“This too shall pass”

— *Medieval Persian proverb*

University of Alberta

Changes in corticospinal excitability induced by neuromuscular electrical stimulation

by

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Abstract

This thesis describes experiments designed to investigate the effects of neuromuscular electrical stimulation (NMES) on corticospinal (CS) excitability in humans. NMES delivered at 100 Hz was more effective for increasing CS excitability than 10-, 50-, or 200-Hz NMES. CS excitability increases occurred after 24 min of 100-Hz NMES, were strongest in the stimulated muscle, and were mediated primarily at a supraspinal level. NMES of the common peroneal nerve of the leg increased CS excitability in multiple leg muscles, whereas NMES of the median nerve of the hand increased CS excitability in only the muscle innervated by that nerve. Additionally, CS excitability for the hand increased after 40 min of relatively high intensity and frequency NMES but not after 2 h of lower intensity and frequency NMES. These results have implications for identifying optimal NMES parameters to augment CS excitability for rehabilitation after central nervous system injury.

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List of Abbreviations

ADM	abductor digiti minimi
ANOVA	analysis of variance
APB	abductor pollicis brevis
BES	brainstem electrical stimulation
CNS	central nervous system
CP	common peroneal
CS	corticospinal
C-T interval	conditioning-test interval
D-wave	direct wave
ECU	extensor carpi ulnaris
EMG	electromyography
FDI	first dorsal interosseus
FES	functional electrical stimulation
fMRI	functional magnetic resonance imaging
GABA	gamma-amino-butyric acid
H_{max}	maximum Hoffman reflex
H-reflex	Hoffman reflex
ISI	inter-stimulus interval
I-wave	indirect wave
LTP	long-term potentiation
M1	primary motor cortex
MEP	motor evoked potential

M_{max}	maximum motor wave
M-wave	motor wave
NMDA	N-methyl-D-aspartate
NMES	neuromuscular electrical stimulation
S1	primary somatosensory cortex
SCI	spinal cord injury
SEP	somatosensory evoked potential
Sol	soleus
SS	somatosensory stimulation
TA	tibialis anterior
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation
VM	vastus medialis

CHAPTER 1: GENERAL INTRODUCTION

1.1 Preface

After trauma to the spinal cord or brain there are reductions in the amount of sensory input transmitted to the areas of the central nervous system (CNS) that control movement. This reduced sensory input occurs initially due to the injury with further reductions often occurring due to prolonged disuse of affected muscles (Chen et al.,, 2002). These reductions in afferent drive lead to maladaptive plasticity in the CNS characterized by decreases in the excitability of motor areas for the affected muscles (Liepert et al., 2000). To combat these reductions in excitability and the associated functional impairments, the afferent volley generated during neuromuscular electrical stimulation (NMES) can be used to induce beneficial plasticity in the CNS whereby motor areas undergo increases in excitability. This NMES-induced plasticity has been linked to functional improvements, such as increased strength (Conforto et al., 2002; Knash et al., 2003) and improved motor learning (Pascual-Leone et al., 1995a). Unfortunately, the wide range of NMES parameters (intensity, frequency, pattern, and duration) and muscle groups tested in previous studies make it difficult to identify the optimal parameters for inducing such plasticity. This thesis consists of two research projects that were designed to better characterize the influence of NMES on plasticity in the CNS. The first project investigates the influence of NMES frequency on corticospinal (CS) excitability for muscles of the leg. The second project extends the findings of the first project by exploring differences in NMES-

induced plasticity between muscles of the hand and leg, and further investigates the effects of altering stimulation parameters on NMES-induced plasticity in the hand. My findings contribute to a growing body of knowledge about how the afferent drive generated during NMES influences CNS plasticity and has implications for identifying the optimal NMES parameters to enhance plasticity for rehabilitation.

This general introduction consists of four main sections. The first two sections include background information on the motor cortex, cortical plasticity, and the measures of cortical plasticity that were employed throughout this thesis. The third section considers the effects of NMES on plasticity in the CNS and describes the motivation for investigating NMES-induced plasticity in this thesis. Finally, the fourth section discusses possible mechanisms that underlie NMES-induced plasticity in the CNS.

1.2 Motor cortex

The motor cortex encompasses regions of the cerebral cortex that play a role in the planning, control, and execution of voluntary movement. In this section I discuss the initial findings of a motor region in the cerebral cortex, changing views regarding the complex and plastic nature of this region, and recent findings characterizing the role of cortical plasticity in motor skill acquisition and injury.

1.2.1 History of the primary motor cortex (M1)

The first evidence of a motor area in the cerebral cortex was discovered in 1870 by Fritsch and Hitzig when they found that electrical stimulation of specific regions of the dog brain could generate movement (new translation 2009). Further

studies identified similar motor regions in the primate (Leyton and Sherrington, 1917) and human brains (Penfield and Boldrey, 1937). It was concluded that the purpose of this motor region was to connect the brain to the contralateral lower motoneurons via the spinal cord, thus allowing for volitional contractions of particular muscles. Initial findings characterized the organization of this cortical motor area as complex and unstable. In 1952, Penfield and Rasmussen published *The Cerebral Cortex of Man* which identified Brodmann's Area 4 of the cerebral cortex in man as the primary motor cortex (M1). At this point in time, the complexity of past experimental findings regarding M1 organization was simplified into an orderly, somatotopic representation map for muscles of the body (homunculus) that bore little relation to the experimental data (Leyton and Sherrinton, 1917; Penfield and Boldrey, 1937). Along with this oversimplification came the common misconception that M1 and the rest of the CNS is an orderly, fixed, and hard-wired entity with little capacity for adaptation.

More recently, studies of M1 organization have shifted the focus back to it having a more complex and unstable nature, as originally described by Leyton and Sherrington (1917) and Penfield and Boldrey (1937). For example, M1 organization is now recognized to have widespread overlap in the representation of different parts of the body (Lemon, 1988; Schieber and Hibbard, 1993; Rathelot and Strick, 2006). Thus, a single M1 neuron projects to more than one muscle and M1 organization is not as simple as the Penfield homunculus (1952). Moreover, mapping of muscle representations in rat M1 with intracortical electrical stimulation demonstrated that M1 maps are capable of rapid (within 4 h)

(Donoghue et al., 1990) and long-lasting (up to 4 months) (Sanes et al., 1990) reorganization. These findings have contributed to a paradigm shift that has resulted in the view of M1 and the rest of the CNS as a plastic, rather than a hard-wired, entity.

1.2.2 Plasticity in M1

Plasticity can be defined as the ability of a system to undergo structural and/or functional modifications under new constraints imposed by the environment (Tyc et al., 2005). Such plasticity is necessary for individuals to adapt to the demands of an ever-changing environment. In M1, plasticity manifests as expansions or reductions in the area that activates specific muscles and as changes in the excitability of cortical circuits associated with specific muscles. Researchers have discovered that the CNS, and specifically M1, is constantly reorganizing and adapting as an individual acquires new skills, after an injury has occurred, and while recovering from injuries.

1.2.2.1 M1 plasticity with motor skill acquisition

During skill acquisition, cortical areas associated with the involved muscle groups expand in size and increase in excitability. For example, experienced Braille readers have expanded cortical map representations in M1 for the fingers involved in Braille reading (Pascual-Leone et al., 1995b). Likewise, short-term repetition of a simple thumb movement induced cortical plasticity for the involved muscles (Classen et al., 1998). Furthermore, practice of a 30 s hand manipulation task (pegboard) three times over induced an increase in performance in conjunction with an increase in cortical excitability for the involved hand

muscles (Halder et al., 2005). This training-induced plasticity is thought to be a product of the simultaneous activity in afferent and efferent pathways that occurs during repeated voluntary movements. Typically these effects of reorganization outlast the practice by 15 min to 1 h, after which the cortical representations and excitability levels return to their original state (McKay et al., 2002a).

1.2.2.2 M1 plasticity with injury

Injuries to the nervous system, which result in reductions in afferent and efferent activity to the affected muscles, also induce plasticity in M1. Following an ischemic nerve-block or amputation, there is expansion of the cortical representation of the muscles just proximal to the site of de-afferentation (Brasil-Neto et al., 1993; Elbert et al., 1997; Chen et al., 1999). There is also "invasion" of the cortical areas representing the de-afferented muscles by regions corresponding to other muscles (Elbert et al., 1997). CNS damage, such as in stroke or spinal cord injury (SCI), and prolonged disuse results in reduced activity in neural circuits that control the affected muscles. The reduced activity leads to maladaptive cortical plasticity where the size of the cortical representations for the affected muscles is reduced (Liepert et al., 2000). In contrast, if individuals attempt to use the affected muscles, increases in cortical excitability and expansion of cortical representations that occur concomitantly with improvements in function, can be promoted (Foltys et al., 2003; Koski et al., 2004).

1.3 Transcranial magnetic stimulation (TMS) of M1

The development of transcranial magnetic stimulation (TMS) has lead to a greater understanding of the plasticity inherent to M1 region in humans. TMS is a non-invasive, safe, and painless technique that allows researchers to explore M1 excitability and organization. This technique was originally developed and tested in rabbits (Harris, 1947) and was modified later for use in humans (Barker et al., 1985). In this section I discuss how TMS is used to activate M1 and the CS tract, as well as different measures of M1 excitability and organization that can be obtained using TMS.

1.3.1 Activation of M1 and CS tract by TMS

1.3.1.1 Electromagnetic induction

TMS is delivered to humans using a coil positioned over the scalp and operates via the process of electromagnetic induction, whereby an electrical current passed through one coil induces a current in a nearby coil. With electromagnetic induction, a brief high current pulse in the first coil produces a magnetic field with lines of flux perpendicular to the coil. An electric field is produced at a right angle to the magnetic field and produces a current that flows in the same plane, but opposite direction in the second coil. When using TMS, the electric field evoked by the current flowing through the coil depolarizes neurons in the individual's brain. By positioning the TMS coil on the scalp over the M1 region of one cerebral hemisphere, one can preferentially activate neurons in M1 directly underneath the coil, which in turn can generate movement in contralateral muscles. Early studies used a circular TMS coil, but more recent variants of this

coil include a figure-of-eight coil that improves the focus of the stimulus, and a larger parabolic or double-cone coil that stimulates the deeper regions of the motor cortex. Both figure-of-eight and parabolic coils were used in the research presented in thesis.

1.3.1.2 CS volleys

Initial studies in animals revealed that stimulation of the exposed motor cortex with a single electrical stimulus gave rise to a series of high frequency waves in the CS tract (Patton and Amassian, 1954). The initial descending waves (D-waves) are produced by direct excitation of the pyramidal tract neurons, whereas later waves originate from indirect, synaptic activation of pyramidal tract neurons (I-waves). Similarly, with TMS, a single magnetic stimulus to the motor cortex activates CS neurons via different populations of excitatory axons, and gives rise to multiple direct and indirect descending volleys in CS tract neurons (Edgley et al., 1990; Burke et al., 1993). A combination of these D-waves and I-waves travel down the CS tract and generate muscle activity. The muscle activity that is generated can be recorded by surface electromyography (EMG) in the form of a motor evoked potential (MEP).

1.3.2 TMS methods for measurement of CS excitability and organization

A variety of measures of CS excitability and organization can be obtained using different TMS techniques. Some common measurements include MEP threshold, MEP amplitude, MEP recruitment curves, area and volume of cortical representations obtained from mapping procedures, and measures of inhibition and facilitation using paired-pulse TMS. In conducting the research for this thesis,

the main measures of CS excitability utilized were MEP threshold and MEP amplitude. Importantly, MEP responses evoked by TMS over M1 are influenced by the excitability of neurons in both M1 and the spinal cord. Thus, TMS techniques that utilize MEPs evoked by TMS over M1 are typically considered to provide a measure of the excitability of the CS pathway, rather than a measure of the excitability of M1 alone.

1.3.2.1 MEP threshold and MEP amplitude

MEP threshold is typically defined as the lowest TMS intensity required to elicit an MEP with a peak-to-peak amplitude of ~50 uV in 50% of successive trials using the same intensity of stimulation (Rossini et al., 1994). The MEP threshold is measured at the site that produces the largest MEP for a given TMS intensity (the "hotspot"). Both of these measures represent the membrane excitability of cortical neurons, as well as the excitability of motoneurons. A decrease in MEP threshold or an increase in the peak-to-peak amplitude of an MEP at a given TMS intensity indicates an increase in CS excitability. These measures can be obtained with the subject at rest or holding a small voluntary contraction of the target muscle. MEPs evoked with a background contraction are facilitated in size because a small percentage of motor units are already active, and others will be sitting closer to threshold compared to when the muscle is at rest. MEPs evoked with a background contraction allow for lower intensities of TMS to evoke measurable responses and thus, are commonly used when individuals have high MEP thresholds or low tolerance for TMS (Amassian et al., 1995). Moderate to good reliability of peak-to-peak MEP amplitude

measurements has been shown for MEPs obtained by TMS in both resting and active conditions (intraclass correlation coefficient~0.70) (Kamen, 2004).

1.3.2.2 MEP recruitment curves

MEP recruitment or stimulus-response curves can also be constructed from MEPs evoked by single TMS pulses and can be used to assess the excitability of CS pathways. Such recruitment curves plot MEP amplitude as a function of TMS intensity (Ridding and Rothwell, 1997). It is believed that as the TMS intensity is increased the slope of the curve may represent the physical spread of the stimulus from the coil to neurons further away from the coil, or the activation of neurons with higher excitatory thresholds, or both (Siebner and Rothwell, 2003).

1.3.2.3 Mapping of M1 organization

TMS can also be used to map the gross somatotopy of the motor homunculus in M1 (Wassermann et al., 1992). To map the representation of a specific muscle within M1, TMS is applied at a given stimulus intensity typically in a grid-like pattern that encompasses the "hotspot" for the target muscle. Cortical mapping procedures provide details of plasticity in the CS system that would not be detected by simply evaluating the peak-to-peak amplitude of an MEP elicited from a single cortical site. Mapping measures include the area of the region from which responses are obtained, the volume or combined amplitude of all of the active sites of the map, and the centre of gravity of the map or the centre of the cortical representation.

1.3.2.4 Paired-pulse TMS

Another TMS technique used to test the excitability of the CS system is the delivery of two successive pulses to the motor cortex, or paired-pulse stimulation. Intracortical inhibitory and facilitatory interactions can be observed depending on the stimulation intensities and the inter-stimulus intervals of the TMS pulses (Kujirai et al., 1993). These measures can provide information on whether intracortical inhibitory and facilitatory circuits mediate net changes in the excitability of CS pathways.

1.4 NMES

NMES involves the delivery of an electrical current through electrodes typically placed on the surface of the skin either over the muscle belly to activate terminal motor axon branches, or over the nerve proximal to the muscle at a site where the nerve runs close to the surface of the skin. Below I discuss the use of NMES for rehabilitation, evidence that NMES induces plasticity in the CNS, evidence that afferent input from NMES applied to muscles of the hand and leg may have differing effects on CS excitability, and finally, the variety of stimulation parameters with which NMES can be delivered and the possible effects on NMES-induced plasticity.

1.4.1 NMES for rehabilitation

Damage to CNS circuits involved in movement control typically disrupts and reduces activity in afferent and efferent pathways for the affected muscles. Depending on the extent of the CNS injury, rehabilitation programs involving repetitive voluntary movement practice to improve function can be somewhat

paradoxical. For example, if an individual has extremely limited voluntary movement, their ability to participate in the training program is compromised and the potential benefits of rehabilitation cannot be realized. Although voluntary movement is often impaired following CNS injury, some efferent and afferent pathways may remain undamaged. If an intact motor unit remains, NMES can be applied to activate motor axons and in turn generate muscular contractions (Popovic et al., 2001; Salmons et al., 2005; Sheffler and Chae, 2007). As a result, NMES applied through electrodes placed on the surface of the skin is a common tool used by individuals with movement impairments following damage to the CNS, such as stroke or SCI (Popovic et al., 2001). In addition to generating contractions to assist with activities of daily living, benefits of NMES include reduced muscle atrophy, increased blood circulation, increased bone density, maintenance of range of motion, reduced spasticity, and improvements in voluntary movement that outlast the stimulation (Baker et al., 2000).

1.4.2 Afferent drive generated by NMES

Similar to the effects of repetitive movement practice (see section 1.2.2.1), functional improvements that outlast the application of NMES are associated with plasticity in CS circuits (Conforto et al., 2002; Knash et al., 2003; Kido and Stein, 2004; Hoffman and Field-Fote, 2007). In addition to activating motor axons, intact afferent pathways are also activated by NMES, and it is the afferent drive generated during the NMES that is crucial to inducing this plasticity. The following information about the afferent drive generated by NMES is summarized

from *The circuitry of the human spinal cord: its role in motor control and movement disorders* by Pierrot-Desseilligny and Burke (2005).

When NMES is applied to a muscle or peripheral nerve, sensory afferents that originate from receptors in the muscle, joints, and the skin and that project to the spinal cord and brain are activated in conjunction with motor pathways. In the muscle, Group Ia and Group II afferents originate from spindle receptors that are activated by changes in muscle length and the rate of muscle length change. Ia afferents have a low electrical threshold and are activated prior to the higher threshold group II afferents which have a strong effect on propriospinal neurons and the modulation of motoneuron excitability during rhythmic motions such as gait. Group Ib afferents originate from Golgi tendon organs that sense muscle tension and have a slightly higher electrical threshold than Ia afferents. Afferents originating from joint and cutaneous mechanoreceptors can also be activated by NMES and play an important role in modulating motoneuron excitability during voluntary movement. Each of these afferent pathways (Ia, II, Ib, etc.) are highly organized and transmit sensory information to areas of the brain based on the location and type of sensory receptor that the afferent innervates.

Sensory information travels along these afferent pathways up the dorsal columns, crosses over to the contralateral side at the medulla, is received and relayed by the thalamus, and eventually arrives in somatosensory cortex (S1). Like M1, S1 is subdivided into distinct regions based upon its anatomical connections and function. S1 and M1 are divided by the central sulcus of the brain, but connections between them allow for the sensory input to S1 to influence

the excitability and organization of M1. Functional magnetic resonance imaging (fMRI) studies show that electrical stimulation of sensory axons in a peripheral nerve results in significant activation of both S1 and M1 (Spiegel et al., 1999; Deuchert et al., 2002). Generally, reducing the afferent drive to S1, such as by CNS injury and prolonged disuse, reduces the excitability of pathways between M1 and muscle, whereas increasing the afferent drive, such as by repetitive movement practice and NMES, increases the excitability of the same pathways.

1.4.3 Evidence of NMES-induced CNS plasticity

The first experimental evidence of plasticity in the CNS induced by NMES was presented by Hamdy and colleagues (1998) when 10 min of pharyngeal nerve stimulation increased the amplitude of MEPs evoked by TMS and reorganized cortical maps for swallowing musculature. Since then, lasting changes in CS excitability and cortical reorganization following NMES have been reported for a variety of muscles including first dorsal interosseus (FDI) (Ridding et al., 2000; McKay et al., 2002a, b; Pitcher et al., 2003), abductor pollicis brevis (APB), abductor digiti minimi (ADM) (Ridding et al., 2000; McKay et al., 2002a), and tibialis anterior (TA) (Khaslavskaia et al., 2002; Knash et al., 2003; Kido and Stein, 2004; Khaslavskaia and Sinkjaer, 2005). Like the plasticity that occurs following repetitive movement practice, these changes have been shown to last for periods of ~15 min to 1 h after the stimulation and for as long as 2 days when NMES is applied on successive days (McKay et al., 2002a, b).

1.4.3.1 NMES-induced plasticity for muscles of the hand versus the leg

The effect of NMES on CS excitability has been studied separately in both the upper and lower limbs. Ridding and colleagues (2000) were the first to show changes in the excitability of CS pathways following NMES of upper limb muscles. Following ulnar nerve stimulation, MEPs evoked in ulnar-innervated muscles, ADM and FDI, were increased by ~50%, whereas MEPs evoked in the median-innervated APB muscle did not change. Likewise, when NMES was applied to activate afferents for FDI, there were increases in MEP amplitude for FDI and no change in MEPs evoked in APB (Ridding et al., 2001). These two studies and others (McKay et al., 2002a; Pitcher et al., 2003) provide evidence that the excitability of cortical projections to hand muscles can be altered specifically by the location of NMES that is applied.

Similar increases in CS excitability have been reported following NMES applied to the lower limb. Following NMES to activate TA, the amplitude of MEPs for TA was increased while the amplitude of soleus (Sol) MEPs did not change (Khaslavskaia et al., 2002; Knash et al., 2003). In another study, common peroneal (CP) nerve stimulation was applied to activate TA during the swing phase of gait and MEPs increased for both TA and Sol suggesting that CS excitability changes may spread to non-stimulated muscles in the leg (Kido and Stein, 2004). However, the increases in Sol MEPs were more variable than those observed for TA.

The previously mentioned studies (Ridding et al., 2000; Ridding et al., 2001; Khaslavskaia et al., 2002; McKay et al., 2002a; Knash et al., 2003; Pitcher

et al., 2003; Kido and Stein, 2004) have evaluated CS excitability changes in the non-stimulated muscles by measuring MEP responses of these muscles when evoked by TMS at the optimal location for the stimulated muscle, rather than the optimal location for the non-stimulated muscles. If there are smaller or more variable effects of NMES on CS excitability for these muscles, as suggested by Kido and Stein (2004), then perhaps more specific measures are necessary to detect these changes. Chapter 3 of this thesis investigates NMES-induced changes in CS excitability in non-stimulated muscles of the hand and leg by measuring MEPs evoked from the respective hotspot for each muscle.

1.4.3.2 Afferent-conditioning of MEPs for muscles of the hand versus the leg

Studies investigating the afferent-conditioning of MEPs by a preceding electrical stimulus to a sensory nerve suggest that CS excitability is affected differently by afferent input received from the hand and leg (Nielsen et al., 1992; Deletis et al., 1992; Kasai et al., 1992; Roy and Gorassini, 2008). MEPs are suppressed by a single peripheral nerve stimulus due to inhibition of cortical circuits for hand muscles (Tokimura et al., 2000), but due to inhibition of spinal circuits for leg muscles (Roy and Gorassini, 2008; Poon et al., 2008). Facilitation of MEPs occurs for both the hand and the leg due to changes in both M1 and spinal excitability (Deletis et al., 1992; Poon et al., 2008; Roy and Gorassini, 2008). This afferent-induced facilitation occurs in muscles innervated by the stimulated nerve and in adjacent muscles (Deletis et al., 1992); however, the facilitation spreads to non-stimulated muscles to a greater degree for muscles of the leg than the hand (Roy and Gorassini, 2008).

These studies suggest that afferent input has both inhibitory and excitatory effects on cortical networks for the hand, but mainly excitatory effects on leg M1. The predominantly excitatory and non-specific effect of a single electrical stimulus on leg M1, as compared to hand M1, agrees with studies that show a specific effect of NMES on CS excitability for muscles of the hand and a less specific effect for muscles of the leg (see 1.4.4.1). However, comparing the amplitude and spread of NMES-induced increases in CS excitability between muscles of the hand and leg is difficult due to differences in stimulation parameters between studies.

1.4.4 NMES parameters

NMES can be applied with a wide range of stimulation parameters (intensity, frequency, pulse width, pattern, and duration). Typically, when used for therapy NMES is applied using high stimulation intensities (above motor threshold), low frequencies (~10-50 Hz), and a short pulse duration (~200 us) (Sheffler and Chae, 2007). Using these parameters NMES can be applied to specific muscles in sequence or in combination to generate or assist with functional movements, such as walking (Liberson et al., 1961), standing (Bajd et al., 1989), grasping (Prochazka et al., 1997), and bladder control (Jezernik et al., 2002). This type of stimulation is often termed functional electrical stimulation (FES) and is most generally defined by the high stimulation intensities that allow for large and functional muscle contractions to be produced. In other instances, NMES is applied at low intensities (near or below motor threshold), high frequencies (up to 200 Hz), and with long pulse durations (up to 1 ms). This type

of stimulation is often termed somatosensory stimulation (SS) and is designed to primarily activate sensory axons and "prime" CNS circuits to increase sensory feedback during rehabilitation sessions without producing large or functional muscle contractions (Hoffman and Field-Fote, 2007).

Within the application of FES and SS, many combinations of stimulation parameters are used. For example, in studies investigating the effect of NMES on CS excitability, NMES intensities have varied from below motor threshold to 50% of a maximal M-wave, frequencies have varied from 1 Hz to 200 Hz, and pulse durations have varied from 200 μs to 1 ms. In addition, NMES has been applied in a variety of different patterns and for differing durations (10 min (Hamdy et al., 1998) to 2 h (Ridding et al., 2000)).

Varying combinations of NMES parameters result in different afferent drive transmitted to the CNS and thus influences the induced changes in CS excitability. For example, CS excitability for swallowing musculature increased the most when NMES was applied at 75% of the maximum tolerated intensity, a frequency of 5 Hz, and a duration of 10 min when compared to other NMES parameters (Fraser et al., 2002). Nevertheless, there is still much to be learned about optimizing stimulation parameters to augment CS excitability. For example, no studies have yet explored the effect of NMES frequency on CS excitability for muscles of the lower limb. Moreover, differences in stimulation parameters used across studies make it difficult to draw comparisons about NMES-induced plasticity between different muscles. Chapters 2 and 3 of this thesis investigated the effect of altering NMES parameters on increasing CS excitability for muscles

in the leg and hand, respectively. Furthermore, Chapter 3 of this thesis explored the effect of NMES on CS excitability for muscles of the leg and hand when using the same stimulation parameters.

1.5 Mechanisms of NMES-induced plasticity

Plasticity in the CNS is induced rapidly by both repetitive movement training and the application of NMES in humans. Although the research conducted in this thesis was not designed to elucidate mechanisms of the plasticity that was measured, an understanding of the basic mechanisms involved is still important. Below I discuss evidence that cortical, rather than spinal, mechanisms underlie increases in CS excitability following movement training and NMES. More specifically, I will present evidence from pharmacological and animal studies that suggest long-term potentiation (LTP) –like and unmasking mechanisms are involved.

1.5.1 Location of NMES-induced CNS plasticity

To understand the mechanisms underlying plasticity in CS circuits following NMES, it is important to first understand at which level of the motor system the changes occur. As the MEP response evoked by TMS over the motor cortex is influenced by the excitability of both the cerebral cortex and the motoneuron pool, this technique alone cannot differentiate between changes occurring at a spinal or a cortical level. The use of TMS applied over the brainstem, transcranial electrical stimulation (TES), or brainstem electrical stimulation (BES) in addition to TMS over motor cortex, can be used to make such distinctions. TES and BES are believed to directly activate CS axons without

involving the soma of the cells, while TMS over the motor cortex is thought to activate CS neurons synaptically (Day et al., 1987a, b). As a result, changes in responses to TES or BES are attributed to changes occurring below the level of the cortex. Findings of increased responsiveness to TMS following voluntary movement training (Perez et al., 2004) and NMES (Stefan et al., 2000; Ridding et al., 2000; Khaslavskaia et al., 2002; Stefan et al., 2002), concomitant with no change or lesser change in responsiveness to TES or BES, suggest that excitability and organizational changes following NMES occur predominantly at a cortical level.

1.5.2 Possible cortical mechanisms of NMES-induced plasticity

One mechanism suggested to underlie activity-dependent plasticity in M1 is the modification of synaptic efficacy by LTP-like mechanisms. Evidence that supports the involvement of LTP-like mechanisms is the blockage of cortical plasticity normally induced by movement training or motor learning by the administration of pharmacological agents known to interfere with LTP, such as lorazepam and dextromethorphan (Butefisch et al., 2000; Ziemann et al., 2001). Furthermore, Hess & Donoghue (1994) reported a similar time course for the development of modifications in synaptic efficacy in the rat motor cortex via the induction of LTP (~25-35 min) as to what has been reported for training-induced plasticity in the human M1 (~30 min). Similarly, changes in CS excitability evoked by NMES of limb muscles occur within ~30-45 min of NMES (Khaslavskaia et al., 2002; McKay et al., 2002a; Knash et al., 2003), suggesting

LTP-like mechanisms may also underlie changes in CS excitability evoked by NMES.

The substrate for rapid plasticity in the motor cortex is thought to be the unmasking of latent, but pre-existing intrinsic horizontal connections, rather than the growth of new connections (for review, see Sanes and Donoghue, 2000). In rats, both LTP-like and unmasking mechanisms are induced by a reduction in local gamma-amino-butyric acid (GABAergic) inhibition and are dependent on pre-existing horizontal connectivity (Hess and Donoghue, 1994; Huntley, 1997). GABA is important for the maintenance of cortical representations in animal models (Jacobs and Donoghue, 1991) and pharmacological agents that up-regulate GABAergic inhibition in humans prevent the induction of cortical plasticity (Ziemann et al., 2001; Werhahn et al., 2002; Kaelin-Lang et al., 2002).

1.6 Thesis outline

CNS plasticity evoked by motor practice and increased afferent drive is associated with improvements in function (Ziemann et al., 2001), skill performance, and further skill learning (Pascual-Leone et al., 1995a). The afferent drive generated during NMES for rehabilitation has yielded similar improvements in function that outlast the stimulation (Conforto et al., 2002; Kido and Stein, 2004), with concomitant increases in CS excitability (Knash et al., 2003; Kido and Stein, 2004). Many studies have investigated the effect of NMES on CS excitability using a variety of NMES parameters and testing a variety of muscles; however, little is known about the effect of altering NMES parameters on this plasticity and whether it is different between muscles. If enhancing CS

excitability is important for improving function, then a better understanding of how NMES parameters can be manipulated to produce maximal increases in CS excitability will be important for the future of NMES therapies.

This thesis had 3 main objectives: 1) To evaluate the effect of frequency of NMES applied over the CP nerve on increases in CS excitability for the TA muscle; 2) To evaluate differences in the amplitude of NMES-induced increases in excitability in stimulated and non-stimulated muscles when NMES is applied to the leg and hand; and 3) To evaluate differences in the amplitude of NMES-induced increases in CS excitability in stimulated and non-stimulated muscles of the hand when NMES was applied with an FES-like and a SS-like protocol.

1.6.1 Chapter 2 outline

I investigated the effect of frequency of NMES applied over the CP nerve on changes in CS excitability for the TA muscle. I hypothesized that the enhanced afferent drive generated by higher frequencies of stimulation would produce larger increases in CS excitability than lower frequencies. This project also explored the time course of increases in CS excitability during NMES and the specificity of increases in CS excitability to the muscles innervated by the stimulated nerve. Finally, this study investigated whether the NMES-induced plasticity occurred at a cortical or spinal level. Based on previous research, I hypothesized that NMES-induced plasticity would occur primarily at a cortical level.

1.6.2 Chapter 3 outline

I investigated differences in NMES-induced plasticity for muscles of the leg and hand, as well as between an FES-like and SS-like protocol applied to the hand. My hypotheses were threefold: 1) I hypothesized that increases in CS excitability would be of greater amplitude for muscles of the hand than muscles of the leg, when NMES parameters were the same; 2) I hypothesized that CP nerve stimulation in the leg would result in a greater spread of increased CS excitability to non-stimulated muscles than median nerve stimulation, when NMES parameters were the same and; 3) I hypothesized that the enhanced afferent drive generated from the FES-like protocol would induce larger changes in MEP amplitude than the SS-like protocol for muscles of the hand.

1.7 References

Amassian, VE, Cracco, RQ, Maccabee, PJ, Cracco, JB, Henry, K (1995) Some positive effects of transcranial magnetic stimulation. Adv Neurol 67: 79-106

Bajd, T, Kralj, A, Turk, R, Benko, H, Sega, J (1989) Use of functional electrical stimulation in the rehabilitation of patients with incomplete spinal cord injuries. J Biomed Eng 11: 96-102

Baker, LL, Wederich, CL, McNeal, DR, Newsam, CJ, Waters, RL (2000) NeuroMuscular Electrical Stimulation: A Pratical Guide. 4: 1-56

Barker, AT, Jalinous, R, Freeston, IL (1985) Non-invasive magnetic stimulation of human motor cortex. Lancet 1: 1106-1107

Brasil-Neto, JP, Valls-Sole, J, Pascual-Leone, A, Cammarota, A, Amassian, VE, Cracco, R, Maccabee, P, Cracco, J, Hallett, M, Cohen, LG (1993) Rapid modulation of human cortical motor outputs following ischaemic nerve block. Brain 116 (Pt 3): 511-525

Burke, D, Hicks, R, Gandevia, SC, Stephen, J, Woodforth, I, Crawford, M (1993) Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. J Physiol 470: 383-393

Butefisch, CM, Davis, BC, Wise, SP, Sawaki, L, Kopylev, L, Classen, J, Cohen,LG (2000) Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci U S A 97: 3661-3665

Chen, R, Cohen, LG, Hallett, M (2002) Nervous system reorganization following injury. Neuroscience 111: 761-773

Chen, R, Corwell, B, Hallett, M (1999) Modulation of motor cortex excitability by median nerve and digit stimulation. Exp Brain Res 129: 77-86

Classen, J, Liepert, J, Wise, SP, Hallett, M, Cohen, LG (1998) Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol 79: 1117-1123

Conforto, AB, Kaelin-Lang, A, Cohen, LG (2002) Increase in hand muscle strength of stroke patients after somatosensory stimulation. Ann Neurol 51: 122-125

Day, BL, Rothwell, JC, Thompson, PD, Dick, JP, Cowan, JM, Berardelli, A, Marsden,CD (1987a) Motor cortex stimulation in intact man. 2. Multiple descending volleys. Brain 110 (Pt 5): 1191-1209

Day, BL, Thompson, PD, Dick, JP, Nakashima, K, Marsden, CD (1987b) Different sites of action of electrical and magnetic stimulation of the human brain. Neurosci Lett 75: 101-106

Deletis, V, Schild, JH, Beric, A, Dimitrijevic, MR (1992) Facilitation of motor evoked potentials by somatosensory afferent stimulation. Electroencephalogr Clin Neurophysiol 85: 302-310

Deuchert, M, Ruben, J, Schwiemann, J, Meyer, R, Thees, S, Krause, T, Blankenburg, F, Villringer, K, Kurth, R, Curio, G, Villringer, A (2002) Event-related fMRI of the somatosensory system using electrical finger stimulation. NeuroReport 13: 365-369

Donoghue, JP, Suner, S, Sanes, JN (1990) Dynamic organization of primary motor cortex output to target muscles in adult rats. II. Rapid reorganization following motor nerve lesions. Exp Brain Res 79: 492-503

Edgley, SA, Eyre, JA, Lemon, RN, Miller, S (1990) Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. J Physiol 425: 301-320

Elbert, T, Sterr, A, Flor, H, Rockstroh, B, Knecht, S, Pantev, C, Wienbruch, C, Taub, E (1997) Input-increase and input-decrease types of cortical reorganization after upper extremity amputation in humans. Exp Brain Res 117: 161-164

Foltys, H, Krings, T, Meister, IG, Sparing, R, Boroojerdi, B, Thron, A, Topper, R (2003) Motor representation in patients rapidly recovering after stroke: a functional magnetic resonance imaging and transcranial magnetic stimulation study. Clin Neurophysiol 114: 2404-2415

Fraser, C, Power, M, Hamdy, S, Rothwell, J, Hobday, D, Hollander, I, Tyrell, P, Hobson, A, Williams, S, Thompson, D (2002) Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. Neuron 34: 831-840

Fritsch, G, Hitzig, E (2009) Electric excitability of the cerebrum (Uber die elektrische erregbarkeit des grosshirns. Epilepsy Behav 15123-130

Halder, P, Sterr, A, Brem, S, Bucher, K, Kollias, S, Brandeis, D (2005) Electrophysiological evidence for cortical plasticity with movement repetition. Eur J Neurosci 21: 2271-2277

Hamdy, S, Rothwell, JC, Aziz, Q, Singh, KD, Thompson, DG (1998) Long-term reorganization of human motor cortex driven by short-term sensory stimulation. Nat Neurosci 1: 64-68

Harris, GW (1947) The innervation and actions of the neuro-hypophysis; an investigation using the method of remote-control stimulation. Philos Trans R Soc Lond B Biol Sci 232: 385-441

Hess, G, Donoghue, JP (1994) Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol 71: 2543-2547

Hoffman, LR, Field-Fote, EC (2007) Cortical reorganization following bimanual training and somatosensory stimulation in cervical spinal cord injury: a case report. Phys Ther 87: 208-223

Huntley, GW (1997) Correlation between patterns of horizontal connectivity and the extend of short-term representational plasticity in rat motor cortex. Cereb Cortex 7: 143-156

Jacobs, KM, Donoghue, JP (1991) Reshaping the cortical motor map by unmasking latent intracortical connections. Science 251: 944-947

Jezernik, S, Craggs, M, Grill, WM, Creasey, G, Rijkhoff, NJ (2002) Electrical stimulation for the treatment of bladder dysfunction: current status and future possibilities. Neurol Res 24: 413-430

Kaelin-Lang, A, Luft, AR, Sawaki, L, Burstein, AH, Sohn, YH, Cohen, LG (2002) Modulation of human corticomotor excitability by somatosensory input. J Physiol 540: 623-633

Kamen, G (2004) Reliability of motor-evoked potentials during resting and active contraction conditions. Med Sci Sports Exerc 36: 1574-1579

Kasai, T, Hayes, KC, Wolfe, DL, Allatt, RD (1992) Afferent conditioning of motor evoked potentials following transcranial magnetic stimulation of motor cortex in normal subjects. Electroencephalogr Clin Neurophysiol 85: 95-101

Khaslavskaia, S, Ladouceur, M, Sinkjaer, T (2002) Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve. Exp Brain Res 145: 309-315

Khaslavskaia, S, Sinkjaer, T (2005) Motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve depends on the voluntary drive. Exp Brain Res 162: 497-502

Kido, TA, Stein, RB (2004) Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. Exp Brain Res 159: 491-500

Knash, ME, Kido, A, Gorassini, M, Chan, KM, Stein, RB (2003) Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. Exp Brain Res 153: 366-377

Koski, L, Mernar, TJ, Dobkin, BH (2004) Immediate and long-term changes in corticomotor output in response to rehabilitation: correlation with functional improvements in chronic stroke. Neurorehabil Neural Repair 18: 230-249

Kujirai, T, Caramia, MD, Rothwell, JC, Day, BL, Thompson, PD, Ferbert, A, Wroe, S, Asselman, P, Marsden, CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471: 501-519

Lemon, R (1988) The output map of the primate motor cortex. Trends Neurosci 11: 501-506

Leyton, SSF, Sherrington, CS (1917) Observations on the excitable cortex of the chimpanzee, orangutang, and gorilla. Q J Exper Physiol 11: 135-222

Liberson, WT, Holmquest, HJ, Scot, D, Dow, M (1961) Functional electrotherapy: stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. Arch Phys Med Rehabil 42: 101-105

Liepert, J, Bauder, H, Wolfgang, HR, Miltner, WH, Taub, E, Weiller, C (2000) Treatment-induced cortical reorganization after stroke in humans. Stroke 31: 1210-1216

McKay, D, Brooker, R, Giacomin, P, Ridding, M, Miles, T (2002a) Time course of induction of increased human motor cortex excitability by nerve stimulation. NeuroReport 13: 1271-1273

McKay, DR, Ridding, MC, Thompson, PD, Miles, TS (2002b) Induction of persistent changes in the organisation of the human motor cortex. Exp Brain Res 143: 342-349

Nielsen, J, Kagamihara, Y, Crone, C, Hultborn, H (1992) Central facilitation of Ia inhibition during tonic ankle dorsiflexion revealed after blockade of peripheral feedback. Exp Brain Res 88: 651-656

Pascual-Leone, A, Nguyet, D, Cohen, LG, Brasil-Neto, JP, Cammarota, A, Hallett, M (1995a) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 74: 1037-1045

Pascual-Leone, A, Wassermann, EM, Sadato, N, Hallett, M (1995b) The role of reading activity on the modulation of motor cortical outputs to the reading hand in Braille readers. Ann Neurol 38: 910-915

Patton, HD, Amassian, VE (1954) Single and multiple-unit analysis of cortical stage of pyramidal tract activation. J Neurophysiol 17: 345-363

Penfield, W, Boldrey, E (1937) Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60: 389-443

Penfield, W, Rasmussen, T (1952) The cerebral cortex of man. Macmillan, New York

Perez, MA, Lungholt, BK, Nyborg, K, Nielsen, JB (2004) Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res 159: 197-205

Pierrot-Deseilligny, E, Burke, D (2005) The circuitry of the human spinal cord: Its role in motor control and movement disorders. Cambride: Cambridge University Press

Pitcher, JB, Ridding, MC, Miles, TS (2003) Frequency-dependent, bi-directional plasticity in motor cortex of human adults. Clin Neurophysiol 114: 1265-1271

Poon, DE, Roy, FD, Gorassini, MA, Stein, RB (2008) Interaction of paired cortical and peripheral nerve stimulation on human motor neurons. Exp Brain Res 188: 13-21

Popovic, MR, Curt, A, Keller, T, Dietz, V (2001) Functional electrical stimulation for grasping and walking: indications and limitations. Spinal Cord 39: 403-412

Prochazka, A, Gauthier, M, Wieler, M, Kenwell, Z (1997) The bionic glove: an electrical stimulator garment that provides controlled grasp and hand opening in quadriplegia. Arch Phys Med Rehabil 78: 608-614

Rathelot, JA, Strick, PL (2006) Muscle representation in the macaque motor cortex: an anatomical perspective. Proc Natl Acad Sci U S A 103: 8257-8262

Ridding, MC, Brouwer, B, Miles, TS, Pitcher, JB, Thompson, PD (2000) Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 131: 135-143

Ridding, MC, McKay, DR, Thompson, PD, Miles, TS (2001) Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 112: 1461-1469

Ridding, MC, Rothwell, JC (1997) Stimulus/response curves as a method of measuring motor cortical excitability in man. Electroencephalogr Clin Neurophysiol 105: 340-344

Rossini, PM, Martino, G, Narici, L, Pasquarelli, A, Peresson, M, Pizzella, V, Tecchio, F, Torrioli, G, Romani, GL (1994) Short-term brain 'plasticity' in humans: transient finger representation changes in sensory cortex somatotopy following ischemic anesthesia. Brain Res 642: 169-177

Roy, FD, Gorassini, MA (2008) Peripheral sensory activation of cortical circuits in the leg motor cortex of man. J Physiol 586 (Pt 17): 4091-105

Salmons, S, Ashley, Z, Sutherland, H, Russold, MF, Li, F, Jarvis, JC (2005) Functional electrical stimulation of denervated muscles: basic issues. Artif Organs 29: 199-202

Sanes, JN, Donoghue, JP (2000) Plasticity and primary motor cortex. Annu Rev Neurosci 23: 393-415

Sanes, JN, Suner, S, Donoghue, JP (1990) Dynamic organization of primary motor cortex output to target muscles in adult rats I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions. Exp Brain Res 79: 479-491

Schieber, MH, Hibbard, LS (1993) How somatotopic is the motor cortex hand area? Science 261: 489-492

Sheffler, LR, Chae, J (2007) Neuromuscular electrical stimulation in neurorehabilitation. Muscle Nerve 35: 562-590

Siebner, HR, Rothwell, J (2003) Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 148: 1-16

Spiegel, J, Tintera, J, Gawehn, J, Stoeter, P, Treede, RD (1999) Functional MRI of human primary somatosensory and motor cortex during median nerve stimulation. Clin Neurophysiol 110: 47-52

Stefan, K, Kunesch, E, Benecke, R, Cohen, LG, Classen, J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543: 699-708

Stefan, K, Kunesch, E, Cohen, LG, Benecke, R, Classen, J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123 Pt 3: 572-584

Tokimura, H, Di,L, V, Tokimura, Y, Oliviero, A, Profice, P, Insola, A, Mazzone, P, Tonali, P, Rothwell, JC (2000) Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol (Lond) 523: 503-513

Tyc, F, Boyadjian, A, Devanne, H (2005) Motor cortex plasticity induced by extensive training revealed by transcranial magnetic stimulation in human. Eur J Neurosci 21: 259-266

Wassermann, EM, McShane, LM, Hallett, M, Cohen, LG (1992) Noninvasive mapping of muscle representations in human motor cortex. Electroencephalogr Clin Neurophysiol 85: 1-8

Werhahn, KJ, Mortensen, J, Kaelin-Lang, A, Boroojerdi, B, Cohen, LG (2002) Cortical excitability changes induced by deafferentation of the contralateral hemisphere. Brain 125: 1402-1413

Ziemann, U, Muellbacher, W, Hallett, M, Cohen, LG (2001) Modulation of practice-dependent plasticity in human motor cortex. Brain 124: 1171-1181

CHAPTER 2: CHANGES IN CORTICOSPINAL EXCITABILITY EVOKED BY COMMON PERONEAL NERVE STIMULATION DEPEND ON STIMULATION FREQUENCY[1]

2.1 Introduction

Trauma to the spinal cord or brain disrupts circuits in the central nervous system (CNS) that control movement. Although some pathways may remain intact, there is a net reduction in activity in neural circuits controlling the affected muscles. Initially, reduced voluntary control after spinal (e.g., spinal cord injury; SCI) or cortical (e.g., stroke) trauma is a direct result of the original injury. However, the CNS has a strong capacity to adapt whereby plasticity in the organization and excitability of synaptic connections between the cortex and muscle occur based on a "use it or lose it" principle (Brasil-Neto et al., 1993; Elbert et al., 1997; Chen et al., 1999). Thus, the prolonged disuse that occurs after CNS trauma can lead to maladaptive CNS plasticity that exacerbates the initial functional impairments and can lead to secondary complications.

Plasticity in the CNS is not always maladaptive, but also can be beneficial, such as the expansion of cortical areas and increases in excitability of neural circuits that are associated with the acquisition of new motor skills (Pascual-Leone et al., 1993; Pascual-Leone et al., 1995; Classen et al., 1998; Butefisch et al., 2000; Ziemann et al., 2001). Such plasticity is thought to stem from the simultaneous activity in afferent and efferent pathways that occurs during

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repeated voluntary movements. Although different from voluntary movement, the sensory feedback generated during neuromuscular electrical stimulation (NMES) also induces plasticity in the CNS (Khaslavskaia, et al., 2002; Ridding, et al., 2000), particularly when combined with voluntary activation (Kido and Stein, 2004; Khaslavskaia and Sinkjaer, 2005; Barsi et al., 2008). Herein we refer to NMES as any prolonged, repetitive, electrical stimulation applied over muscle or peripheral nerve. NMES is often used after SCI or stroke to generate contractions to assist in performing functional movements (Liberson et al., 1961; Prochazka et al., 1997; Chae et al., 2008). From a rehabilitation perspective, it is interesting to note that improvements in function can persist after the stimulation is turned-off, and these improvements are thought to be mediated at least in part by plasticity in CNS circuits (Conforto et al., 2002; Hoffman and Field-Fote, 2007; Celnik et al., 2007).

NMES-induced plasticity in the CNS manifests as changes in synaptic organization and excitability. Such plasticity can be detected experimentally as changes in the size of motor evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS). Increases in MEP amplitude induced by NMES have been shown for a range of muscles and have been evoked using a variety of stimulation parameters (intensity, frequency, pulse width, pattern and duration). One approach has been to deliver NMES at intensities near motor threshold to activate primarily sensory axons (similar to NMES protocols known as "somatosensory stimulation"). For increasing the excitability of corticospinal (CS) pathways to hand muscles, NMES has typically been delivered at these low

intensities and at frequencies between 3 and 30 Hz (Ridding et al., 2000; McKay et al., 2002a, b; Pitcher et al., 2003). This type of stimulation is designed to "prime" CNS circuits and enhance sensory feedback for rehabilitation training sessions without producing large or functional muscle contractions (Hoffman and Field-Fote, 2007). Another approach is to deliver NMES at intensities above motor threshold to activate both sensory and motor fibers and generate functional contractions. This approach has been used to increase the excitability of the CS pathway to tibialis anterior (TA) by delivering NMES at intensities ranging from two times motor threshold (Khaslavskaia and Sinkjaer, 2005) to intensities that evoked an M-wave that was 50% of maximal (M_{max}) (Knash et al., 2003; Kido and Stein, 2004). While stimulating the leg at these intensities, frequencies have ranged from 25 Hz (Knash et al., 2003) to 200 Hz (Khaslavskaia et al., 2002). This type of NMES is intended to be delivered during rehabilitation sessions to generate and assist with functional movements. The advantages of this approach are that higher intensity stimulation will produce a larger afferent volley, the NMES may have beneficial effects at the level of the muscle, and the influences on the CNS are enhanced when NMES is combined with voluntary movements (Kido and Stein, 2004; Khaslavskaia and Sinkjaer, 2005; Barsi et al., 2008). Unfortunately, the wide range of NMES parameters and muscles tested between different studies makes it difficult to identify the optimal parameters for augmenting CS excitability.

The influence of NMES frequency on CS excitability is not yet well defined. Research with stimulation of the pharynx has explored a range of

combinations of stimulation intensity, frequency, and duration on changes in CS excitability for swallowing muscles. Excitability increased the most with stimulation applied at 75% of the maximum tolerated intensity, a frequency of 5 Hz, and a duration of 10 min, suggesting that NMES-driven cortical plasticity is dependent on stimulation parameters (Fraser et al., 2002) and the effect is most likely related to the strength of the afferent volley sent to the CNS. To our knowledge, only one study has investigated how changes in NMES frequency affect CS excitability for limb muscles. For the first dorsal interosseus (FDI) muscle of the hand, CS excitability was depressed by 3-Hz NMES and enhanced by 30-Hz NMES (Pitcher et al., 2003). To date, no studies have explored the effect of NMES frequency on CS excitability for muscles of the lower limb. We have shown previously that high frequency (100 Hz) NMES that increases the afferent volley to the spinal cord can enhance the central or "reflexive" contribution to electrically-evoked contractions of ankle musculature compared to NMES at lower frequencies (Collins et al., 2002; Klakowicz et al., 2006; Dean et al., 2007). The present experiments are based on the rationale that higher frequencies of stimulation would also enhance afferent input sent to the brain and thus be optimal for increasing CS excitability.

The purpose of this study was to quantify the effect of frequency of common peroneal (CP) nerve stimulation (10, 50, 100, 200 Hz) on CS excitability for TA. An additional goal was to characterize the time course of changes in CS excitability during the stimulation with a higher temporal resolution than in previous studies. The TA muscle was chosen because reduced function in the

ankle dorsiflexors is common following CNS trauma and NMES is often used for rehabilitation of that muscle (Liberson et al., 1961; Merletti et al., 1978; Chae et al., 2008). We delivered NMES in a 20 s on, 20 s off cycle for 40 min to evoke the repetitive type of movements that would be suitable for rehabilitation. In past studies CS excitability has been quantified in a minimum of 10- or 15-min increments (Khaslavskaia et al., 2002; McKay et al., 2002a; Knash et al., 2003). In the present study, changes in CS excitability during the stimulation were quantified in 2-min intervals. We hypothesized that higher frequencies of stimulation would increase TA MEPs to a greater extent than lower frequencies. MEPs evoked concurrently in soleus (Sol) and vastus medialis (VM) were analysed to determine whether changes in CS excitability were specific to the homonymous muscle (TA) or were more generalizable to heteronymous muscles. To discern whether changes in spinal excitability were induced by NMES, the ratios of maximal H-reflex (H_{max}) to M_{max} were determined for Sol and TA before and after stimulation. The results of this study provide insight into the optimal NMES frequency for increasing CS excitability for rehabilitation of impaired dorsiflexion after CNS injury.

2.2 Methods

2.2.1 Participants

Six men and 2 women ranging in age from 22-46 years with no known neurological disorders volunteered for this study. All subjects gave written, informed consent prior to testing. The experiments were conducted according to the Human Research Ethics Committee at the University of Alberta. Subjects

were seated with their backs and necks supported, and hip, knee, and ankle angles at ~110°, 100° and 90°, respectively. Padded restraints were secured around the right foot to minimize movement, and the left foot was placed on a foot rest. Subjects were instructed to not consume caffeine within 12 h prior to experimental sessions or during a session to eliminate the influence of caffeine on CNS excitability (Walton et al., 2003) and to refrain from intense physical activity within 12 h before the testing sessions.

2.2.2 Experimental procedure

All subjects participated in three 2- to -3 h testing sessions at least 48 h apart in which NMES frequencies of 10, 50 and 100 Hz were tested on different days. The order in which the different frequencies were tested was randomized for each subject. Six subjects returned, and two were unavailable, for a fourth session during which NMES was applied at 200 Hz. The time of day of each session was the same for each subject to reduce the potential confounding effect of diurnal changes in CNS excitability (Lagerquist et al., 2006; Tamm et al., 2009).

2.2.3 Electromyography (EMG)

Electromyography (EMG) was recorded from TA, Sol, and VM of the right leg using bipolar (2.25 cm^2) surface-recording electrodes (Vermed Medical, Bellow Falls, Vermont). EMG signals were pre-amplified (1,000x) and band pass filtered at 30-1,000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England). Data were sampled at 2,000 Hz for maximal voluntary isometric contractions and 5,000 Hz for all evoked potentials with a 12-bit A/D

converter (National Instruments, Austin, Texas). During the collection of MEPs, data were recorded in 450-ms sweeps from 100 ms before to 350 ms after stimulus delivery.

2.2.4 NMES

NMES was applied over the right CP nerve using bipolar (2.25 cm^2) surface electrodes (Vermed Medical Inc.) placed near the fibular head at the site that evoked a response (M-wave or H-reflex) in TA at the lowest stimulation intensity. Rectangular pulses of 1-ms duration were delivered from a Digitimer (DS7A, Hertfordshire, England) constant current stimulator at an intensity at which a single stimulus evoked an M-wave that was ~15% of M_{max} in TA. The stimulation was delivered for 40 min at either 10, 50, 100 or 200 Hz in a 20 s on, 20 s off cycle.

2.2.5 TMS

To test the excitability of the CS pathway, MEPs were evoked in the right TA using TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) applied using a figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota). All MEPs were evoked while subjects remained relaxed. MEPs were recorded before (control), during, and after each 40-min period of NMES, as depicted in Figure 2-1. The optimal stimulation site for right TA was found by moving the coil over the left motor cortex to find the site that elicited the largest amplitude MEP in TA at the lowest intensity of stimulation. Using a Brainsight image-guided stimulation system (Rogue Research, Montreal, Quebec) this site was recorded

and the coil was manually held in place to maintain position and orientation (precision: ± 3 mm) for all trials. MEP threshold for TA was then determined by finding the lowest intensity that produced MEPs of at least 50 uV in 4 out of 8 trials. The intensity of TMS was then set at 120% of this threshold for the remainder of the experiment. Six MEPs were recorded immediately before and after each 40 min bout of NMES at an inter-stimulus interval (ISI) that varied randomly between 5 and 8 s. To quantify the time course of any changes in CS excitability during the 40 min of NMES, 3 MEPs were evoked in each 20 s "off" period of the stimulation at an ISI of 8 s (see Figure 2-1).

2.2.6 M-wave/H-reflex (M/H) recruitment curves

To assess changes in spinal excitability, we calculated the ratio of H_{max}:M_{max} using data from M-wave/H-reflex (M/H) recruitment curves collected before and after the NMES from TA in all 8 subjects and Sol in 6 subjects (see Figure 2-1). The right CP and tibial nerves were stimulated (Digitimer, DS7A, Hertfordshire, England; 1 ms pulse width) using bipolar (2.25 cm^2) surface electrodes (Vermed Medical Inc.) placed near the fibular head and over the popliteal fossa, respectively. In many subjects, it was difficult to evoke consistent H-reflexes in the TA muscle at rest, therefore all M/H recruitment curves for TA were collected while subjects held a background contraction of 5% maximal EMG output using visual feedback of TA EMG low-pass filtered at 1 Hz. Sol M/H recruitment curves were collected at rest. Each recruitment curve was constructed from responses to 40 stimuli delivered with an ISI that varied randomly between 3 and 5 s. Stimulation intensity was varied pseudo-randomly

from below M-wave and H-reflex threshold to 1.5-2 times the minimum current required to evoke M_{max}.

2.2.7 Data analyses

MEPs recorded from TA were measured peak-to-peak and normalized to M_{max}. M_{max} was calculated as the largest M-wave in TA from each M/H recruitment curve. All MEP data (TA, Sol, VM) were visually inspected *post-hoc* and responses evoked when there was background EMG activity prior to the stimulation were removed from the analysis. MEPs were discarded if the EMG during the 1 s prior to the TMS stimuli exceeded two standard deviations of the average baseline signal recorded at rest before the stimulation. Of the 16,200 MEPs that were evoked from eight subjects, 86 MEPs (<1% of total responses) were removed from the analyses based on this criteria.

Changes in CS excitability during the 40 min of NMES were quantified by averaging MEPs over 2-min intervals. In this way, the 3 MEPs evoked in each of 3 successive "off" periods were averaged together (n=9). A two-way repeated measures analysis of variance (ANOVA) was used to compare the effect of different frequencies of NMES on the MEP amplitude for TA. Using the data available for all eight subjects, the factors for the ANOVA were "Frequency" (three levels: 10, 50, 100 Hz) and "Time" (22 levels: pre-NMES, post-NMES, each 2-min interval during NMES). The same analysis was used to compare changes in MEP amplitude for TA evoked by 100- and 200-Hz stimulation for the six subjects who received the NMES at 200 Hz, but with only two levels of "Frequency." Because our main interest was in the "Frequency x Time"

interaction, main effects of "Frequency" and "Time" are only reported when the interaction was not significant.

MEPs were also evoked in Sol and VM each time a TA MEP was elicited. Sol and VM MEPs were measured peak-to-peak as described above for TA. However, because we did not measure M_{max} in these muscles in all subjects, Sol and VM MEP measurements were not converted to a percentage of M_{max} and were left in mV. Because the amplitude of a MEP as measured in mV could differ between days due to changes at the recording site, comparisons between data collected on separate days (i.e., at different frequencies) were not appropriate for analyzing these data. Thus, separate one-way repeated measures ANOVAs with 22 levels of time were used to test the influence of each NMES frequency on MEPs recorded from Sol and VM. This analysis enabled us to evaluate the effect of time of stimulation, but not the effect of frequency, on changes in MEP amplitude for these heteronymous, non-stimulated muscles.

Our measure of spinal excitability was the ratio of H_{max}:M_{max}. H_{max} and M_{max} were measured from peak-to-peak, and their ratio was calculated from the average of the three largest H-reflexes and the single largest M-wave from each M/H recruitment curve for both TA and Sol muscles. A two-way repeated measures ANOVA with "Frequency" (3 levels: 10, 50, 100 Hz) and "Time" (2 levels: pre-NMES, post-NMES) as factors was used to analyze changes in H_{max}:M_{max} ratios. The same analysis was used to compare changes in H_{max}:M_{max} ratios evoked by 100- and 200-Hz NMES for the six subjects who participated in both sessions, but with only two levels of "Frequency."

For all tests, the significance level was set at $p<0.05$. *Post-hoc* analyses (Tukey HSD tests) were performed when appropriate. All descriptive statistics are reported as mean ± standard error.

2.3 Results

Significant increases in TA MEPs were induced by NMES delivered at 100 Hz but not 10, 50, or 200 Hz. MEPs became significantly larger than control (pre) values at the 24th min of 100-Hz NMES and remained elevated throughout and immediately following the 40-min stimulation period. Changes in MEPs of other, non-stimulated, leg muscles (Sol, VM) followed similar patterns as those observed for TA, but these changes were mostly not of statistical significance. There were no statistically significant changes in H_{max}:M_{max} ratios in either TA or Sol for any of the NMES protocols.

2.3.1 MEPs in TA

Figure 2-2 shows data recorded from one subject before, during, and after 40 min of NMES of the CP nerve at 10, 50, 100 and 200 Hz. *Panel a* shows MEPs recorded before and after each NMES protocol. In this individual MEPs increased by 20% after 40 min of 10-Hz NMES, decreased by 9% after NMES at 50 Hz, increased by 169% NMES at 100 Hz, and increased by 38% after 200-Hz NMES; however, changes in MEP amplitude for individual subjects were not tested for statistical significance. *Panel b* shows that MEPs evoked throughout 40 min of NMES at 100 Hz were consistently larger than those evoked during NMES at the other three frequencies.

The amplitude of MEPs averaged across all subjects (*n*=8) who received the 10-, 50-, and 100-Hz NMES are shown in Figure 2-3. The data in *Panel a* show that MEPs evoked during the 100-Hz NMES became larger than those evoked during NMES at the other frequencies approximately halfway through the stimulation. The ANOVA analyses identified a significant interaction between "Frequency" and "Time" [$F_{(42, 294)}$=2.25, p<0.01]. *Post-hoc* comparisons showed that 40 min of NMES at 100 Hz, but not 10 or 50 Hz, increased TA MEPs significantly from control. After the 100-Hz stimulation MEPs were significantly elevated by 101% while MEPs evoked after the 10-Hz (27 % increase) and 50-Hz (54 % increase) stimulation were not significantly different from control. The mean amplitude of MEPs recorded before and after each NMES protocol for each subject are shown in *Panel b*. The significant increases in MEP amplitude from control during NMES at 100 Hz began 24 min into the stimulation and MEPs remained elevated during and after the 100-Hz stimulation, as shown by the open triangles in *Panel a*. MEPs recorded before the stimulation (i.e., control) were not significantly different between frequencies. In contrast, starting 28 min into the stimulation, MEPs evoked during the 100-Hz NMES became significantly elevated from MEPs recorded at the same time point during NMES at 10 and 50 Hz as shown by the asterisks. There were no significant increases in TA MEPs from control at any time during or after the 10- or 50-Hz stimulation.

Mean data averaged over the 6 participants who received both 100- and 200-Hz NMES are shown in Figure 2-4. There was a significant interaction between "Frequency" and "Time" [$F_{(21, 105)}$=2.24, p<0.01]. *Post-hoc* comparisons

showed that 40 min of 200-Hz NMES did not significantly alter TA MEPs whereas 100-Hz NMES increased TA MEPs significantly from control. The open symbols in Figure 2-4a show that MEPs started to become significantly elevated from control at the 28th min of NMES. The asterisks in Figure 2-4a indicate significant differences between MEPs evoked at the same time point during 100- and 200-Hz NMES. Mean pre-NMES and post-NMES MEP amplitudes for each subject are shown in Figure 2-4b.

2.3.2 MEPs in Sol and VM

MEPs were also evoked in Sol and VM by the same stimulus that evoked MEPs in TA. There was no significant main effect of "Time" of stimulation for Sol MEPs recorded during 10-Hz [$F_{(21, 147)}$=1.22, p=0.24], 100-Hz [$F_{(21, 147)}$=1.05, p=0.41] or 200-Hz NMES [$F_{(21, 105)}$=0.60, p=0.91], but there was a significant main effect of "Time" during 50-Hz stimulation [$F_{(21, 147)}$=1.78, p=0.03]. *Post hoc* comparisons showed that Sol MEP amplitude was significantly elevated from control at the 38th and 40th min during the 50-Hz stimulation as denoted by the open symbols in Figure 2-5a but were not significantly elevated immediately after the NMES. There was no significant effect of "Time" of stimulation for VM MEP amplitude during 10-Hz [$F_{(21, 147)}$=1.51, p=0.08], 50-Hz [$F_{(21,147)}$=1.27, p=0.21], 100-Hz [$F_{(21, 147)}$=1.13, p=0.32] or 200-Hz [$F_{(21, 105)}$=1.09, p=0.37] NMES.

2.3.3 M/H recruitment curves

Stimulation applied at 10, 50, 100, and 200 Hz produced no significant changes in H_{max}:M_{max} ratio for TA or Sol. The 4 two-way repeated measures

ANOVAs used to analyse these data showed that all main effects and interaction effects had *p*-values greater than 0.2. Averaged across the four stimulation frequencies, H_{max}:M_{max} ratios for TA were 9 ± 4 before and 9 ± 5 after stimulation and for Sol were 55 ± 24 before and 50 ± 22 after stimulation.

2.4 Discussion

The present experiments were designed to test the hypothesis that higher frequencies of CP nerve stimulation would increase CS excitability for TA to a greater extent than lower frequencies of stimulation. Our main finding was that 100-Hz NMES applied over the CP nerve was more effective than 10-, 50-, and 200-Hz NMES at increasing CS excitability for TA. Changes in CS excitability for heteronymous non-stimulated muscles (Sol, VM) were mostly not of statistical significance, suggesting that the effect of NMES on CS excitability was strongest in the stimulated muscle. The H_{max}:M_{max} ratio in TA and Sol was not altered by NMES at any frequency, suggesting that there were no changes in spinal excitability for either muscle.

2.4.1 Frequency-dependent changes in CS excitability

Increases in CS excitability can be evoked by NMES and the electrically evoked afferent drive transmitted to the cortex is crucial for inducing these changes (Ridding et al., 2000, 2001; Khaslavskaia et al., 2002; McKay et al., 2002b; Knash et al., 2003; Kido and Stein 2004; Khaslavskaia and Sinkjaer, 2005). Stimulating the pharynx at a fairly high intensity Fraser and colleagues (2002) found that 5-Hz NMES was most effective for increasing CS excitability. Stimulating muscles of the hand at a fairly low intensity, 3-Hz NMES depressed

CS excitability and 30-Hz stimulation facilitated CS excitability (Pitcher et al., 2003). Despite the difference in stimulation intensity between these studies, they suggest that changes in CS excitability driven by NMES are dependent on stimulation frequency and that the relationship between NMES frequency and CS excitability changes may be different for different muscle groups. The present study is the first to investigate the relationship between NMES frequency and CS excitability for muscles of the lower limb. Consistent with our hypothesis, 100-Hz stimulation was more effective for increasing CS excitability than lower frequencies (10 and 50 Hz). CS excitability increased significantly (a twofold increase) following 100-Hz NMES, but the changes were smaller and not significant after 10- and 50-Hz NMES (27 and 54% increases, respectively). Contrary to our hypothesis, 200-Hz NMES was less effective than 100 Hz for increasing CS excitability and did not significantly alter MEP amplitudes from control. By the 28^{th} min of stimulation, CS excitability for TA during 100-Hz CP nerve stimulation was significantly higher than CS excitability for TA at the same time point of the 10-, 50- and 200-Hz stimulation protocols. As shown in Figs. 3b and 4b, some subjects had particularly robust increases in CS excitability following 100-Hz NMES while others did not. Similar inter-subject variability was found for the effect of NMES on CS excitability for hand muscles (Kaelin-Lang, et al., 2002), suggesting that the sensory volley evoked by NMES has a greater effect on CS excitability in some individuals than in others. Nevertheless, our data show a frequency-dependent effect of NMES for increasing CS excitability for TA and show that when NMES is delivered under the conditions

of the present study (i.e., stimulus intensity, pulse width, pattern and duration), 100-Hz stimulation was more effective than 10, 50, or 200 Hz. Whether this effect of frequency would remain for different combinations of stimulation parameters is presently unknown and is beyond the scope of the present study.

The observed changes in CS excitability may depend on the number of pulses delivered rather than the frequency of NMES. Longer periods of 10- and 50-Hz stimulation might produce similar changes in CS excitability to those observed during 100-Hz stimulation. However, if CS excitability depended only on the number of pulses it would have increased the most during our 200-Hz stimulation and this did not occur. When using NMES for rehabilitation, determining the number of pulses needed to evoke changes in CS excitability is less important than determining which NMES frequency induces the changes to the greatest extent and the fastest. Hence, our aim was to determine an effect of frequency on inducing CS excitability changes rather than exploring the effect of number of pulses.

Contrary to our results, past research has shown significant increases in CS excitability for limb muscles following frequencies of NMES $\leq$30 Hz. In the upper limb, ulnar nerve stimulation at 10 Hz increased CS excitability for the FDI and abductor digiti minimi muscles after 45 min of stimulation (Ridding et al., 2000; McKay et al., 2002a). However, this is outside the range of our 40-min stimulation protocol, stimulation intensities were lower than those used in the present study, and differences in cortical organization between the upper and lower limbs (Kurusu and Kitamura 1999) could influence how NMES affects CS

excitability. In the lower limb, the significant increases in CS excitability reported by Khaslavskaia, and Sinkjaer (2005) and Knash, and colleagues (2003) of 38 and 50%, respectively, following 25-30-Hz NMES are comparable to the non-significant changes in MEPs evoked by 10- and 50-Hz stimulation in the present study (27 and 54% increases). Hence, it is important to note that we do not propose that lower frequency NMES does not affect CS excitability, but rather that NMES at 100 Hz has a stronger effect on CS excitability than NMES at lower frequencies.

NMES at 200 Hz did not significantly increase CS excitability in the present study. The lack of change in CS excitability when NMES was applied at 200 Hz may be due to the hyperpolarization of sensory axons beneath the stimulating electrodes. Immediately after an action potential travels along a human axon, fluctuations in axonal excitability include a period of hyperpolarization (Burke et al., 2001). Prolonged trains of NMES with short ISIs, such as our 20 s trains of 200-Hz stimulation, result in deeper and longer periods of hyperpolarization (Burke et al., 2001). When axons are hyperpolarized, they will be more difficult to recruit with NMES, hence decreasing the afferent volley transmitted to the CNS. Khaslavskaia and colleagues (2002) used 200-Hz stimulation of the CP nerve and found significant increases in TA MEP amplitude; however, their stimulation was delivered for 20 ms (i.e., 5 pulses) once every second for 30 min, rather than the 20 s trains used in the present study. Hyperpolarization is maximal with a train of 10-20 impulses, and the delivery of more pulses lengthens the sub-normal excitability period, but if the stimulation

stops when the axon first reaches maximal hyperpolarization then resting state is re-established in ~100 ms (Burke et al., 2001). Thus, the pattern of stimulation utilized by Khaslavskaia and colleagues (2002) may have resulted in less hyperpolarization than the pattern used in this study. Nevertheless, our results suggest that prolonged stimulation trains delivered in an on-off cycle, similar to when NMES is used for rehabilitation, are more effective for increasing CS excitability when delivered at 100 Hz than at 10, 50, or 200 Hz.

2.4.2 Time course of CS excitability increases

CS excitability has been shown to remain elevated after 30 min of NMES in leg muscles (Khaslavskaia et al., 2002) and 45 min in arm muscles (McKay et al., 2002a). Although CP nerve stimulation transiently increased CS excitability for TA after just 10 min, the increase did not persist to the 20th min and only by the 30th min was a lasting increase observed (Knash et al., 2003). The present study charts the time course of MEP changes during NMES with a higher resolution (2 min) than has previously been documented and shows that sustained increases in CS excitability can occur after 24 min during NMES of the lower limb, somewhat earlier than the 30 min that has been reported previously. Although this time course provides no direct evidence for mechanisms underlying CS excitability changes evoked by NMES, it is consistent with past research suggesting that mechanisms such as long-term potentiation may play a role (Hess and Donoghue 1994; Butefisch et al., 2000; Kaelin-Lang et al., 2002; McKay et al., 2002a).

2.4.3 Generalizability of the effects of NMES

Stimulating the ulnar nerve in the upper limb, Ridding and colleagues (2000) found significant changes in cortical excitability in muscles innervated by the ulnar nerve, but not in a heteronymous non-stimulated muscle. Khaslavskaia and colleagues (2002) showed that NMES of the CP nerve increased TA MEP amplitude two-fold, but did not alter MEPs of the antagonist muscle (Sol). These observations suggest that CS excitability changes are specific to the muscles innervated by the stimulated nerve. Similarly, we observed changes in CS excitability that were primarily restricted to TA. Somewhat surprisingly, 50-Hz NMES did not induce significant changes in TA MEPs, but increased MEPs in Sol at the 38^{th} and 40^{th} min (but not immediately thereafter). Research exploring the effect of a single electrical pulse to the tibial nerve shows that MEPs were altered in Sol and TA (the heteronymous muscle) and thus supports a more global effect of afferent input on CS excitability (Roy and Gorassini, 2008). Thus, NMES applied to the leg may have an effect on CS excitability for heteronymous muscles, but our data suggests that the effects are not as strong as for the stimulated muscles. This study and past studies have evaluated CS excitability changes in non-stimulated muscles by measuring MEP responses of these muscles when evoked by TMS at the optimal location for the stimulated muscle, rather than the optimal location for the heteronymous muscles. If there are smaller effects of NMES on CS excitability for heteronymous muscles, then perhaps more specific measures are necessary to detect these changes. Further research focusing

specifically on the effects of NMES on CS excitability for non-stimulated muscles might clarify the extent to which such changes occur.

2.4.4 Location of excitability changes

Increases in TMS-evoked MEPs could be a result of changes in neural excitability in the brain or spinal cord. Because of their different sites of activation, transcranial electrical stimulation (TES), brainstem electrical stimulation (BES) and F-wave measures have been utilized to differentiate the location of the excitability changes. Many studies show increases in TMS-evoked MEPs following NMES without concomitant changes in TES or BES-evoked MEPs, or F-wave amplitude, and therefore conclude that excitability changes occur in the cortex and not in the spinal cord (Stefan et al., 2000; Ridding et al., 2000; Stefan et al., 2002). Conversely, Khaslavskaia and colleagues (2002) show changes in TMS-evoked MEPs and smaller changes in TES-evoked MEPs following CP nerve stimulation, suggesting that excitability changes do occur in the spinal cord. Nevertheless, the same motor neurons may not be activated by TMS as are activated by TES, BES and F-waves, and therefore these data should be interpreted with caution. We measured spinal excitability for TA and Sol with the ratio of H_{max}:M_{max}, whereby increases in the ratio suggest increases in spinal excitability. There was no change in this ratio for either TA or Sol from before to after stimulation, suggesting that changes in MEPs were of cortical origin.

2.4.5 Implications

NMES is commonly used to treat impaired ankle dorsiflexion that often develops after CNS trauma (Liberson et al., 1961; Merletti et al., 1978; Chae et al., 2008). In addition to the obvious improvements in dorsiflexion that occur during the stimulation, it became evident early on that benefits can persist even after the stimulation was turned-off (Liberson et al., 1961). We now know that these persistent benefits include improvements in walking speed (Ladouceur and Barbeau, 2000), reduced spasticity (Stefanovska et al., 1989) and increased dorsiflexor strength (Merletti et al., 1978) and these occur concomitantly with increased CS excitability (Knash et al., 2003; Kido and Stein, 2004). Persistent increases in hand muscle strength have been induced by peripheral nerve stimulation applied at a low intensity (no visible twitch) to preferentially activate sensory fibers (Conforto, et al., 2002). Studies using similar electrical stimulation protocols to Conforto and colleagues have found increases in CS excitability (Ridding, et al., 2000; Mckay, et al., 2002a, b; Pitcher, et al., 2003), suggesting that the functional improvements in the upper limb (Conforto, et al., 2002) also coincide with increases in CS excitability. Moreover, increases in MEPs of the biceps muscle by combined motor practice and increased sensory input have been associated with improvements in elbow flexion (Ziemann et al., 2001) and cortical plasticity evoked during motor skill acquisition has been related to marked improvements for skill performance and further skill learning (Pascual-Leone et al., 1995). These results suggest that increased CS excitability is involved in lasting functional improvements. If enhancing CS excitability leads to

functional improvements, then development of methods to further enhance CS excitability will be important for maximizing the efficacy of NMES therapies. However, inter-subject variability in the responses to NMES suggests that increases in CS excitability induced by NMES, and any associated benefits for rehabilitation, may be greater in some individuals than others. Nonetheless, our data show that NMES applied at 100 Hz is more effective than NMES at 10, 50 and 200 Hz for increasing CS excitability of the dorsiflexors.

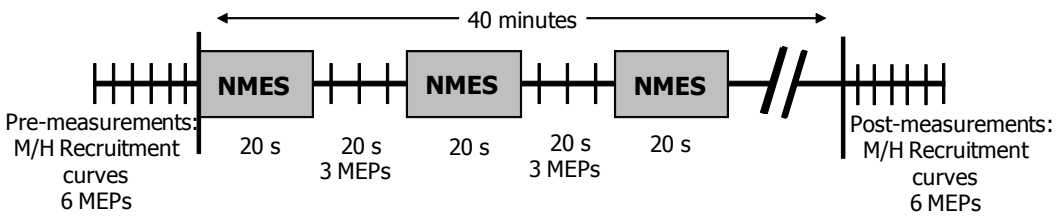

Figure 2-1 Schematic of timeline for one experimental session. NMES was delivered at 10, 50, 100, or 200 Hz over the CP nerve on separate days. Each *vertical line* represents the timing of delivery of the TMS. MEPs were evoked by TMS delivered at 120% of resting MEP threshold determined before each 40-min period of NMES.

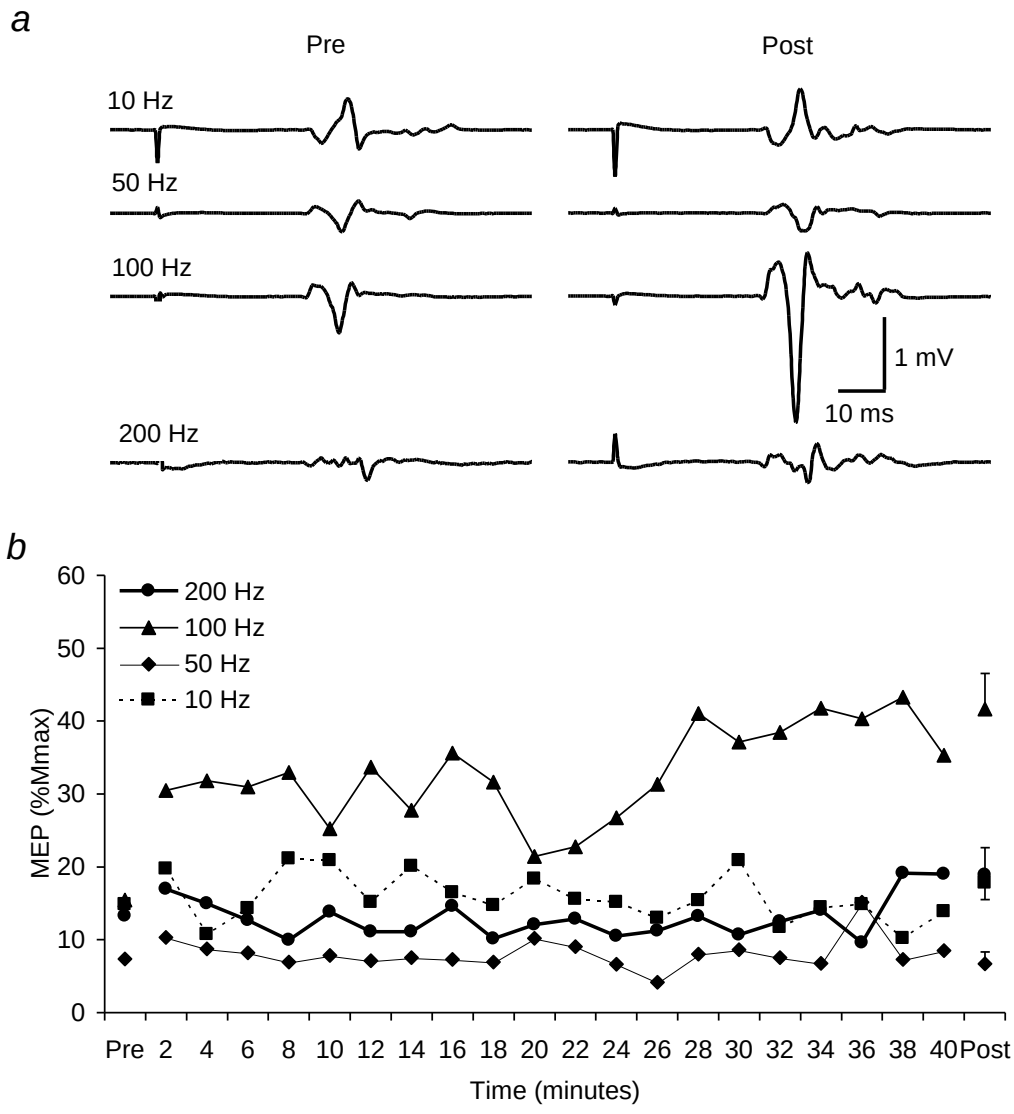

Figure 2-2 Changes in TA MEP amplitude induced by NMES delivered at four frequencies in a single subject. *Panel a* shows the mean waveforms of MEPs (*n*=6) evoked before (*control*; left panel) and after (*post*; right panel) each frequency. *Panel b* shows the mean amplitude of MEPs recorded before (*pre*), during (2-40) and after (*post*) 40 min of NMES at each frequency. Data collected during the stimulation are an average of 9 MEPs. *Error bars* represent one standard error.

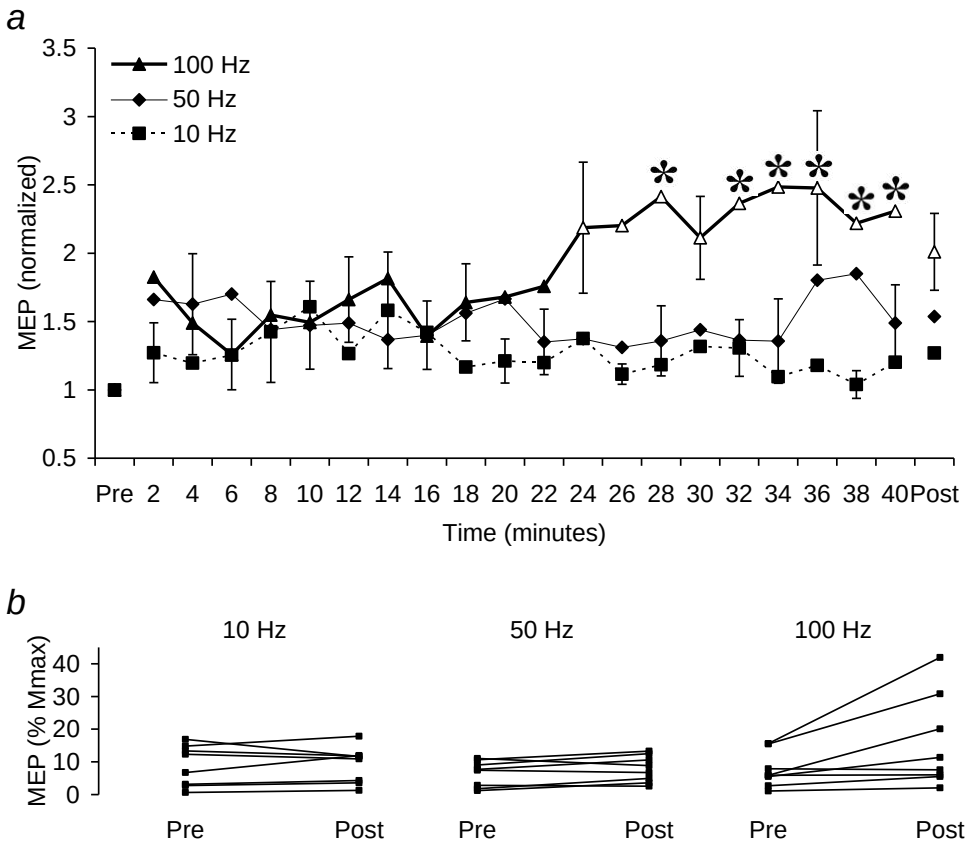

Figure 2-3 Amplitude of MEPs recorded from TA averaged across the group (*n*=8) before (*pre*), during (2-40) and after (*post*) 10-, 50- and 100-Hz NMES. All data were normalised to control MEP amplitude recorded before the NMES. *Open data symbols* in *Panel a* indicate significant differences from control (*pre*). *Asterisks* indicate significant differences from MEPs recorded at the same time point during 10- and 50-Hz NMES. *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than all three data points, at each time point. *Panel b* shows mean MEP amplitude recorded before (*pre*) and after (*post*) each stimulation frequency for each subject.

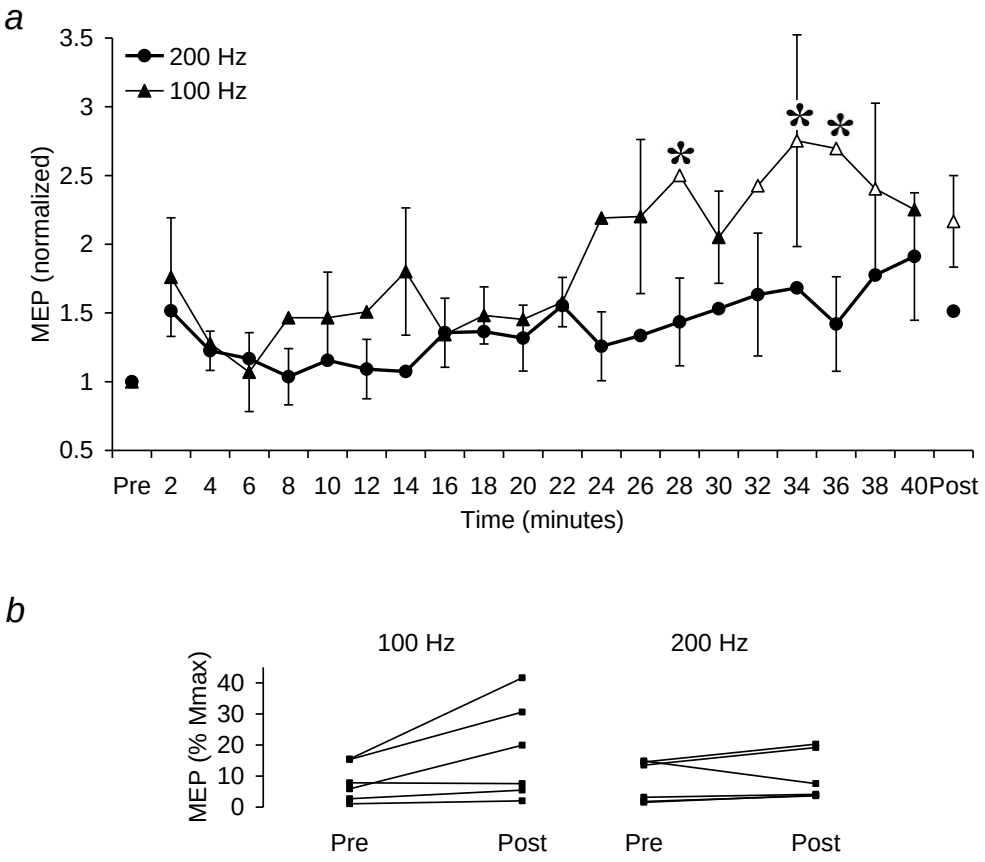

Figure 2-4 Amplitude of MEPs recorded from TA averaged across the group (*n*=6) before (*pre*), during (2-40) and after (*post*) 100- and 200-Hz NMES. All data were normalised to MEP amplitude recorded before the NMES. *Open data symbols* in *Panel a* indicate significant differences from control (*pre*). *Asterisks* indicate significant differences from MEPs recorded at the same time point during 200-Hz NMES. *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than both data points, at each time point. *Panel b* shows mean MEP amplitude recorded before (*pre*) and after (*post*) stimulation at 100 and 200 Hz for each subject.

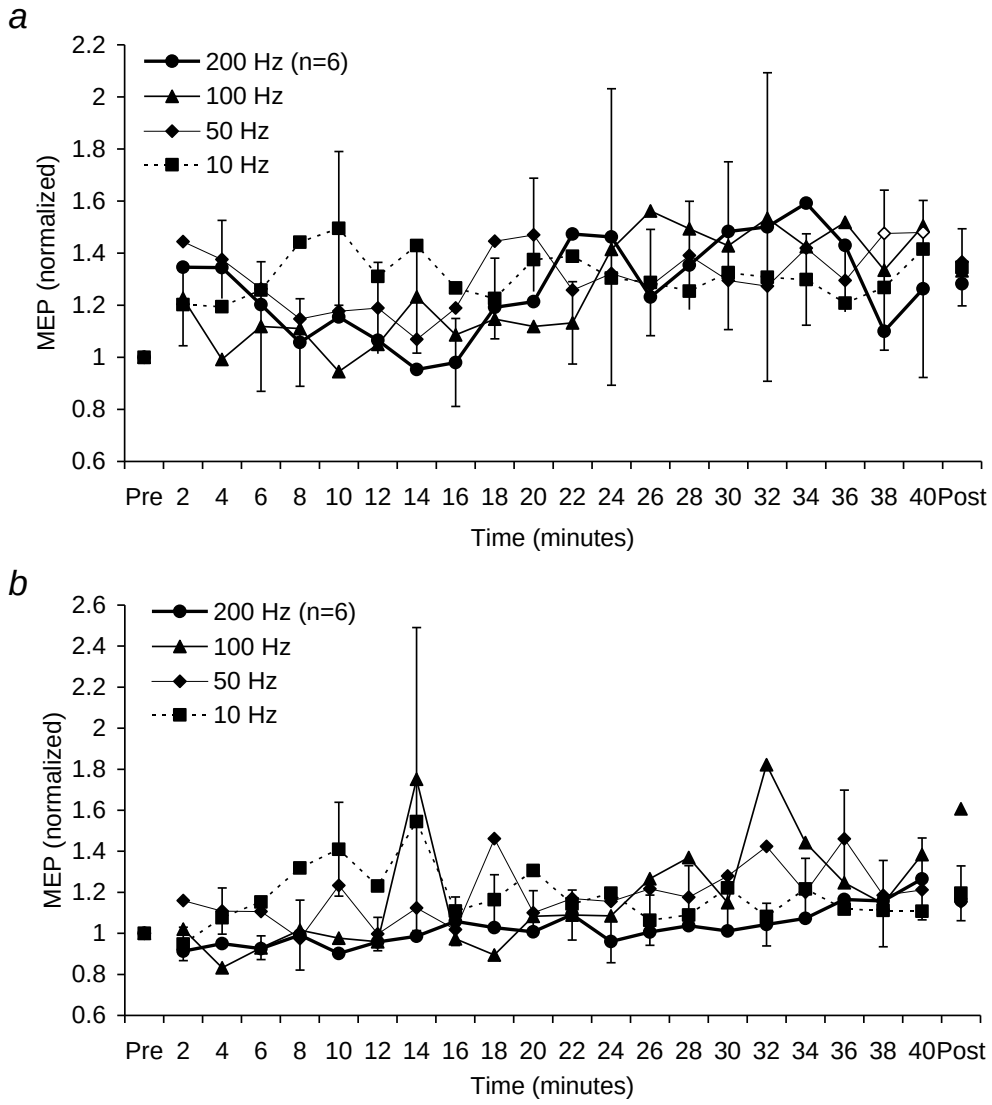

Figure 2-5 Time course of changes in MEP amplitude for Sol (*Panel a*) and VM (*Panel b*) averaged across the group during 10-, 50-, 100-, and 200-Hz NMES. *Open data points* indicate significant differences from control (*pre*). *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than all four data points, at each time point.

2.5 References

Barsi GI, Popovic DB, Tarkka IM, Sinkjaer T, Grey MJ (2008) Cortical excitability changes following grasping exercise augmented with electrical stimulation. Exp Brain Res 191 (1): 57-66

Brasil-Neto JP, Valls-Sole J, Pascual-Leone A, Cammarota A, Amassian VE, Cracco R, Maccabee P, Cracco J, Hallett M, Cohen LG (1993) Rapid modulation of human cortical motor outputs following ischaemic nerve block. Brain 116 (Pt 3): 511-525

Burke D, Kiernan MC, Bostock H (2001) Excitability of human axons. Clin Neurophysiol 112: 1575-1585

Butefisch CM, Davis BC, Wise SP, Sawaki L, Kopylev L, Classen J, Cohen LG (2000) Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci USA 97: 3661-3665

Celnik P, Hummel F, Harris-Love M, Wolk R, Cohen LG (2007) Somatosensory stimulation enhances the effects of training functional hand tasks in patients with chronic stroke. Arch Phys Med Rehabil 88: 1369-1376

Chae J, Sheffler L, Knutson J (2008) Neuromuscular electrical stimulation for motor restoration in hemiplegia. Top Stroke Rehabil 15: 412-426

Chen R, Corwell B, Hallett M (1999) Modulation of motor cortex excitability by median nerve and digit stimulation. Exp Brain Res 129: 77-86

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG (1998) Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol 79: 1117-1123

Collins DF, Burke D, Gandevia SC (2002) Sustained contractions produced by plateau-like behaviour in human motoneurones. J Physiol 538: 289-301

Conforto AB, Kaelin-Lang A, Cohen LG (2002) Increase in hand muscle strength of stroke patients after somatosensory stimulation. Ann Neurol 51: 122-125

Dean JC, Yates LM, Collins DF (2007) Turning on the central contribution to contractions evoked by neuromuscular electrical stimulation. J Appl Physiol 103 (1): 170-6

Elbert T, Sterr A, Flor H, Rockstroh B, Knecht S, Pantev C, Wienbruch C, Taub E (1997) Input-increase and input-decrease types of cortical reorganization after upper extremity amputation in humans. Exp Brain Res 117: 161-164

Fraser C, Power M, Hamdy S, Rothwell J, Hobday D, Hollander I, Tyrell P, Hobson A, Williams S, Thompson D (2002) Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. Neuron 34: 831-840

Hess G, Donoghue JP (1994) Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol 71: 2543-2547

Hoffman LR, Field-Fote EC (2007) Cortical reorganization following bimanual training and somatosensory stimulation in cervical spinal cord injury: a case report. Phys Ther 87: 208-223

Kaelin-Lang A, Luft AR, Sawaki L, Burstein AH, Sohn YH, Cohen LG (2002) Modulation of human corticomotor excitability by somatosensory input. J Physiol 540: 623-633

Khaslavskaia S, Ladouceur M, Sinkjaer T (2002) Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve. Exp Brain Res 145: 309-315

Khaslavskaia S, Sinkjaer T (2005) Motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve depends on the voluntary drive. Exp Brain Res 162: 497-502

Kido TA, Stein RB (2004) Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. Exp Brain Res 159: 491-500

Klakowicz PM, Baldwin ER, Collins DF (2006) Contribution of m-waves and h-reflexes to contractions evoked by tetanic nerve stimulation in humans. J Neurophysiol 96: 1293-1302

Knash ME, Kido A, Gorassini M, Chan KM, Stein RB (2003) Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. Exp Brain Res 153: 366-377

Kurusu K, Kitamura J (1999) Long-latency reflexes in contracted hand and foot muscles and their relations to somatosensory evoked potentials and transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol 110: 2014-2019

Ladouceur M, Barbeau H (2000) Functional electrical stimulation-assisted walking for persons with incomplete spinal injuries: longitudinal changes in maximal overground walking speed. Scand J Rehabil Med 32: 28-36

Lagerquist O, Zehr EP, Baldwin ER, Klakowicz PM, Collins DF (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170: 1-6

Liberson WT, Holmquest HJ, Scot D, Dow M (1961) Functional electrotherapy: stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. Arch Phys Med Rehabil 42: 101-105

McKay D, Brooker R, Giacomin P, Ridding M, Miles T (2002a) Time course of induction of increased human motor cortex excitability by nerve stimulation. NeuroReport 13: 1271-1273

McKay DR, Ridding MC, Thompson PD, Miles TS (2002b) Induction of persistent changes in the organisation of the human motor cortex. Exp Brain Res 143: 342-349

Merletti R, Zelaschi F, Latella D, Galli M, Angeli S, Sessa MB (1978) A control study of muscle force recovery in hemiparetic patients during treatment with functional electrical stimulation. Scand J Rehabil Med 10: 147-154

Pascual-Leone A, Cammarota A, Wassermann EM, Brasil-Neto JP, Cohen LG, Hallett M (1993) Modulation of motor cortical outputs to the reading hand of braille readers. Ann Neurol 34: 33-37

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M (1995) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 74: 1037-1045

Pitcher JB, Ridding MC, Miles TS (2003) Frequency-dependent, bi-directional plasticity in motor cortex of human adults. Clin Neurophysiol 114: 1265-1271

Prochazka A, Gauthier M, Wieler M, Kenwell Z (1997) The bionic glove: an electrical stimulator garment that provides controlled grasp and hand opening in quadriplegia. Arch Phys Med Rehabil 78: 608-614

Ridding MC, Brouwer B, Miles TS, Pitcher JB, Thompson PD (2000) Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 131: 135-143

Ridding MC, McKay DR, Thompson PD, Miles TS (2001) Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 112: 1461-1469

Roy FD, Gorassini MA (2008) Peripheral sensory activation of cortical circuits in the leg motor cortex of man. J Physiol 586 (Pt 17): 4091-105

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543: 699-708

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123 Pt 3: 572-584

Stefanovska A, Vodovnik L, Gros N, Rebersek S, Acimovic-Janezic R (1989) FES and spasticity. IEEE Trans Biomed Eng 36: 738-745

Tamm AS, Lagerquist O, Ley AL, Collins DF (2009) Chronotype influences diurnal variations in the excitability of the human motor cortex and the ability to generate torque during a maximum voluntary contraction. J Biol Rhythms 24: 211-224

Walton C, Kalmar J, Cafarelli E (2003) Caffeine increases spinal excitability in humans. Muscle Nerve 28: 359-364

Ziemann U, Muellbacher W, Hallett M, Cohen LG (2001) Modulation of practice-dependent plasticity in human motor cortex. Brain 124: 1171-1181

CHAPTER 3: NEUROMUSCULAR ELECTRICAL STIMULATION HAS A GLOBAL EFFECT ON CORTICOSPINAL EXCITABILITY FOR LEG MUSCLES IN CONTRAST TO A MORE FOCUSED EFFECT FOR HAND MUSCLES[2]

3.1 Introduction

Neuromuscular electrical stimulation (NMES) is a common rehabilitation tool used to help restore movement following damage to central motor pathways (Baker et al., 2000). Functional improvements following NMES applied to leg and hand muscles occur concomitantly with increased corticospinal (CS) excitability and are driven by the electrically-evoked afferent volley; however, CS excitability may be affected differently for leg and hand muscles. CS excitability increased for the leg muscle innervated by the stimulated nerve (the *target* muscle) and for leg muscles not innervated by the stimulated nerve (*non-target* muscles), suggesting that there may be a "global" effect of NMES on CS excitability for leg muscles (Kido and Stein, 2004; Mang et al., 2010). However, other studies have shown CS excitability changes in the *target* leg muscle only (Khaslavskaia et al., 2002; Knash et al., 2003). In contrast, NMES of hand muscles had a "focused" effect on CS excitability, whereby excitability increases have only been shown for the *target* muscle (Ridding et al., 2000; 2001; Mckay et al., 2002; Pitcher et al., 2003). Additionally, NMES may increase CS excitability more for the *target* muscle of the hand compared to the leg. Such a limb-dependent effect of NMES on CS excitability may reflect differences in afferent

[2] The contributing authors to the work presented in this chapter were: Mang CS, Clair JM, and Collins DF.

projections to the central nervous system (CNS) (Kurusu and Kitamura, 1999) or differences between limbs in mechanisms underlying CNS plasticity; however, inconsistencies in NMES parameters between studies make it difficult to draw such conclusions. The present experiments were designed to discern whether apparent differences in the effect of NMES on CS excitability between the leg and hand are due to physiological differences in how the electrically-evoked afferent volley during NMES affects CS excitability or are simply due to differing methodology between studies.

NMES-induced increases in CS excitability for muscles of the leg and hand have not yet been compared in the same study. Typically, when studying leg muscles, NMES is delivered at relatively high intensities that generate M-waves up to 50% of the maximal M-wave (M_{max}), frequencies up to 200 Hz, a range of different patterns, and durations of ~30-45 min. This intensity of stimulation generates functional contractions and thus is similar to stimulation protocols often termed functional electrical stimulation (FES) (Sheffler and Chae, 2007). In contrast, when investigating the effect of NMES on CS excitability for hand muscles, NMES is typically delivered with low intensities (near motor threshold), low frequencies (10-30 Hz), patterns involving short bursts of stimulation (ie, 500 ms on – 500 ms off), and long durations (2 hr). Such low intensity stimulation is similar to NMES protocols often termed somatosensory stimulation (SS), as it is designed to activate primarily sensory axons to prime the CNS before rehabilitation training without generating large or functional muscle contractions (Hoffman and Field-Fote, 2007).

Generally, increases in stimulation intensity and frequency enhance the afferent drive generated by NMES and thus, one might expect FES-like NMES to increase CS excitability more than SS-like NMES. Despite the use of FES-like NMES when stimulating muscles of the leg as compared to SS-like NMES for the hand, CS excitability increased similarly (range: ~40-100% increase) for the *target* muscle; therefore, it may be predicted that NMES would increase CS excitability more for a hand muscle compared to a leg muscle if parameters were the same. Moreover, NMES applied to the leg appears to have a somewhat global effect on CS excitability for leg muscles, versus a more focused effect for hand muscles. For example, common peroneal (CP) nerve stimulation in the leg significantly increased CS excitability for both the *target* muscle, tibialis anterior (TA), and a *non-target* muscle, soleus (Sol) by ~50% (Kido and Stein, 2004; Mang et al., 2010). In contrast, stimulation of the ulnar nerve in the hand increased CS excitability for only the *target* muscle, first dorsal interosseus (FDI), and not for *non-target* muscles in close proximity (Ridding et al., 2000; Ridding et al., 2001; McKay et al., 2002; Pitcher et al., 2003). Presently, it is unclear whether these differing results are due to physiological differences in neural circuits that control the leg and hand or are due to methodological differences in the stimulation parameters used between studies.

The purpose of this study was twofold. First, to determine whether there are physiological differences in the central effect of NMES for leg and hand muscles, we investigated the effect of FES-like NMES of the CP nerve (leg) and the median nerve (hand) on CS excitability for *target* and *non-target* muscles. The

CP and median nerves were chosen because they have been studied previously and are commonly stimulated for rehabilitation of dorsiflexion and grasping, respectively. We hypothesized that median nerve stimulation would increase motor evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS) over the motor cortex for the *target* muscle more than CP nerve stimulation. We also hypothesized that CP nerve stimulation would increase MEPs for *non-target* muscles more than median nerve stimulation, consistent with a more global effect of NMES on CS excitability for leg muscles versus a more focused effect for hand muscles. Secondly, to assess the influence of different stimulation parameters on CS excitability for hand muscles, we compared CS excitability increases following FES-like and SS-like NMES of the median nerve. We hypothesized that the larger afferent volley generated by FES-like NMES would induce larger increases in MEPs in the *target* muscle than the SS-like NMES. We also hypothesized that the FES-like NMES would have a larger effect in *non-target* muscles compared to SS-like NMES. The results of this study contribute to the understanding of how to apply NMES to increase CS excitability for muscles of the leg and hand.

3.2 Methods

3.2.1 Participants

Eight men and 2 women ranging in age from 20 to 47 years old with no known neurological disorders participated in this study. All participants gave written, informed consent prior to testing. The experiments were conducted according to the Human Research Ethics Board at the University of Alberta.

Subjects were seated with their backs and necks supported. When NMES was applied over the CP nerve, the hip, knee, and ankle angles were maintained at ~110°, 100° and 90°, respectively. Padded restraints were secured around the right foot to minimize movement and the left foot was placed on a foot rest. When NMES was applied over the median nerve, the shoulder, elbow, and wrist angles were maintained at ~15°, 120° and 180°, respectively. The elbow was supported by an arm support and the hand was rested on a hand rest in a relaxed position. Subjects were instructed to not consume caffeine within 12 h prior to experimental sessions or during a session to eliminate the influence of caffeine on CNS excitability (Walton et al., 2003) and to refrain from intense physical activity within 12 h before the testing sessions.

3.2.2 Experimental procedure

All subjects participated in 3 separate ~3 h testing sessions at least 48 h apart in which NMES was applied to the CP nerve to activate TA in the leg on one occasion and the median nerve to activate abductor pollicis brevis (APB) in the hand on two occasions. The order of testing sessions was randomized for each participant. The time of day of each session was the same for each subject to reduce the potential confounding effect of diurnal changes in CNS excitability (Lagerquist et al., 2006; Tamm et al., 2009).

3.2.3 Electromyography (EMG)

When NMES was applied to the CP nerve, electromyography (EMG) was recorded from TA, Sol and vastus medialis (VM) of the right leg. EMG was

recorded from APB, FDI, and extensor carpi ulnaris (ECU) of the right hand in experimental sessions when NMES was applied to the median nerve (see Figure 3-1). All EMG was recorded using bipolar (2.25 cm^2) surface recording electrodes (Vermed Medical, Bellow Falls, Vermont) with the exception of EMG from FDI, which was recorded with electrodes trimmed down to ~1cm^2. EMG signals were pre-amplified (1,000x) and band-pass filtered at 10-1,000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England). Data were sampled at 2,000 Hz for all evoked potentials with a 12-bit A/D converter (National Instruments, Austin, Texas). During the collection of MEPs, data were recorded in 450 ms sweeps from 100 ms before to 350 ms after stimulus delivery.

3.2.4 NMES

NMES was applied over the right CP nerve near the fibular head or the right median nerve at the wrist using bipolar (3.2 cm) round neurostimulation electrodes (Axelgaard manufacturing co., ltd.) placed at the site that evoked a response (M-wave or H-reflex) at the lowest stimulation intensity in TA and APB, respectively. With these stimulation sites TA and APB were considered to be the *target* muscles because they are innervated by the stimulated nerves. Sol and FDI were considered to be *non-target* muscles *adjacent* to the *target* muscles, and VM and ECU were considered to be *non-target* muscles *remote* to the NMES applied to the CP and median nerves, respectively (see Figure 3-1). Rectangular pulses of 1 ms duration were delivered from a Digitimer (DS7A, Hertfordshire, England) constant current stimulator for all experimental sessions. Participants were

instructed to relax while NMES was applied to minimize the effect of voluntary contraction on CS excitability.

3.2.4.1 FES-like stimulation

For the session involving CP nerve stimulation and one session involving median nerve stimulation NMES was applied at an intensity at which a single stimulus evoked an M-wave that was ~15% M_{max} in TA or APB, respectively. The stimulation frequency was 100 Hz and was delivered for 40 min in a 20 s on, 20 s off cycle (adapted from Mang et al., 2010; see Figure 3-2).

3.2.4.2 SS-like stimulation

For the other experimental session involving median nerve stimulation, the NMES was applied at an intensity that just evoked a small but visible twitch in APB (~3-5% M_{max}) and a frequency of 10 Hz. The stimulation was applied for a total duration of 2 hrs in a 500 ms on, 500 ms off cycle (adapted from Ridding et al., 2000; see Figure 3-2).

3.2.5 TMS

To test the excitability of the CS pathway, MEPs were evoked using TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) applied with a parabolic or figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota). The parabolic coil was used when there were difficulties evoking MEPs in leg muscles with the figure-of-eight coil (n=6). For a given subject, the same coil was used in all sessions. All MEPs were evoked while subjects were relaxed. In the experimental session when the right CP nerve was stimulated, MEPs were evoked from the optimal stimulation sites ("hotspots") for right TA, Sol, and VM. In the

experimental sessions when the right median nerve was stimulated, MEPs were evoked from the hotspots for right APB, FDI, and ECU. The hotspots were found by moving the coil over the left motor cortex to find the site that elicited the largest amplitude MEP in the muscle of interest at the lowest intensity of stimulation before the application of NMES. Using a Brainsight image-guided stimulation system (Rogue Research, Montreal, Quebec) the sites were recorded and the coil was manually held in place to maintain position and orientation (precision: ± 3 mm) during measurement trials. Resting MEP thresholds were then determined by finding the lowest intensity that produced MEPs of at least 50 uV in 4 out of 8 trials. The intensity of TMS was then set at 120% of the threshold for each muscle for the measurements. Ten MEPs were evoked from each hotspot and recorded from each muscle immediately before (control) and after the prolonged period of NMES in each session at an inter-stimulus interval that varied randomly between 4-6 s. The order of muscles tested was randomized before and after the NMES for each session.

3.2.6 M_{max}

Stimulation intensity was varied pseudorandomly from below motor threshold to 1.5-2 times the minimum current required to evoke M_{max} over 5 stimuli. M_{max} was calculated as the largest M-wave evoked in the *target* muscle. The amplitude of M_{max} from the *target* muscles were tested on 6 occasions evenly spaced throughout the experimental sessions. Occasionally the amplitude of M_{max} would be reduced in APB following the NMES, likely due to a loss of adhesion of electrodes to the skin over time. In these instances the recording electrodes were

replaced and M_{max} was re-collected. In some instances there were still small variations in M_{max} despite replacement of the electrodes. Thus, all MEPs were normalized to the M_{max} taken nearest to the collection of the MEPs.

3.2.7 Data analyses

Changes in CS excitability induced by NMES were determined by quantifying and comparing the averages of the 10 MEPs evoked before and after NMES. MEPs recorded from TA and APB were measured peak-to-peak and normalized to M_{max}. As we did not measure M_{max} in the other muscles (Sol, VM, FDI, and ECU) these MEP measurements were not converted to a percentage of M_{max} and were left in mV. All MEP data (TA, Sol, VM, and APB, FDI, ECU) were visually inspected *post-hoc* and responses evoked when there was background EMG activity prior to the stimulation were removed from the analysis. MEPs were discarded if the EMG during the 1 s prior to the TMS stimuli exceeded 2 standard deviations of the average baseline signal recorded at rest before the stimulation.

Two-tailed paired *t-tests* were used to test whether there was a significant change in MEP amplitude from before to after the NMES protocol for each muscle. A two-way repeated measures analysis of variance (ANOVA) was used to compare the percent change in MEP amplitude for each muscle following the FES-like NMES applied to the leg and hand. Using the data available for all 10 subjects, the factors were Limb (2 levels: leg and hand) and Muscle (3 levels: *target, adjacent,* and *remote*). Another two-way repeated measures ANOVA was used to compare the percent change in MEP amplitude for each muscle following

the SS-like and FES-like NMES protocols applied to the hand. For this ANOVA the factors were NMES protocol (2 levels: SS-like and FES-like) and Muscle (3 levels: *target*, *adjacent*, and *remote*). For all tests the significance level was set at $p<0.05$. *Post-hoc* analyses (Tukey HSD tests) were performed when appropriate. All descriptive statistics are reported as mean ± standard error.

3.3 Results

FES-like CP nerve stimulation significantly increased MEPs for the *target* (TA), *adjacent* (Sol), and *remote* (VM) muscles. In contrast, MEPs significantly increased for only the *target* (APB), but not other muscles (FDI and ECU) following FES-like median nerve stimulation. The amplitude of change in MEPs between muscles of the leg and hand following FES-like NMES were not significantly different for any muscle (*target*, *adjacent*, and *remote*). SS-like median nerve stimulation did not change MEPs for any of the tested muscles (APB, FDI, ECU). Likewise, there was no difference between the amplitude of change in MEPs for any muscle of the hand when NMES was applied with FES-like and SS-like parameters.

3.3.1 Changes in MEPs induced by FES-like NMES of muscles of the leg and hand

Panels a and *b* of Figure 3-3 show mean MEP waveforms (n=10) recorded from one subject before and after FES-like NMES applied to the CP and median nerve. MEPs are shown for the *target* muscle (TA or APB), the *adjacent* muscle (Sol or FDI), and the *remote* muscle (VM or ECU). After FES-like CP nerve stimulation MEPs increased by 69% for the *target* muscle (TA), 98% for the

adjacent muscle (Sol), and 2% for the *remote* muscle (VM) in this individual. After FES-like median nerve stimulation, MEPs for this individual increased by 81% for the *target* muscle (APB), 15% for the *adjacent* muscle (FDI), and decreased by 9% for the *remote* muscle (ECU). In this individual there appeared to be a larger increase in MEPs for the *target* muscle in the hand than the leg, but a larger increase in MEPs for the *adjacent* muscle in the leg (Sol) than for the hand (FDI). MEPs for the *remote* muscle in both the leg and hand did not change markedly in this individual. Changes in MEP amplitude were not tested for statistical significance in individual subjects.

The percent change in MEP amplitude averaged across the group following FES-like NMES applied to the CP and median nerves are shown in Figure 3-4a. MEP amplitude before and after CP nerve stimulation increased significantly for the *target* muscle (TA) [$t_{(9)}$=-2.36, p=0.043], the *adjacent* muscle (Sol) [$t_{(9)}$=-2.29, p=0.047], and the *remote* muscle (VM) [$t_{(9)}$=-2.26, p=0.049]. In contrast, FES-like median nerve stimulation significantly increased MEPs for the *target* muscle (APB) [$t_{(9)}$=-2.78, p=0.021], but not for the *adjacent* muscle (FDI) [$t_{(9)}$=0.38, p=0.715] and the *remote* muscle (ECU) [$t_{(9)}$=-1.14, p=0.283]. The ANOVA analyses revealed no main effects of "Limb" [$F_{(1,9)}$=0.005, p=0.95] or "Muscle" [$F_{(1,9)}$=1.39, p=0.27] and no interaction [$F_{(2,18)}$=0.49, p=0.62] on the percent changes in MEPs following FES-like NMES, suggesting that the magnitude of increases in MEPs were not different between the leg and hand or between *target* and *non-target* muscles. The percent change in MEPs for each

muscle from before to after NMES applied to the leg and hand for each subject is shown in *Panels b* and *c* of Figure 3-4.

3.3.2 Changes in MEPs induced by FES-like and SS-like NMES of the hand

Panels b and *c* of Figure 3-3 show MEPs recorded from one subject before and after FES-like and SS-like median nerve stimulation. MEPs are shown for the *target* muscle (APB), the *adjacent* muscle (FDI), and the *remote* muscle (ECU). In contrast to the increase in MEPs in the *target* muscle (APB) by FES-like median nerve stimulation (81% increase), MEPs in APB increased by only 55% after SS-like median nerve stimulation. Following SS-like median nerve stimulation, MEPs in the *adjacent* muscle (FDI) decreased by 59% and MEPs for the *remote* muscle (ECU) increased by just 5%. The large decrease in MEPs in the *adjacent* muscle is in contrast to a 15% increase in MEPs in the *adjacent* muscle following FES-like median nerve stimulation. Nevertheless, in this individual MEP increases following FES-like and SS-like median nerve stimulation appear to be mostly focused to the *target* muscle and were larger following FES-like NMES than following SS-like NMES. However, changes in MEP amplitude were not tested for statistical significance in individual subjects.

The percent change in MEP amplitude averaged across the group following FES-like and SS-like median nerve stimulation are shown in Figure 3-4a. *T-tests* comparing MEP amplitude before and after FES-like median nerve stimulation identified a significant increase in MEPs for the *target* muscle (APB) [$t_{(9)}$=-2.78, p=0.021], but no change for the *adjacent* muscle (FDI) [$t_{(9)}$=0.38, p=0.715] and the *remote* muscle (ECU) [$t_{(9)}$=-1.14, p=0.283]. In contrast, SS-like

median nerve stimulation did not significantly change MEPs in any of the recorded muscles (APB [$t_{(9)}$=-1.32, p=0.22]; FDI [$t_{(9)}$=0.11, p=0.92]; ECU [$t_{(9)}$=0.42, p=0.69]). The ANOVA analyses revealed no main effects of "NMES protocol" [$F_{(1,9)}$=0.22, p=0.65] or "Muscle" [$F_{(1,9)}$=1.81, p=0.19] and no interaction [$F_{(2,18)}$=0.39, p=0.68] on the percent changes in MEPs following NMES applied to the median nerve, suggesting that the magnitude of increases in MEPs were not different between FES-like and SS-like NMES or between *target* and *non-target* muscles. The percent change in MEPs for each muscle from before to after FES-like and SS-like median nerve stimulation for each subject are shown in *Panels c* and *d* of Figure 3-4.

3.4 Discussion

The present experiments were designed to compare changes in CS excitability when FES-like NMES was applied to the leg versus the hand, as well as changes in CS excitability of muscles of the hand following FES-like versus SS-like NMES. The main finding was that FES-like NMES increased CS excitability for *target* and *non-target* muscles in the leg, but only for the *target* muscle in the hand. Furthermore, SS-like NMES of the hand did not change CS excitability for any muscles of the hand.

3.4.1 CS excitability changes following FES-like NMES of the leg versus the hand

3.4.1.1 Target Muscle

Previous studies have shown that NMES increases CS excitability for the *target* muscle in both the leg and hand. CP nerve stimulation in the leg increased

CS excitability for the *target* muscle (TA) by ~40-50% (Knash et al., 2003; Kido and Stein, 2004; Khaslavskaia and Sinkjaer, 2005) and by ~100% in other studies (Khaslavskaia et al., 2002; Mang et al., 2010). Similarly, ulnar nerve stimulation in the hand increased CS excitability for the *target* muscle (FDI) by ~50% (Ridding et al., 2000; McKay et al., 2002) and by ~100% in another study (Ridding et al., 2001). Although the CS excitability increases induced by NMES for muscles of the leg and hand are similar, the stimulation parameters used in these studies were vastly different. NMES of the leg was applied with FES-like parameters, such that the intensity and frequency of the stimulation were high (intensity: up to 50% maximal M-wave; frequency: up to 200 Hz) and the duration was ~30-45 min. In contrast, NMES of the hand was applied with SS-like parameters whereby the intensity of the stimulation was near motor threshold, the frequency was 10 Hz, and the duration was long (2 h). Khaslavskaia and colleagues (2002) suggest that higher intensities of NMES increase CS excitability more than lower intensities; however, this assertion was based on data collected from only one participant. Likewise, higher frequency NMES increased CS excitability more than lower frequency NMES for hand muscles (Pitcher et al., 2003). These results suggest that the larger afferent volley generated from NMES delivered at high intensities and frequencies increases CS excitability more than lower intensity and frequency NMES. Thus, if stimulation parameters were the same, CS excitability of the *target* muscle might increase more following NMES of the hand than the leg. Conversely, there is likely a limit in the extent to which CS excitability can be increased by NMES. Where this "ceiling effect" lies and

whether it has been reached in the present or previous studies has not been explored.

The present study is the first to compare NMES-induced increases in CS excitability between muscles of the leg and hand in the same subjects using the same stimulation parameters. With 10 participants the data do not support our hypothesis that CS excitability increases in the *target* muscles would be greater in the hand than the leg; however, there is a non-significant trend for larger increases in CS excitability in the *target* muscle of the hand (76% increase) than the leg (49% increase). Greater increases in CS excitability for hand muscles than leg muscles could be reflective of stronger afferent projections to the cortex from the hand or differences between limbs in the cortical mechanisms that mediate such changes. Moreover, such a difference would suggest that NMES applied to strengthen CS pathways for rehabilitation would be more effective for muscles of the hand than the leg.

3.4.1.2 Non-target (adjacent and remote) muscles

Previously, some studies have shown that CP nerve stimulation in the leg increased CS excitability for TA, the *target* muscle, and did not affect CS excitability for a *non-target* muscle (Sol) (Khaslavskaia et al., 2002; Knash et al., 2003). However, other studies found that CP nerve stimulation did significantly increase CS excitability for Sol, as well as TA (Kido and Stein, 2004; Mang et al., 2010). In contrast, when NMES was applied to the hand, CS excitability increases have only been reported for the *target* muscle and not for *non-target* muscles in close proximity (Ridding et al., 2000; Ridding et al., 2001; McKay et al., 2002;

Pitcher et al., 2003). These results suggest that the afferent drive generated by NMES can increase CS excitability for *target* and *non-target* muscles of the leg, but for only *target* muscles in the hand. However, these differences could be due to the higher intensity FES-like NMES used when stimulating the leg compared to the lower intensity SS-like NMES used when stimulating the hand.

The present study is the first to investigate NMES-induced CS excitability increases in *non-target* muscles of the leg and hand when stimulation parameters were the same. FES-like NMES of the CP nerve in the leg increased CS excitability significantly for the *target* muscle (TA) and *non-target* muscles (Sol and VM). In contrast, FES-like NMES of the median nerve at the wrist increased CS excitability significantly for only the *target* muscle (APB) and not for *non-target* muscles (FDI, ECU). CS excitability increases for *non-target* muscles could be due to spread of the electrical current from the stimulation; however, this is unlikely because CS excitability increased for *non-target* muscles in the leg but not in the hand, despite further distance between the stimulation site and the *non-target* muscles for the leg compared to the hand. Furthermore, NMES generated an M-wave of ~15% maximal in the *target* muscle, but little or no M-wave in the *non-target* muscles. Instead, we suggest that CS excitability increases in *non-target* leg muscles but not in *non-target* hand muscles may reflect differences in the organization of afferent projections to the cortex between the leg and hand.

Studies investigating the conditioning of MEPs by a preceding stimulus applied to a peripheral sensory nerve suggest that CNS excitability is affected differently by afferent input received from the leg and hand (Nielsen et al., 1992;

Deletis et al., 1992; Kasai et al., 1992; Roy and Gorassini, 2008). In leg muscles, MEPs elicited by stimulating motor cortex are suppressed by a single peripheral nerve stimulus due to spinal inhibition (Roy and Gorassini, 2008; Poon et al., 2008), whereas MEPs for hand muscles are suppressed due to inhibition of cortical networks (Tokimura et al., 2000). In contrast, MEPs are facilitated for both leg and hand muscles due to changes in both cortical and spinal excitability (Deletis et al., 1992; Poon et al., 2008; Roy and Gorassini, 2008). This afferent-induced facilitation occurs in muscles innervated by the stimulated nerve and in adjacent muscles (Deletis et al., 1992); however, the facilitation spreads to non-stimulated muscles to a greater degree for muscles of the leg than the hand (Roy and Gorassini, 2008). These results suggest that afferent input has predominantly excitatory effects on leg motor cortex, but both inhibitory and excitatory effects on hand motor cortex. The primarily non-specific and excitatory effect of a single electrical stimulus on leg motor cortex as compared to hand motor cortex is consistent with NMES-induced increases in CS excitability for multiple muscles of the leg compared to only the *target* muscle in the hand. Alternatively, a global effect of NMES on CS excitability for leg muscles compared to a more focused effect for hand muscles could be explained by differences in the mechanisms underlying plasticity for hand and leg muscles; however, such mechanistic differences have not been addressed experimentally.

Nevertheless, non-significant trends in the data suggest that CS excitability increases in *non-target* hand muscles following FES-like NMES (37±10% and 22±6% increases in *adjacent* and *remote* muscles, respectively). A

larger sample size may provide greater statistical power to observe CS excitability increases in *non-target* hand muscles; however, it is likely that such increases occur to a lesser extent in the hand than the leg. Such global CS excitability increases following NMES could be advantageous for rehabilitation as NMES applied to one muscle could increase CS excitability for multiple muscles.

3.4.2 CS excitability changes following FES-like versus SS-like NMES of the hand

3.4.2.1 Target Muscle

The afferent drive generated by NMES depends on stimulation parameters and is crucial for increasing CS excitability. A functional magnetic resonance imaging study found a clear relationship between NMES intensity and hemodynamic activity in sensorimotor cortex, suggesting that higher intensities of NMES generated larger afferent drive to the cortex than lower intensities (Smith et al., 2003). Likewise, NMES-induced CS excitability changes for hand and leg muscles depend on stimulation frequency (Pitcher et al., 2003; Mang et al., 2010). NMES applied with low intensities and frequencies (SS-like NMES) have increased CS excitability by ~50-100% in the *target* muscle; however, no studies have tested whether CS excitability could be increased even more for hand muscles by higher intensity and frequency FES-like NMES.

The present study tested the hypothesis that the larger afferent drive generated by FES-like NMES will increase CS excitability for the *target* muscle more than SS-like NMES for muscles of the hand. FES-like median nerve stimulation increased CS excitability significantly for the *target* muscle (APB) by

~75% while CS excitability did not change following SS-like NMES. These data suggest that just 40 min of FES-like NMES would be more effective than 2 h of SS-like NMES when applied therapeutically to enhance CS excitability for hand muscles. However, the lack of any significant increase in CS excitability following SS-like NMES is inconsistent with the previous literature and was unexpected. Although non-significant, it is important to note that CS excitability for APB following SS-like NMES was 47±10% larger than pre-NMES values, which is comparable to past research reporting significant CS excitability increases of ~55% for FDI following SS-like ulnar nerve stimulation (Ridding et al., 2000). Further data collection is necessary to increase statistical power and determine whether SS-like NMES increases CS excitability for APB.

3.4.2.2 Non-target (adjacent and remote) muscles

Neither the FES-like or SS-like NMES significantly increased CS excitability for *non-target* muscles of the hand. These data are consistent with previous literature which suggests a focused effect of NMES on CS excitability for hand muscles. Nevertheless, non-significant trends for increased CS excitability for the *adjacent* (FDI; 37±11% increase) and *remote* (ECU; 22±6% increase) hand muscles are apparent following FES-like NMES and for the *adjacent* muscle (FDI; 48±15% increase), but not the *remote* muscle (ECU; <1±4% increase), following SS-like NMES. These data suggest that FES-like NMES elicits more focused CS excitability increases in the hand than the leg, but may have a larger effect on *non-target* hand muscles than SS-like NMES.

3.4.3 Implications

FES-like NMES, similar to that used in the present study, is commonly used for treatment of impaired dorsiflexion and grasping following CNS injury. Improvements in dorsiflexion (Knash et al., 2003) and grasping (Conforto et al., 2002) that outlast the stimulation are associated with increases in CS excitability for the involved muscles. Other studies suggest an important role of CS excitability changes in improvements in motor performance (Ziemann et al., 2001), the acquisition of motor skills, and improvements in motor learning (Pascual-Leone et al., 1995) in healthy, neurologically-intact individuals. If enhancing CS excitability leads to functional improvements, then a better understanding of how NMES can be applied to maximize CS excitability increases in different muscles will be important for maximizing the efficacy of NMES therapies. Our data supports the idea that NMES applied to the leg produces a more global increase in CS excitability compared to a more focused increase induced in hand muscles, and suggest that the stronger FES-like NMES delivered for a relatively short period of time is more effective for increasing CS excitability for hand muscles than extended periods of the weaker SS-like NMES.

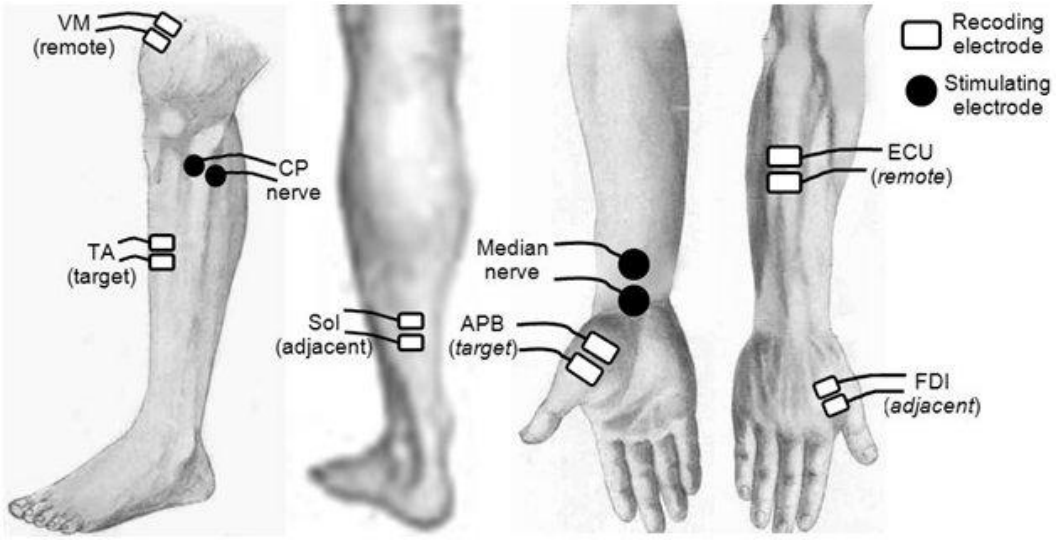

Figure 3-1 Schematic of stimulating and recording electrode placements for experimental sessions involving CP nerve stimulation and median nerve stimulation. When CP nerve stimulation was applied TA was considered to be the *target* muscle, Sol the *adjacent* muscle, and VM the *remote* muscle. When median nerve stimulation was applied APB was considered to be the *target* muscle, FDI the *adjacent* muscle, and ECU the *remote* muscle.

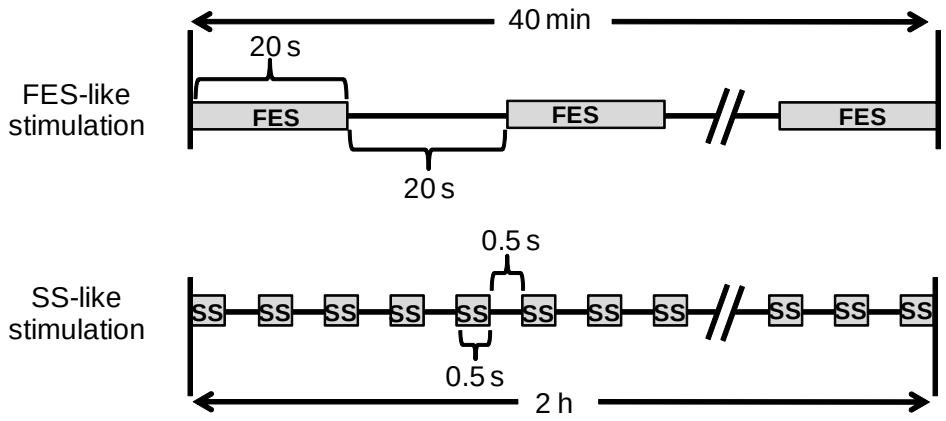

Figure 3-2 Schematic of stimulation patterns delivered for FES-like and SS-like NMES. Each grey box represents a period of NMES followed by a black line representing a rest period. FES-like stimulation was delivered at an intensity of 15% M_{max}, a frequency of 100 Hz, a pulse width of 1 ms, and a total duration of 40 min. SS-like stimulation was delivered at an intensity near motor threshold, a frequency of 10 Hz, a pulse width of 1 ms, and a total duration of 2 h.

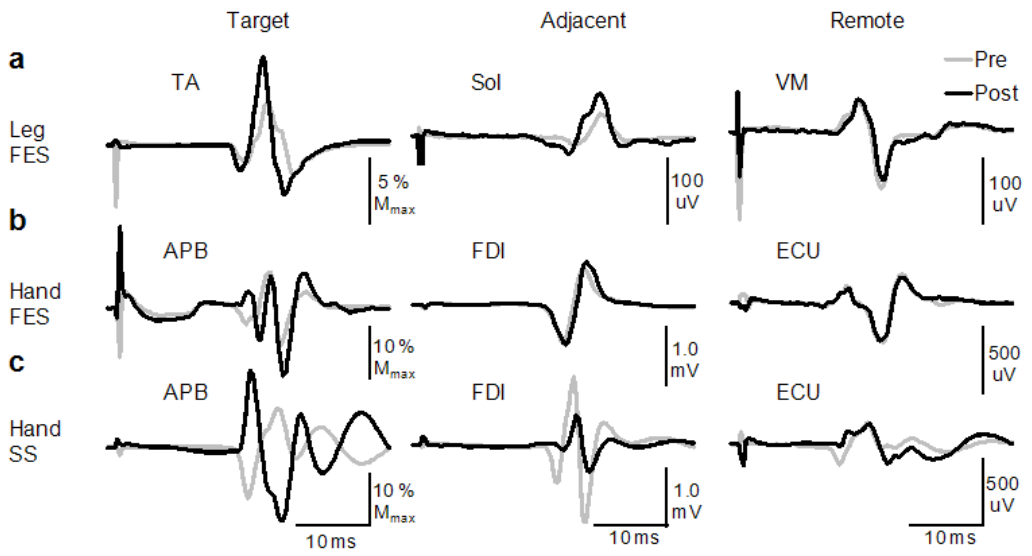

Figure 3-3 Changes in MEP amplitude in the *target*, *adjacent*, and *remote* muscles following FES-like NMES of the leg and hand, and SS-like NMES of the hand in a single subject. *Panel a* shows the mean MEP waveforms (*n*=10) before (pre; grey line) and after (post; black line) FES-like CP nerve stimulation. *Panel b* shows the mean MEP waveforms (*n*=10) before (pre; grey line) and after (post; black line) FES-like median nerve stimulation. *Panel c* shows the mean MEP waveforms (*n*=10) before (pre; grey line) and after (post; black line) SS-like median nerve stimulation.

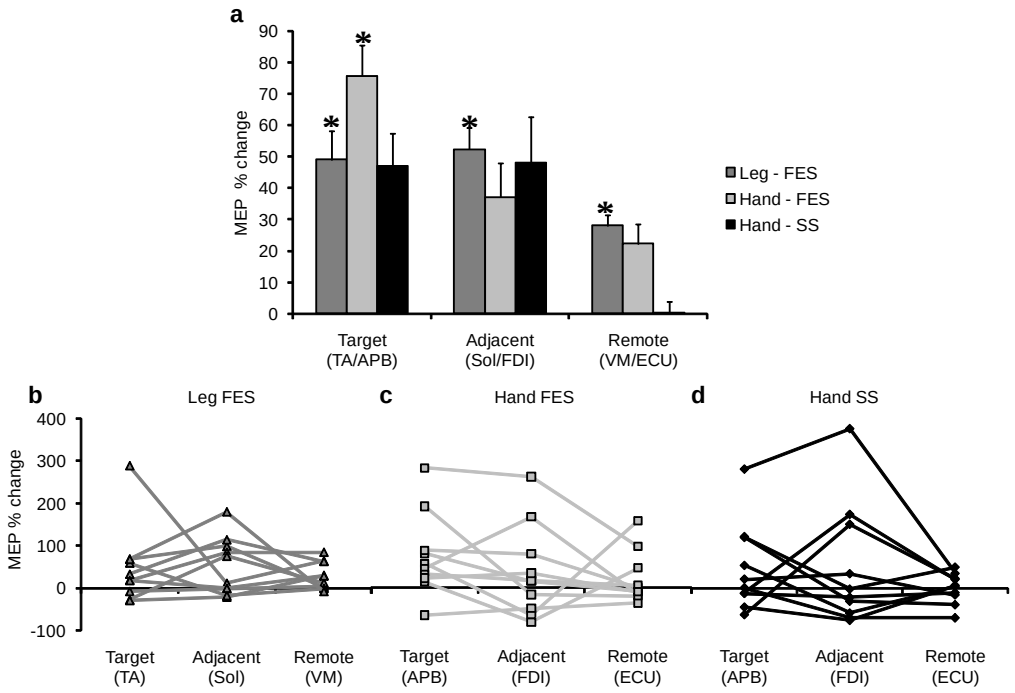

Figure 3-4 Percent change of MEPs averaged across the group for the *target*, *adjacent*, and *remote* muscles following FES-like NMES of the leg (dark grey) and hand (light grey), and SS-like NMES of the hand (black). *Asterisks* in *Panel a* represent significant increases from control (pre-NMES). *Error bars* represent one standard error. *Panels b, c, and d* show mean percent changes in MEPs following FES-like CP nerve stimulation, FES-like median nerve stimulation, and SS-like median nerve stimulation for each subject.

3.5 References

Baker, LL, Wederich, CL, McNeal, DR, Newsam, CJ, Waters, RL (2000) NeuroMuscular Electrical Stimulation: A Pratical Guide. 4: 1-56

Conforto, AB, Kaelin-Lang, A, Cohen, LG (2002) Increase in hand muscle strength of stroke patients after somatosensory stimulation. Ann Neurol 51: 122-125

Deletis, V, Schild, JH, Beric, A, Dimitrijevic, MR (1992) Facilitation of motor evoked potentials by somatosensory afferent stimulation. Electroencephalogr Clin Neurophysiol 85: 302-310

Fraser, C, Power, M, Hamdy, S, Rothwell, J, Hobday, D, Hollander, I, Tyrell, P, Hobson, A, Williams, S, Thompson, D (2002) Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. Neuron 34: 831-840

Hoffman, LR, Field-Fote, EC (2007) Cortical reorganization following bimanual training and somatosensory stimulation in cervical spinal cord injury: a case report. Phys Ther 87: 208-223

Kasai, T, Hayes, KC, Wolfe, DL, Allatt, RD (1992) Afferent conditioning of motor evoked potentials following transcranial magnetic stimulation of motor cortex in normal subjects. Electroencephalogr Clin Neurophysiol 85: 95-101

Khaslavskaia, S, Ladouceur, M, Sinkjaer, T (2002) Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve. Exp Brain Res 145: 309-315

Khaslavskaia, S, Sinkjaer, T (2005) Motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve depends on the voluntary drive. Exp Brain Res 162: 497-502

Kido, TA, Stein, RB (2004) Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. Exp Brain Res 159: 491-500

Knash, ME, Kido, A, Gorassini, M, Chan, KM, Stein, RB (2003) Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. Exp Brain Res 153: 366-377

Kurusu, K, Kitamura, J (1999) Long-latency reflexes in contracted hand and foot muscles and their relations to somatosensory evoked potentials and transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol 110: 2014-2019

Lagerquist, O, Zehr, EP, Baldwin, ER, Klakowicz, PM, Collins, DF (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170: 1-6

Mang, CS, Lagerquist, O, Collins, DF (2010) Changes in corticospinal excitability evoked by common peroneal nerve stimulation depend on stimulation frequency. Exp Brain Res 203: 11-20

McKay, D, Brooker, R, Giacomin, P, Ridding, M, Miles, T (2002) Time course of induction of increased human motor cortex excitability by nerve stimulation. NeuroReport 13: 1271-1273

Nielsen, J, Kagamihara, Y, Crone, C, Hultborn, H (1992) Central facilitation of Ia inhibition during tonic ankle dorsiflexion revealed after blockade of peripheral feedback. Exp Brain Res 88: 651-656

Pascual-Leone, A, Nguyet, D, Cohen, LG, Brasil-Neto, JP, Cammarota, A, Hallett, M (1995) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 74: 1037-1045

Pitcher, JB, Ridding, MC, Miles, TS (2003) Frequency-dependent, bi-directional plasticity in motor cortex of human adults. Clin Neurophysiol 114: 1265-1271

Poon, DE, Roy, FD, Gorassini, MA, Stein, RB (2008) Interaction of paired cortical and peripheral nerve stimulation on human motor neurons. Exp Brain Res 188: 13-21

Ridding, MC, Brouwer, B, Miles, TS, Pitcher, JB, Thompson, PD (2000) Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 131: 135-143

Ridding, MC, McKay, DR, Thompson, PD, Miles, TS (2001) Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 112: 1461-1469

Roy, FD, Gorassini, MA (2008) Peripheral sensory activation of cortical circuits in the leg motor cortex of man. J Physiol 586 (Pt 17): 4091-105

Sheffler, LR, Chae, J (2007) Neuromuscular electrical stimulation in neurorehabilitation. Muscle Nerve 35: 562-590

Smith, GV, Alon, G, Roys, SR, Gullapalli, RP (2003) Functional MRI determination of a dose-response relationship to lower extremity neuromuscular electrical stimulation in healthy subjects. Exp Brain Res 150: 33-39

Tamm, AS, Lagerquist, O, Ley, AL, Collins, DF (2009) Chronotype influences diurnal variations in the excitability of the human motor cortex and the ability to generate torque during a maximum voluntary contraction. J Biol Rhythms 24: 211-224

Tokimura, H, Di, L, V, Tokimura, Y, Oliviero, A, Profice, P, Insola, A, Mazzone, P, Tonali, P, Rothwell, JC (2000) Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol (Lond) 523: 503-513

Walton, C, Kalmar, J, Cafarelli, E (2003) Caffeine increases spinal excitability in humans. Muscle Nerve 28: 359-364

Ziemann, U, Muellbacher, W, Hallett, M, Cohen, LG (2001) Modulation of practice-dependent plasticity in human motor cortex. Brain 124: 1171-1181

CHAPTER 4: GENERAL DISCUSSION

The experiments described in this thesis were designed to investigate the effect of neuromuscular electrical stimulation (NMES) on corticospinal (CS) excitability in healthy, neurologically-intact humans. Experiments were conducted to explore the influence of stimulation parameters on changes in CS excitability induced by NMES and to determine whether NMES affects CS excitability differently for muscles of the hand versus the leg. First, I discuss the findings in each chapter related to the effect of NMES parameters on NMES-induced changes in CS excitability. Next, I discuss the results related to the application of NMES to muscles of the hand versus the leg on CS excitability. Finally, I discuss the findings related to mechanisms of NMES-induced plasticity in the central nervous system (CNS).

4.1 The effect of NMES parameters on CS excitability changes

An overarching hypothesis in this thesis was that NMES would increase CS excitability the most when applied with the parameters that produced the largest afferent drive. Thus, a major focus was to characterize the influence of NMES parameters on CS excitability changes. In this thesis, the size of the afferent drive was considered to be the amount of afferent activation per unit of time. For instance, increases in NMES intensity and frequency, but not increases in NMES duration, were considered to increase the size of the afferent volley generated by NMES.

A primary goal of the work described in this thesis was to determine the effect of stimulation frequency on changes in CS excitability. I hypothesized that

higher frequencies of stimulation would produce larger increases in CS excitability than lower frequencies. Common peroneal (CP) nerve stimulation delivered at 100 Hz significantly increased CS excitability for the tibialis anterior (TA) muscle while 10- and 50-Hz NMES did not. Moreover, increases in CS excitability for TA occurred by the 24th min of 100-Hz NMES. Surprisingly, 200-Hz NMES did not increase CS excitability. I suggested that the lack of change in CS excitability following 200-Hz NMES might have been due to hyperpolarization of sensory axons underneath the stimulating electrodes (Burke et al., 2001). Although some sensory receptors can fire as fast as 600 Hz, such high firing rates are maintained for only very brief periods of time. Prolonged trains of stimuli, such as a 20 s train of NMES delivered at 200 Hz, result in a summation of the late sub-excitable period that an axon goes through after a stimulus, leading to longer and deeper periods of hyperpolarization (Burke et al., 2001), and thus leading to a reduction in the afferent drive generated by the NMES. If increases in CS excitability are dependent on the size of the afferent volley generated, then these data suggest that increases in stimulation frequency may not necessarily result in an increased afferent volley. Future experiments using surface or microneurography techniques could be conducted to measure the electrically-evoked afferent volley generated at varying stimulation frequencies, followed by further experiments to determine whether CS excitability increases evoked by NMES scale with the afferent drive that is generated. The results from the research presented in this thesis suggest that the NMES frequency that generates the largest afferent drive to increase CS excitability is likely greater

than 50 Hz and less than 200 Hz, but do not conclusively determine that 100 Hz is optimal. From the results presented in this thesis, I proposed that prolonged stimulation trains delivered to the CP nerve in an on-off cycle, similar to how NMES is often used for rehabilitation, are more effective for increasing CS excitability for TA when delivered at 100 Hz, than at 10, 50, or 200 Hz. Also, increases in CS excitability occurred after 24 min of 100-Hz NMES, suggesting that improvements in motor function that are associated with increased CS excitability could also be realized after just 24 min of 100-Hz NMES.

Experiments were also conducted to compare changes in CS excitability of hand muscles following functional electrical stimulation (FES)- and somatosensory stimulation (SS)-like NMES of the median nerve. Abductor pollicis brevis (APB) was considered to be the *target* muscle for the NMES, as APB is innervated by the median nerve. Likewise, first dorsal interosseus (FDI) and extensor carpi ulnaris (ECU) were considered to be *non-target* muscles for the NMES, as they are innervated by the ulnar nerve. More specifically, FDI was considered to be an *adjacent* muscle and ECU a *remote* muscle based on their proximity to the *target* muscle, APB. The FES-like NMES was strong enough to evoke contractions in the *target* muscle and was delivered in a pattern of 20 s on-20 s off for 40 min. The SS-like NMES was delivered near motor threshold with a pattern of 500 ms on-500 ms off for 2 h and evoked relatively small contractions for the *target* muscle. These two protocols were compared because their effect on CS excitability is commonly tested experimentally, but they have never before been directly compared. I hypothesized that the FES-like NMES would increase

CS excitability for the *target* muscle (APB) more than the SS-like NMES. I also hypothesized that FES-like NMES would have a stronger effect on *non-target* muscles (FDI and ECU) than SS-like NMES.

FES-like NMES significantly increased CS excitability by 76±10% but SS-like NMES did not significantly alter CS excitability from control for the *target* muscle (APB). These data supported my hypothesis, but the lack of any significant increase in CS excitability following SS-like NMES was inconsistent with the previous literature and unexpected. Compared to the previous literature the SS-like NMES in these experiments were applied to the same number of subjects with the same parameters (intensity, frequency, pulse-width, pattern, and duration). However, Ridding and colleagues (2000) applied SS-like NMES to the ulnar nerve to *target* FDI, rather than to the median nerve to *target* APB, and measured CS excitability by the area, rather than the amplitude, of motor evoked potential (MEP) responses. Although MEP area and amplitude are believed to yield similar results, these methodological differences could explain different outcomes between the experiments described in this thesis and the past literature (Ridding et al., 2000). Also, it is important to note that CS excitability increases in the *target* muscle following SS-like NMES were apparent in these data (47±10% increase), albeit not statistically significant, and comparable to the increases in MEP area of ~55% described by Ridding and colleagues (2000). Thus, further data collection is necessary to obtain sufficient statistical power to conclusively determine whether there is an effect of SS-like median nerve stimulation on CS excitability for APB. Nevertheless, I proposed that FES-like NMES delivered for

40 min would be more effective than SS-like NMES delivered for 2 h when applied therapeutically to enhance CS excitability for APB. Contrary to my hypothesis, CS excitability did not significantly change CS excitability for *non-target* hand muscles (FDI and ECU) following either FES-like or SS-like NMES. However, there were non-significant trends for greater changes in *non-target* muscles of the hand following FES-like NMES compared to SS-like NMES. For example, the *remote* muscle (ECU) increased by 22±6% following FES-like NMES but by less than 1% following SS-like NMES.

While NMES-induced plasticity in the CNS is an ever-growing research area, little research has focused on the influence of NMES parameters on inducing such plasticity. One study investigated the influence of stimulation frequency on inducing changes in CS excitability for hand muscles and found that 30-Hz NMES increased CS excitability while 3-Hz NMES decreased CS excitability (Pitcher et al., 2003). For leg muscles, one study investigated the effect of stimulation intensity on CS excitability changes and found that higher stimulation intensities increased CS excitability more than lower intensities (Khaslavskaia et al., 2002). Both of these studies, as well as the research presented in this thesis, suggest that NMES parameters influence changes in CS excitability induced by NMES. As many different combinations of stimulation parameters (intensity, frequency, pulse width, pattern, and duration) can be utilized, there are many future investigations that could stem from altering NMES parameters and testing their effectiveness for increasing CS excitability; however, these experiments would likely involve prolonged periods of NMES, require multiple trials, and may

not be the most efficient way to determine the optimal NMES parameters for increasing CS excitability. Alternatively, it is likely that the effectiveness of certain sets of NMES parameters for increasing CS excitability are dependent on the size of the afferent volley that reaches the sensorimotor cortex during the NMES. Thus, evaluation of the amplitude of somatosensory evoked potentials (SEPs), or the degree of activation of sensorimotor cortex using functional magnetic resonance imaging (fMRI), evoked by single pulses or trains of electrical stimuli delivered with varying parameters would be useful. Such experiments would help to predict optimal NMES parameters for increasing CS excitability without having to deliver NMES for the long durations necessary to elicit CS excitability increases. Past research found a dose-response relationship between NMES intensity and hemodynamic activity in sensorimotor cortex for the quadriceps femoris muscle using fMRI (Smith et al., 2003), but other parameters such as frequency, pulse width, and pattern are yet to be investigated.

4.2 The effect of NMES on CS excitability for muscles of the hand and leg

The effect of NMES on CS excitability has also been tested experimentally in a variety of muscles, ranging from throat muscles (Fraser et al., 2002) to limb muscles (Ridding et al., 2000; Khaslavskaia et al., 2002). However, no studies thus far have directly compared changes in CS excitability induced by NMES in different muscles. In the research conducted for this thesis, CS excitability changes for hand and leg muscles were compared following NMES applied to the median and CP nerves, respectively. As in the previously described experiment, APB was the *target* muscle when median nerve stimulation was

applied, and FDI and ECU were *non-target* muscles. TA, the muscle innervated by the CP nerve, was considered to be the *target* muscle during NMES of the leg, while soleus and vastus medialis were the *non-target* muscles. I hypothesized that NMES would increase CS excitability for the *target* muscle more when applied to the hand versus the leg. I also hypothesized that NMES would have a more focused versus global effect on CS excitability for hand and leg muscles, respectively. Thus, I predicted that CP nerve stimulation would increase MEPs for *non-target* leg muscles more than median nerve stimulation would increase MEPs for *non-target* hand muscles. Although CS excitability increased by 76±10% for the *target* hand muscle compared to 49±9% for the *target* leg muscle, CS excitability increases in hand and leg muscles were not significantly different following NMES. CS excitability increased significantly for the *non-target* muscles only following NMES applied to the leg; however, the size of the increases were not different from non-significant increases in *non-target* hand muscles. Nevertheless, I proposed that significant increases in CS excitability in *non-target* leg muscles but not in *non-target* hand muscles suggest a more global effect of NMES on CS excitability for the leg than the hand. I suggested that a global effect of NMES on CS excitability in the leg may reflect differences in the organization of afferent projections to the cortex between the hand and leg (Kurusu and Kitamura, 1999). Such a difference would also be consistent with studies investigating afferent-conditioning of MEPs with electrical stimuli delivered to a sensory nerve, which suggest that afferent-induced facilitation of MEPs spreads to non-stimulated muscles to a greater degree for muscles of the

leg than for muscles of the hand (Deletis et al., 1992; Roy and Gorassini, 2008). A higher degree of specificity of sensory projections from the hand, as compared to the leg, could explain the greater skillfulness of hand muscles in performing finer and more discrete movements than leg muscles. For example, greater specificity of sensory projections would provide more specific sensory feedback and might aid in the selection of appropriate neuronal responses and the execution of specific or fine movements. In addition, these data suggest that NMES applied to one muscle of the leg for rehabilitation could have rehabilitative benefits for multiple leg muscles.

Based on non-significant trends within the data, further investigations into changes in CS excitability evoked by NMES of hand and leg muscles are warranted. I plan to collect data from 5 more subjects to increase statistical power and further evaluate trends for larger increases in CS excitability for *target* muscles following NMES of the hand compared to the leg, as well as for possible changes in CS excitability for *non-target* hand muscles following FES-like NMES. Initially, experiments conducted for this thesis investigating differences in NMES-induced plasticity between *target* and *non-target* muscles of the hand and leg included cortical mapping procedures to more thoroughly evaluate possible differences in the induced plasticity. Mapping measures such as cortical map area, volume, and centre of gravity provide details of plasticity that can not be detected by evaluating the peak-to-peak amplitude of an MEP elicited from a single cortical site. However, the application of mapping procedures to evaluate the organization and excitability of leg motor cortex was found to be problematic and

thus mapping procedures were removed from the protocol. Participant discomfort was a common problem as higher intensities of transcranial magnetic stimulation (TMS) are often necessary to activate the relatively deep regions of the leg motor cortex and many stimuli are necessary to create cortical maps. Furthermore, the measures obtained from mapping leg motor cortex were found to be quite variable and difficult to interpret. It seems that the leg motor cortex is not conducive to motor mapping with TMS, likely due to its location in deeper regions of the cortex and its relatively concentrated organization, compared to the surface location and more diffuse organization of hand motor cortex (Penfield and Rasmussen, 1952).

In addition, inter-subject variability in responses to NMES in the data suggests that increases in CS excitability induced by NMES, and any associated benefits for rehabilitation, may be greater in some individuals than others. Further investigation into why some individuals have particularly robust increases in CS excitability following NMES, while others show no change, would be useful to determine if some individuals are better candidates for NMES therapy than others. Kleim and colleagues (2006) suggest that training-dependent plasticity in the human motor cortex is reduced in individuals with a polymorphism in the brain-derived neurotrophic factor gene. Another explanation might be that individuals with particularly strong afferent projections from the stimulated muscle to the sensorimotor cortex experience a larger effect of NMES on CS excitability. If this were the case, testing involving SEPs could be used to identify individuals who would be particularly receptive to NMES treatment for movement impairments.

4.3 Mechanisms of NMES-induced CNS plasticity

Additional objectives of this thesis were to discern whether CS excitability changes induced by NMES were mediated in the cortex or the spinal cord and to chart a time course of CS excitability changes during NMES. MEPs evoked by TMS over the motor cortex increased significantly with no concomitant change in maximal H-reflex to M-wave ratio (H_{max}:M_{max} ratio), suggesting that CS excitability increases were cortical in nature. Although changes in spinal excitability could occur with no concurrent change in H_{max}:M_{max} ratio, these data are consistent with past research that found no change in spinal excitability as evaluated by transcranial electrical stimulation, brainstem electrical stimulation, and F-waves following NMES (Stefan et al., 2000; Ridding et al., 2000; Stefan et al., 2002). The time course of CS excitability changes during NMES showed sustained increases in CS excitability starting after 24 min of 100-Hz NMES. Although no direct evidence of the mechanisms underlying CS excitability changes induced by NMES was provided in this thesis, I proposed that the time course is consistent with past research suggesting long-term potentiation (LTP) plays an important role in cortical plasticity (Hess and Donoghue, 1994; Butefisch et al., 2000; McKay et al., 2002; Kaelin-Lang et al., 2002).

Mechanisms of NMES-induced CNS plasticity are not well understood. Pharmacological studies exploring CNS plasticity induced by SS, which primarily activates afferents, suggest that gamma-amino-butyric acid (GABA) mechanisms involved in LTP are important for plasticity in the upper limb induced by SS; however, the blocking of N-methyl D-aspartate (NMDA) receptors important for

LTP had no effect (Kaelin-Lang et al., 2002). In contrast, increased CS excitability evoked by NMES combined with motor cortex activation by TMS (paired associative stimulation) was blocked by pharmacological agents known to interfere with NMDA receptors, and the effect of agents affecting GABA receptors were not tested (Stefan et al., 2002). It is possible that NMES combined with motor cortex activation results in a combination of plasticity-inducing mechanisms from GABA and NMDA receptors and thus enhances plasticity; however, these studies do not provide direct evidence and this is only speculation. Moreover, voluntary contractions combined with NMES enhance CNS plasticity for muscles of the lower limb more than NMES alone (Knash et al., 2003; Khaslavskaia and Sinkjaer, 2005). These protocols might be considered similar to paired associative stimulation protocols, as both involve combined afferent and motor cortex activation, and thus may share a common mechanism. A better understanding of the mechanisms triggered by the combination of afferent and motor cortex activation as compared to the stimulation of afferents alone might help to determine how CS excitability can be maximally increased. A study testing the effects of lorazepam, a $GABA_A$ receptor agonist, and dextromethorphan, an NMDA receptor antagonist, on CS excitability changes induced by NMES alone and NMES combined with voluntary contractions would help to better characterize the mechanisms underlying both protocols.

4.4 Summary

The research presented in this thesis contributes to a growing body of knowledge about how NMES influences plasticity in the CNS. Previous research

has studied the effect of NMES on plasticity when using a variety of stimulation parameters and in a variety of muscles; however, systematic comparisons of different stimulation parameter combinations and between muscles have rarely been conducted. NMES-induced increases in CS excitability are associated with recovery of motor function after CNS injury (Conforto et al., 2002; Hoffman and Field-Fote, 2007), and thus an understanding of how to best apply NMES to induce such excitability increases will be important for maximizing the efficacy of NMES therapies. This thesis emphasizes that the careful selection of stimulation parameters is important when applying NMES to maximally increase CS excitability and that NMES affects CS excitability differently for muscles of the hand and leg. More specifically, NMES delivered in an on-off cycle of prolonged stimulation trains is more effective for increasing CS excitability for the dorsiflexors when delivered at 100 Hz than at 10, 50, or 200 Hz and FES-like NMES has a greater effect on CS excitability for hand muscles than SS-like NMES. Furthermore, NMES has a more focused effect on CS excitability for hand muscles, compared to a more global effect on CS excitability for leg muscles. The results of this thesis and previous research indicate that NMES combined with other rehabilitation interventions holds great promise for enhancing recovery for individuals living with the consequences of a CNS injury or disease. Continued research into the optimal parameters and combination of therapies and the underlying mechanisms will be important to optimize treatment interventions and provide the maximum benefits for these individuals.

4.5 References

Burke, D, Kiernan, MC, Bostock, H (2001) Excitability of human axons. Clin Neurophysiol 112: 1575-1585

Butefisch, CM, Davis, BC, Wise, SP, Sawaki, L, Kopylev, L, Classen, J, Cohen, LG (2000) Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci U S A 97: 3661-3665

Conforto, AB, Kaelin-Lang, A, Cohen, LG (2002) Increase in hand muscle strength of stroke patients after somatosensory stimulation. Ann Neurol 51: 122-125

Deletis, V, Schild, JH, Beric, A, Dimitrijevic, MR (1992) Facilitation of motor evoked potentials by somatosensory afferent stimulation. Electroencephalogr Clin Neurophysiol 85: 302-310

Fraser, C, Power, M, Hamdy, S, Rothwell, J, Hobday, D, Hollander, I, Tyrell, P, Hobson, A, Williams, S, Thompson, D (2002) Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. Neuron 34: 831-840

Hess, G, Donoghue, JP (1994) Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol 71: 2543-2547

Hoffman, LR, Field-Fote, EC (2007) Cortical reorganization following bimanual training and somatosensory stimulation in cervical spinal cord injury: a case report. Phys Ther 87: 208-223

Kaelin-Lang, A, Luft, AR, Sawaki, L, Burstein, AH, Sohn, YH, Cohen, LG (2002) Modulation of human corticomotor excitability by somatosensory input. J Physiol 540: 623-633

Khaslavskaia, S, Ladouceur, M, Sinkjaer, T (2002) Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve. Exp Brain Res 145: 309-315

Khaslavskaia, S, Sinkjaer, T (2005) Motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve depends on the voluntary drive. Exp Brain Res 162: 497-502

Kleim, JA, Chan, S, Pringle, E, Schallert, K, Procaccio, V, Jiminez, R, Cramer, SC (2006) BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neuroscience 9(6):735-737

Knash, ME, Kido, A, Gorassini, M, Chan, KM, Stein, RB (2003) Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. Exp Brain Res 153: 366-377

Kurusu, K, Kitamura, J (1999) Long-latency reflexes in contracted hand and foot muscles and their relations to somatosensory evoked potentials and transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol 110: 2014-2019

McKay, D, Brooker, R, Giacomin, P, Ridding, M, Miles, T (2002) Time course of induction of increased human motor cortex excitability by nerve stimulation. NeuroReport 13: 1271-1273

Penfield, W, Rasmussen, T (1952) The cerebral cortex of man. Macmillan, New York

Pitcher, JB, Ridding, MC, Miles, TS (2003) Frequency-dependent, bi-directional plasticity in motor cortex of human adults. Clin Neurophysiol 114: 1265-1271

Ridding, MC, Brouwer, B, Miles, TS, Pitcher, JB, Thompson, PD (2000) Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 131: 135-143

Roy, FD, Gorassini, MA (2008) Peripheral sensory activation of cortical circuits in the leg motor cortex of man. J Physiol 586 (Pt 17): 4091-105

Smith, GV, Alon, G, Roys, SR, Gullapalli, RP (2003) Functional MRI determination of a dose-response relationship to lower extremity neuromuscular electrical stimulation in healthy subjects. Exp Brain Res 150: 33-39

Stefan, K, Kunesch, E, Benecke, R, Cohen, LG, Classen, J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543: 699-708

Stefan, K, Kunesch, E, Cohen, LG, Benecke, R, Classen, J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123 Pt 3: 572-584

APPENDIX A: AFFERENT CONDITIONING OF MOTOR EVOKED POTENTIALS BEFORE AND AFTER NEUROMUSCULAR ELECTRICAL STIMULATION[3]

A.1 Introduction

Sensory inputs to the central nervous system (CNS) play an important role in motor control and help shape motor commands from the primary motor cortex (M1). The influence of afferent input on the human cortex has been demonstrated by functional magnetic resonance imaging studies which show that the afferent volley evoked by electrical stimulation of a peripheral nerve activates both somatosensory cortex and M1 (Spiegel et al., 1999; Deuchert et al., 2002). Also, increasing sensory input to the CNS by a prolonged period of neuromuscular electrical stimulation (NMES) increases corticospinal (CS) excitability for up to 1 h after the stimulation (McKay et al., 2002). Such CS excitability increases are associated with improvements in motor function following CNS injury (Conforto et al., 2002; Foltys et al., 2003; Koski et al., 2004; Hoffman and Field-Fote, 2007) and are believed to occur primarily at the level of M1 (Ridding et al., 2000).

Afferent inputs influence both inhibitory and excitatory circuitry in the CNS. For instance, when afferent inputs first arrive at M1, motor evoked potentials (MEPs) from transcranial magnetic stimulation (TMS) applied over M1 are depressed. This depression is termed short-latency afferent inhibition (Tokimura et al., 2000). Comparisons of MEPs evoked from TMS over M1 and the brainstem, as well as with H-reflexes, suggest that this inhibition occurs

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through supraspinal mechanisms for hand muscles (Tokimura et al., 2000), but likely through spinal mechanisms for leg muscles (Roy and Gorassini, 2008). Afferent inputs can also access excitatory CNS circuitry to facilitate the MEP. Such facilitation is thought to occur through both cortical and spinal mechanisms for hand and leg muscles (Deletis et al., 1992; Rosenkranz and Rothwell, 2003; Rothwell and Rosenkranz, 2005; Poon et al., 2008; Roy and Gorassini, 2008). Although it is well known that a prolonged period of NMES can produce lasting increases in M1 excitability, how NMES influences afferent-induced inhibition and facilitation has not yet been explored. This information may improve our understanding of how NMES increases CNS excitability.

This study was designed to investigate the effect of a prolonged period of NMES on the afferent-conditioning of MEPs for muscles of the hand and the leg. I hypothesized that increases in CS excitability induced by NMES would occur concomitantly with reduced afferent-induced inhibition and enhanced afferent-induced facilitation of MEPs.

A.2 Methods

A.2.1 Subjects

The data described in this Appendix were collected during the same experimental sessions as those described in Chapter 3 and the same ten neurologically-intact subjects (age range: 20 to 47 years) took part. All subjects gave written, informed consent prior to testing. The experiments were conducted according to the Human Research Ethics Board at the University of Alberta. Subjects were seated with their backs and necks supported. During experimental

sessions testing muscles of the hand, the shoulder, elbow, and wrist angles were maintained at ~15°, 120° and 180°, respectively. The elbow was supported by an arm support and the hand was rested on a hand rest in a relaxed position. During sessions testing muscles of the leg, the hip, knee, and ankle angles were maintained at ~110°, 100° and 90°, respectively. Padded restraints were secured around the right foot to minimize movement and the left foot was placed on a foot rest.

A.2.2 Experimental procedure

All subjects participated in two experimental sessions at least 48 h apart in which NMES was applied to either the median nerve in the hand or the common peroneal (CP) nerve in the leg. The order of testing sessions was randomized for each subject.

A.2.3 Electromyography (EMG)

Electromyography (EMG) was recorded from abductor pollicis brevis (APB) in the right hand when NMES was applied to the median nerve. When NMES was applied to the CP nerve, EMG was recorded from tibialis anterior (TA) in the right leg. All EMG was recorded using bipolar (2.25 cm^2) surface recording electrodes (Vermed Medical, Bellow Falls, Vermont). EMG signals were pre-amplified (1,000x) and band pass filtered at 10-1,000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England). Data were sampled at 2,000 Hz with a 12-bit A/D converter (National Instruments, Austin,

Texas). During the collection of MEPs, data were recorded in 450 ms sweeps from 100 ms before to 350 ms after stimulus delivery.

A.2.4 NMES

NMES was applied over the right median nerve at the wrist or the right CP nerve near the fibular head using bipolar (3.2 cm) round neurostimulation electrodes (Axelgaard manufacturing co., ltd.) placed at the site that evoked a response (M-wave or H-reflex) at the lowest stimulation intensity in APB and TA, respectively. Rectangular pulses of 1 ms duration were delivered from a Digitimer (DS7A, Hertfordshire, England) constant current stimulator for all experimental sessions. The maximal M-wave (M_{max}) was determined in a trial which involved the delivery of 5 electrical stimuli varying from below motor threshold to 1.5-2 times the minimum current required to evoke M_{max} and was calculated as the largest M-wave evoked in this trial. NMES was applied at an intensity at which a single stimulus evoked an M-wave that was ~15% of M_{max} in APB or TA and was delivered at100 Hz for 40 min in a 20 s on, 20 s off cycle. Subjects were instructed to remain relaxed while NMES was applied to minimize the effect of voluntary contraction on CS excitability.

A.2.5 Afferent-conditioning of MEPs

Afferent-conditioning of MEPs was tested before and after NMES for both the hand and the leg. Subjects were instructed to remain relaxed during data collection.

A.2.5.1 Conditioning stimulus (peripheral nerve)

Rectangular pulses of 1 ms duration were delivered from a Digitimer (DS7A, Hertfordshire, England) constant current stimulator. For the session involving NMES of the median nerve, the conditioning stimulus was delivered to the median nerve. For the session involving the NMES of CP nerve, the conditioning stimulus was delivered to the CP nerve. The conditioning stimulus intensity was adjusted to elicit ~15% of M_{max} in APB for the median nerve stimulus and in TA for the CP nerve stimulus.

A.2.5.2 Test stimulus (TMS)

To test the excitability of the CS pathway, MEPs were evoked using TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) applied with a parabolic or figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota). The parabolic coil was used when there were difficulties evoking MEPs in leg muscles with the figure-of-eight coil. For a given subject, the same coil was used in all sessions. Using a Brainsight image-guided stimulation system (Rogue Research, Montreal, Quebec) the coil was placed over the hotspot for APB in trials where the median nerve was stimulated and over the hotspot for TA in trials where the CP nerve was stimulated. The hotspots were found by moving the coil over the left M1 to find the site that elicited the largest amplitude MEP in the muscle of interest at the lowest stimulation intensity. MEP thresholds for the muscle of interest were determined by finding the lowest intensity that produced MEPs of at least 50 uV in 4 out of 8 trials. The TMS intensity was set to 120% of the resting threshold of the muscle of interest before NMES.

A.2.5.3 Testing Paradigm

A magnetic stimulus was delivered to M1 at seven different intervals following a conditioning stimulus to the peripheral nerve. Conditioning-test (C-T) intervals were chosen based on evidence of peak MEP inhibition and facilitation from past investigations of the afferent-conditioning of MEPs (Deletis et al., 1992; Tokimura et al., 2000; Roy and Gorassini, 2008). In the experimental session where muscles of the hand were of interest, a single median nerve stimulus preceded the magnetic stimulus by 15, 17, 18, 22, 25, 30, or 35 ms. In the experimental session where muscles of the leg were of interest, a single CP nerve stimulus preceded the magnetic stimulus by 30, 32, 35, 37, 40, 45, or 50 ms. The C-T intervals were presented randomly during the study. Ten MEPs were elicited at each C-T interval for a total of 70 conditioned MEPs with 21 control MEPs randomly intermixed throughout the recording session. The test stimuli were delivered at an inter-stimulus interval (ISI) that varied randomly between 4-6 s. All stimulus triggering and delays were controlled via a digital timing device (Grass instruments S-88, Quincy, Massachusetts).

A.2.6 Data analyses

MEPs were measured peak-to-peak. All conditioned MEPs were normalized to the unconditioned control MEPs from the same trial. Normalized MEP amplitude at each ISI was averaged across the group (n=10) and the ISIs which yielded the largest facilitation and inhibition from control were identified. This method was used instead of calculating the peak facilitation and inhibition for each subject and then averaging across the group because of larger than

expected variability in the ISIs at which peak facilitation and inhibition occurred. The ISIs for peak inhibition ranged from 17-35 ms for APB and from 30-50 ms for TA and for peak facilitation ranged from 15-35 ms for APB and from 30-45 ms for TA. These ranges are larger than would be expected to account for differences in nerve conduction velocity and height between subjects, suggesting that the observed effects at such varying intervals may have been due more to the inherent variability of MEP responses than to an effect of the peripheral nerve stimulus. Nevertheless, two separate 2-way repeated measures analysis of variance (ANOVAs) were used to compare peak facilitation and peak inhibition before and after NMES applied to the median and CP nerves. Using the data from the 2 selected ISIs for all 10 subjects, the factors were Time (2 levels: pre and post-NMES) and Condition (2 levels: peak facilitation and peak inhibition).

Changes in CS excitability induced by NMES were determined by quantifying and comparing the average amplitudes of the unconditioned control MEPs as a percentage of M_{max} evoked before and after NMES. Separate two tailed paired *t-tests* were used to test whether the amplitude of unconditioned MEPs were significantly different from before to after NMES for APB and TA. For all tests the significance level was set at $p<0.05$. *Post-hoc* analyses (Tukey HSD tests) were performed when appropriate. All descriptive statistics are reported as mean ± standard deviation.

A.3 Results

A.3.1 Median nerve stimulation

Panel a of Figure A-1 shows the time course of afferent-conditioning of MEPs elicited in APB following an electrical stimulus applied to the median nerve in one subject. The grey and black lines are representative of measurements obtained before and after NMES over the median nerve, respectively. In this individual, inhibition of ~ 50% occurred at ~15-17 ms and 22-25 ms and facilitation of ~40-90% occurred at ~17-18 ms and ~30-35 ms. Although the time course appears to shift slightly to the right for this individual following NMES, the amplitudes of peak inhibition and facilitation appear to be similar before and after NMES, even though the amplitude of unconditioned MEPs elicited in APB for this individual increased by ~300%.

The average time course across the group for the afferent-conditioning of MEPs elicited in APB by a median nerve stimulus is depicted in *Panel c* of Figure A-1. These data show that inhibition and facilitation of MEPs were much less robust when averaged across the group as compared to the individual shown in *Panel a*. Nonetheless a 2-way repeated measures ANOVA identified a significant main effect of Condition [$F_{(1,9)}$=6.1, p=0.035] but no effect of Time [$F_{(1,9)}$=3.6, p=0.088] and no interaction [$F_{(1,9)}$=0.048, p=0.831]. MEPs were greater at the peak facilitatory ISI (30 ms for pre and 15 ms for post) than at the peak inhibitory ISI (18 ms for pre and 22 ms for post).

There was a significant increase in the amplitude of unconditioned APB MEPs from before to after NMES. The mean amplitude of unconditioned APB

MEPs across the group increased from 9±7 % M_{max} before NMES to 15±11 % M_{max} after NMES.

A.3.2 CP nerve stimulation

Panel b of Figure A-1 shows the time course of afferent-conditioning of MEPs elicited in TA following an electrical stimulus applied to the CP nerve in one subject. The grey and black lines are representative of measurements obtained before and after a prolonged period of NMES over the CP nerve, respectively. In this individual conditioned MEPs were lowest (~25-50% of unconditioned) at ISIs of ~35-40 ms and highest (~75-90% of unconditioned) at ISIs of ~32 ms and ~45-50 ms, and were similar before and after NMES despite an increase in unconditioned TA MEPs of ~15%.

The average time course across the group for the afferent-conditioning of MEPs elicited in TA by a CP nerve stimulus is depicted in *Panel d* of Figure A-1. Conditioned MEPs were more similar to unconditioned MEPs when averaged across the group as compared to the individual shown in *Panel a*. Nonetheless, a 2-way repeated measures ANOVA identified a significant main effect of Condition [$F_{(1,9)}$=8.1, p=0.022] but no effect of Time [$F_{(1,9)}$= 0.624, p=0.452] and no interaction [$F_{(1,9)}$= 0.227, p=0.646]. MEPs were greater at the peak facilitatory ISI (30 ms) than at the peak inhibitory ISI (45 ms).

A two-tailed paired *t-test* revealed that the amplitude of unconditioned TA MEPs did not change from before to after NMES. The mean amplitude of unconditioned TA MEPs across the group were 17±9 % M_{max} and 17±5 % M_{max} before and after NMES, respectively.

A.4 Discussion

The present experiments were designed to test the hypothesis that a prolonged period of NMES would reduce afferent-induced inhibition and enhance afferent-induced facilitation of MEPs evoked by TMS over M1. Our main finding was that the amplitude of afferent-induced inhibition and facilitation for APB did not change following a prolonged period of NMES, despite increases in CS excitability for APB. Although afferent-induced inhibition and facilitation for TA also did not change following NMES, such changes may not have been expected as CS excitability for TA was not significantly elevated during the collection of afferent-conditioning data post-NMES.

A.4.1 Time course of afferent-conditioning of MEPs

Previous research has demonstrated afferent-induced inhibition of MEPs by ~50% for APB following a median nerve stimulus delivered ~22 ms before a TMS pulse (Tokimura et al., 2000) and afferent-induced facilitation for APB of more than 100% at ISIs between 25 and 60 ms (Deletis et al., 1992). MEP responses in TA were generally depressed by a preceding CP nerve stimulus (Roy et al., 2010), potentially due to a post-synaptic effect on the motoneuron, such as recurrent inhibition, caused by stimulating the homonymous nerve (Poon et al., 2008). Due to the general depression of TA MEPs, the facilitatory effect of CP nerve stimulation on TA MEPs was observed as a weakening of the inhibition (i.e., "disinhibition") (Roy et al., 2010). Peak inhibition of ~50 % occurred at an ISI of ~30 ms and peak facilitation or "disinhibition" up to ~70% of resting unconditioned MEPs occurred at an ISI of ~40 ms (Roy et al., 2010).

In the present study, the data from individual subjects presented in Figures A-1a and A-1c are consistent with previous literature regarding the amplitude and ISIs of afferent-induced inhibition and facilitation of MEPs. However, when averaged across the group (Figures A-1b and A-1d) the amplitude of conditioned MEPs were similar to those of the unconditioned test MEPs and peak facilitation and inhibition were much less robust than previous research, suggesting that across the group the afferent inputs may have had a limited effect on inhibitory and excitatory CNS circuitry.

The weak conditioning effect of the afferent input on the MEPs may be due to the intensity of the peripheral nerve stimulus that preceded the TMS pulse. Previous research has typically used stimulus intensities near motor threshold (Tokimura et al., 2000; Roy et al., 2010), whereas the intensity used in the present study evoked an M-wave that was ~15% M_{max} to match that used during the prolonged period of NMES. While the higher intensity nerve stimulus may have produced large facilitation at the level of M1, it may also have triggered inhibitory mechanisms, such as recurrent inhibition, in the spinal cord at the same time, resulting in a cancelation of any net facilitatory or inhibitory effect on the MEP. Thus, this intensity of nerve stimulus could have interfered with our ability to observe the afferent-induced inhibition and facilitation reported in previous research. Despite all of this, across the group the MEPs evoked at the peak facilitatory ISI were significantly larger than the MEPs evoked at the peak inhibitory window in all instances, indicating that there was an effect of the afferent stimulus on MEPs. Furthermore, when MEP amplitude for each ISI was

averaged across the group, the ISIs yielding peak inhibition and facilitation were mostly consistent with past research for hand muscles. Similar to the ISIs reported in past research, peak inhibition for APB MEPs occurred at ~18-22 ms, and peak facilitation occurred at ~30 ms. For leg muscles, the inhibitory and facilitatory windows appeared to be reversed compared to previous research (Roy et al., 2010). Peak inhibition of TA MEPs occurred at ~45 ms and facilitation ("disinhibition") occurred at ~30 ms.

A.4.2 Afferent-conditioning of MEPs before and after NMES

As NMES increases M1 excitability, I hypothesized that it may also alter the effect of afferent inputs on M1 inhibitory and excitatory circuitry. The data did not support our hypothesis and suggests that NMES-induced CS excitability increases for APB do not occur concomitantly with changes in afferent-induced inhibition and facilitation of M1 and spinal circuitry for APB. However, the increase in the amplitude of unconditioned MEPs for APB following NMES may have obscured the comparison of afferent-induced inhibition and facilitation before and after NMES. The larger MEP indicates that the TMS activated additional CS neurons after the NMES than before it and thus the afferent-conditioning effect was tested in different CS neurons before and after the NMES. If the TMS intensity had been turned down following the NMES to elicit unconditioned MEPs of similar amplitude to those observed before NMES, then it would be more likely that a similar subset of cortical neurons was tested before and after NMES, and this may have provided a more sensitive measure of the afferent-conditioning.

In contrast to CS excitability increases observed for APB, evaluation of the amplitude of unconditioned MEPs suggests that CS excitability for TA was not changed following NMES at the time when the conditioning trials were conducted. The experiments described in Chapter 3 were conducted in the same sessions as these experiments and showed increases in CS excitability for TA following NMES; however, the data for this study was collected after other measures that were included in Chapter 3 and the effect of NMES on CS excitability for TA may have been reduced by the time these data were collected. Thus, if CS excitability for TA was no longer enhanced when the post-NMES afferent-conditioning data was collected, then differences in afferent-induced inhibition and facilitation for TA compared to the pre-NMES measures would not be expected.

A.4.3 Conclusions

The present study suggests that when a prolonged period of NMES increases CS excitability for APB, it does not alter the effect of afferent input on CNS circuitry. It is important to note that the amplitude of peak afferent-induced inhibition and facilitation were less robust than previous research and thus the ability to see such changes may have been compromised. However, even in individual subjects that showed more robust afferent-induced inhibition and facilitation, changes following NMES were not apparent (see Figure A-1). Further studies using lower peripheral nerve stimulus intensities, testing afferent-conditioning immediately following NMES, and controlling the amplitude of unconditioned MEPs before and after NMES, are necessary to conclusively

determine the effect or lack of effect of NMES on the afferent-conditioning of MEPs. An increase in CS excitability with no concomitant change in afferent-induced inhibition or facilitation following NMES would suggest that NMES increases the excitability of motor pathways without altering the influence of sensory input on CNS circuitry.

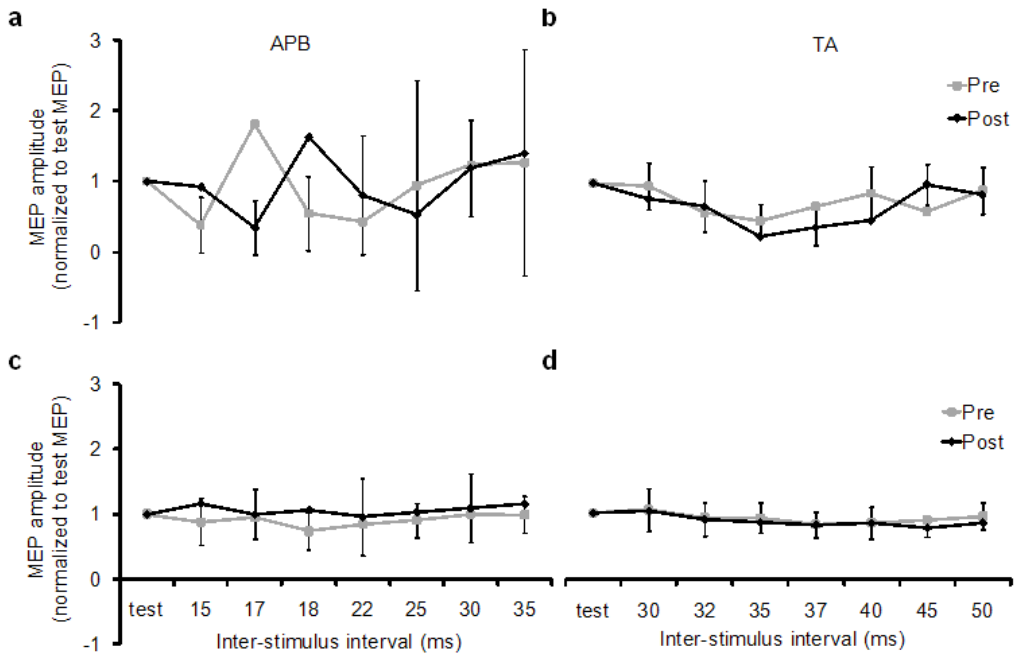

Figure A-1 Afferent-conditioning of MEPs before and after NMES. *Panels a* and *b* show the amplitude of MEPs elicited in APB and TA in a single subject when a median or CP nerve stimulus precedes the TMS pulse by varying ISIs. *Panels c* and *D* show the amplitude of MEPs in APB and TA averaged across the group, when a median or CP nerve stimulus precedes the TMS pulse by varying ISIs. All MEPs are normalized to the amplitude of unconditioned (*test*) MEPs. Grey and black lines depict afferent-conditioning data collected before (*pre)* and after (*post)* NMES. *Error bars* represent one standard deviation. For clarity, error bars have been staggered and are shown for the data point at every second ISI for data collected before (*pre)* and after (*post)* NMES.

A.5 References

Conforto, AB, Kaelin-Lang, A, Cohen, LG (2002) Increase in hand muscle strength of stroke patients after somatosensory stimulation. Ann Neurol 51: 122-125

Deletis, V, Schild, JH, Beric, A, Dimitrijevic, MR (1992) Facilitation of motor evoked potentials by somatosensory afferent stimulation. Electroencephalogr Clin Neurophysiol 85: 302-310

Deuchert, M, Ruben, J, Schwiemann, J, Meyer, R, Thees, S, Krause, T, Blankenburg, F, Villringer, K, Kurth, R, Curio, G, Villringer, A (2002) Event-related fMRI of the somatosensory system using electrical finger stimulation. NeuroReport 13: 365-369

Foltys, H, Krings, T, Meister, IG, Sparing, R, Boroojerdi, B, Thron, A, Topper, R (2003) Motor representation in patients rapidly recovering after stroke: a functional magnetic resonance imaging and transcranial magnetic stimulation study. Clin Neurophysiol 114: 2404-2415

Hoffman, LR, Field-Fote, EC (2007) Cortical reorganization following bimanual training and somatosensory stimulation in cervical spinal cord injury: a case report. Phys Ther 87: 208-223

Koski, L, Mernar, TJ, Dobkin, BH (2004) Immediate and long-term changes in corticomotor output in response to rehabilitation: correlation with functional improvements in chronic stroke. Neurorehabil Neural Repair 18: 230-249

McKay, DR, Ridding, MC, Thompson, PD, Miles, TS (2002) Induction of persistent changes in the organisation of the human motor cortex. Exp Brain Res 143: 342-349

Poon, DE, Roy, FD, Gorassini, MA, Stein, RB (2008) Interaction of paired cortical and peripheral nerve stimulation on human motor neurons. Exp Brain Res 188: 13-21

Ridding, MC, Brouwer, B, Miles, TS, Pitcher, JB, Thompson, PD (2000) Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 131: 135-143

Rosenkranz, K, Rothwell, JC (2003) Differential effect of muscle vibration on intracortical inhibitory circuits in humans. J Physiol 551: 649-660

Rothwell, JC, Rosenkranz, K (2005) Role of afferent input in motor organization in health and disease. IEEE Eng Med Biol Mag 24: 40-44

Roy, FD, Gorassini, MA (2008) Peripheral sensory activation of cortical circuits in the leg motor cortex of man. J Physiol 586 (Pt 17): 4091-105

Roy, FD, Yang, JF, Gorassini, MA (2010) Afferent regulation of leg motor cortex excitability after incomplete spinal cord injury. J Neurophysiol 103: 2222-2233

Spiegel, J, Tintera, J, Gawehn, J, Stoeter, P, Treede, RD (1999) Functional MRI of human primary somatosensory and motor cortex during median nerve stimulation. Clin Neurophysiol 110: 47-52

Tokimura, H, Di, L, V, Tokimura, Y, Oliviero, A, Profice, P, Insola, A, Mazzone, P, Tonali, P, Rothwell, JC (2000) Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol (Lond) 523: 503-513