Deletis et al. 1992; Nielsen et al. 1997; Roy and Gorassini 2008).

One study showed that resting MEPs were facilitated in response to activation of the tibial nerve 45-50 ms earlier, whereas they were inhibited at inter-stimulus intervals of 32.5-37.5 ms (Roy and Gorassini 2008). Thus, accurate conditioning-test intervals are important because applying the TMS when the corticospinal neurons may not be influenced by the afferent input will provide inaccurate measures of excitability that do not represent the influence of afferent feedback on the motor cortex. Our goal was to apply a cortical stimulus at the time when it was feasible timewise for the cutaneous volley to have reached the motor cortex, allowing us to investigate the influence of cutaneous feedback on the cortical output.

4.3 Applications, implications, and future directions of the current work

Cutaneous reflexes have been shown to play a large role in balance and postural control (Thoumie and Do 1996; Zehr et al. 2001; Day and Cole 2002). In fact, long-latency cutaneous reflexes have been shown to be isolated to specific regions of the foot sole (Nakajima et al. 2006; Nakajima et al. 2009). The current study has demonstrated that a transcortical loop, which is likely inhibitory, is generated from cutaneous afferents of the foot sole to plantar flexor muscles of the lower limb. To understand the specific intra-cortical networks which are activated with cutaneous afferents of the foot sole, future studies are required to investigate the modification of short interval intracortical inhibition and intracortical facilitation by afferent inputs from the foot. This information would allow us to conclude which networks are involved in shaping the changes when cutaneous afferent input is provided from the foot sole. Future studies are also required to determine if activation of plantar cutaneous afferents by vibration yields activation of

cortical networks as vibration of the palmar surface of the hand has been shown to modulate cortical excitability of the upper limb (Christova et al. 2011). Recently vibration of the foot sole has become an important tool in postural control and motor improvement (Priplata et al. 2003; Perry et al. 2008). By determining if central structures in addition to musculoskeletal structures are also modulated with vibration, we would be able to apply this information to improving rehabilitation practices.

4.1 Conclusion

In the current thesis we found that the cutaneous reflexes in the lower limb evoked from foot sole stimulation during standing are partly modulated through a transcortical loop. We suggest that stimulation to distinct regions of the foot sole causes a decrease in cortical drive to the motoneurons of the lower limb. It is often assumed that transcortical reflex pathways play little or no role in the control of leg muscles (Christensen et al. 2000). This work provides support that in fact cutaneous afferents from the foot sole through transcortical pathways play a substantial role in regulation of posture and generation of functional responses. Overall this work has expanded our knowledge of the existence of transcortical reflex pathways in the lower limb in humans.

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