University of Alberta

A comparison of neuromuscular electrical stimulation parameters on increasing corticospinal excitability

by

Alyssa R. Hindle

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Abstract

In this thesis, experiments that investigated the effects of neuromuscular electrical stimulation (NMES) parameters on corticospinal (CS) excitability are described. The NMES-induced afferent volley can increase CS excitability, which can facilitate neurorehabilitation. However, literature outlining the optimal NMES parameters for increasing CS excitability is limited. In the first project, we found that functional electrical stimulation (NMES with high stimulus intensity and frequency) increased CS excitability, while somatosensory stimulation (NMES with low stimulus intensity and frequency) did not. In the second project, longer pulse durations increased the magnitude of H-reflexes from tibialis anterior, but we found no difference in their effects on CS excitability between pulse durations. These results provide insight for which parameters of NMES best increase CS excitability. Improvements in function resulting from NMES are attributed to the NMES-induced increases in CS excitability, thus identifying how to best increase CS excitability may be important for rehabilitative applications.

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List of Symbols and Abbreviations

τ_{SD}	strength duration time constant
AMT	active motor evoked potential threshold
APB	abductor pollicis brevis
CS	corticospinal
CNS	central nervous system
CP	common peroneal
D-wave	direct wave
ECU	extensor carpi ulnaris
EMG	electromyography
FDI	first dorsal interosseous
FES	functional electrical stimulation
GABA	gamma-aminobutyric acid
H-reflex	Hoffmann reflex
$H_{6\%Mmax}$	H-reflex amplitude when the M-wave is ~6% of maximal
H_{max}	maximal H-reflex amplitude
H_{max}:M_{max}	ratio of maximal H-reflex amplitude to maximal M-wave amplitude
H_{Mmax}	H-reflex amplitude when M-wave is maximal
I-wave	indirect wave
I_h	hyperpolarization activated current
LSD	least significant difference
LTD	long term depression
LTP	long term potentiation
NMES	neuromuscular electrical stimulation
NMDAR	*N*-methyl-D-aspartate receptor
M-wave	motor wave
MEP	motor evoked potential
MEP-RC	motor evoked potential recruitment curve
MEP_{max}	maximal motor evoked potential amplitude
MH-RC	M-wave/H-reflex recruitment curve
M_{Hmax}	M-wave amplitude when H-reflex amplitudes are maximal
M_{max}	maximal M-wave amplitude

MVC	maximum voluntary contraction
PAS	paired associative stimulation
RM-ANOVA	repeated measures analysis of variance
RMT	resting motor evoked potential threshold
rTMS	repetitive transcranial magnetic stimulation
SS	somatosensory stimulation
STDP	spike timing dependent plasticity
TA	tibialis anterior
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation

CHAPTER 1: General Introduction

1.1 Preface

"Neuroplasticity" refers to the ability of the nervous system to adapt both anatomically and functionally to changes in neuronal activity (Cohen et al. 1991; Ridding et al. 2001). Neuroplasticity can be adaptive, and is part of normal development and motor learning (Buonomano and Merzenich 1998; Kleim et al. 2004). However, after damage to the nervous system, neuroplasticity can be maladaptive and may result in decreased ability to control movement (Pascual-Leone et al. 2005). Either way, these changes occur in a use dependent manner (Bayona et al. 2005) and can be conceptualized as a "use it or lose it" principle. To counteract maladaptive changes and promote beneficial neuroplasticity, rehabilitative techniques have been designed to increase the excitability of corticospinal (CS) pathways that control movement, and evidence suggests that increases in excitability are linked with lasting improvements in function (Everaert et al. 2010; Fraser et al. 2002). Modification of the excitability in the CS pathway has been attributed to long-term changes in synaptic efficiency in the cortex, known as long-term potentiation (LTP) and long-term depression ([LTD]Ridding and Rothwell 2007). The aim of many rehabilitative techniques is to exploit these LTP-like and LTD-like mechanisms for therapeutic applications (Ridding and Rothwell 2007).

It is important to identify how to manipulate CS excitability to enable the development of efficient rehabilitative programs for improving motor control. The excitability of the sensorimotor cortex can be influenced by changes in peripheral and central activity. Accordingly, one way to alter CS excitability is by the application of neuromuscular electrical stimulation ([NMES] Chipchase et al. 2011; Mang et al. 2010; Ridding et al. 2000). NMES involves the application of an electrical current over a peripheral nerve or muscle belly. The parameters of NMES used (i.e. pulse amplitude, duration, and frequency) can be manipulated in a variety of ways, and have a direct influence on the sensory volley that is sent to the central nervous system (Bergquist et al. 2011; Chipchase et al. 2011; Fraser et al. 2002). Although a direct comparison between the parameters of NMES and their respective effects on CS excitability would be beneficial to develop more efficient rehabilitative programs, it has not been done

and the parameters used throughout the literature are varied (Bergquist et al. 2011; Chipchase et al. 2011). Further, as NMES is already used in clinical settings to reduce muscle atrophy (Bergquist et al. 2011), maintain bone density and improve cardiovascular health (Sheffler and Chae 2007), the identification of optimal parameters for increasing CS excitability may provide an opportunity to enhance the benefits gained from current rehabilitative NMES procedures. The overarching theme of this thesis is to investigate how different parameters of NMES influence CS excitability. Accordingly, this introductory chapter provides a brief overview of the neural connectivity between sensory input and motor output (Section 1.2) and the mechanisms behind changes in CS excitability (Section 1.3) as well as how CS excitability is measured (Section 1.4). In Section 1.5.1, as the effect of NMES parameters on CS excitability is the focus of this thesis, an in-depth discussion of NMES and how the parameters affect the sensory volley and CS excitability is provided. Further, given other techniques using electrical stimulation and transcranial magnetic stimulation (TMS) also influence CS excitability, a selection of these approaches is also outlined in Sections 1.5.2 through 1.5.4 of this chapter. Finally, an overview will be provided of the subsequent two research chapters that investigate NMES parameters and their influence on CS excitability.

1.2 Sensorimotor Pathways that Control Movement

A goal of many rehabilitative techniques is to strengthen connections between sensory (ascending) and motor (descending) pathways in the nervous system. Thus, it is important to understand the structural connectivity between these pathways. The following provides a brief overview of the major pathways involved, although other areas in the nervous system have roles in sensorimotor processing that are not discussed herein.

Somatosensory information from receptors in skin, muscles and joints of the trunk and limbs is propagated along ascending pathways to the brain. From these receptors, somatosensory information travels through the dorsal root ganglion into the spinal cord where the sensory fibers terminate on interneurons and motoneurons, providing the basis for simple reflex pathways, or ascend toward the brain along the

ascending pathways (see Figure 1-1). Several pathways transmit sensory information to the brain. For somatosensory information, the most important pathways are the dorsal column-medial lemniscal system, or, more simply, the "dorsal columns" which conveys proprioceptive information. En route to the brain, the dorsal columns cross the midline at the medullary pyramid and continue to travel through the pons and midbrain to the thalamus (Chang and Ruch 1947).

Another important ascending pathway is the spinothalamic pathway, which conveys both nociceptive and thermal sensory information (Bowsher 1957). The spinothalamic pathway crosses the midline at the level of the spinal cord, and then ascends ispilaterally towards the brain, terminating on the ventral posterior lateral nucleus of the thalamus (Bowsher 1957). This information is then sent to the somatosensory cortex, the dorsal anterior insular cortex, and to the anterior cingulate gyrus (Kandel et al. 2000).

After synapsing in the thalamus, the afferent volley transmitted from the dorsal column-medial lemniscus pathway is sent to the cerebral cortex where it can modulate the output of the descending pathways (Kandel et al. 2000). The thalamus relays these afferent volleys directly to the primary somatosensory cortex (Broadmann areas 3, 1 and 2) and, to a lesser extent, the primary motor cortex (Broadmann area 4), as well as the somatosensory association cortex (Broadmann area 5) and the premotor cortex ([Broadmann area 6] Jones 1986). The primary pathway by which low threshold group 1 afferents influence motor output is indirect: first from area 3a/3b, then posteriorly to areas 1 and 2, the latter having strong connections to area 4 (Jones et al. 1978). Additionally, areas 1, 2, 4 and 5 project to area 6, and area 6 projects back to area 3a, 4 and 5 (Jones et al. 1978). Thus, through a brief relay or direct connection, information from the dorsal columns is transmitted to the primary motor cortex.

Once input reaches the primary motor cortex (area 4), the descending pathways are activated for motor commands to exit the brain. In the CS pathway, the descending pathway that is most directly involved in the control of movement, the axons of neurons originating in the motor cortex descend through the white matter of the brain, where the lateral CS tract crosses the midline at the medulla (Yeo and Jang 2011) while the

anterior CS tract descend ipsilaterally down the spinal cord. Upon reaching the target level of the spinal cord, these fibers synapse with motoneurons and interneurons responsible for controlling movement. Given the structural connectivity between sensory and motor areas of the nervous system, it is clear that sensory input can influence motor output.

1.3 Cortical Mechanisms of Changes in Corticospinal Excitability

Although outside the scope of the research within this thesis, the mechanisms of change in CS excitability are important to consider to best develop techniques that influence CS excitability. Lasting changes in CS excitability are attributed to the plastic nature of synaptic connections. LTP is a type of plasticity that results in long-lasting increases in synaptic strength, while LTD has the opposite result (Bliss and Cooke 2011). Both types of plasticity are activity dependent and input specific (Bliss and Cooke 2011). Spike timing-dependent plasticity (STDP) is one type of plasticity that is characterized by the reliance on the timing of paired activity between pre- and postsynaptic cells to elicit either LTP or LTD (Bliss and Cooke 2011). LTP is induced when activity occurs in the presynaptic cell before the postsynaptic cell within a specific window of time, and LTD is induced when the opposite occurs (Markram et al. 1997). The cellular mechanism responsible for these changes is the binding of glutamate to the *N*-methyl-D-aspartate receptors (NMDARs) and the level of calcium ions in the postsynaptic cell, which is influenced by the activity of the postsynaptic dendrite (Cooke and Bliss 2006; Hoogendam et al. 2010). As such, both LTP and LTD involve the influx of calcium into the postsynaptic cell, however the difference lies in the fact that LTP is dependent on a large and fast influx of calcium and LTD is dependent on a small and slow influx of calcium (Hoogendam et al. 2010).

The mechanisms of LTP and LTD are not restricted to glutamatergic synapses, as is shown in hippocampal slices, and different mechanisms have been implicated for different locations in the central nervous system (Bliss and Cooke 2011). For example, modulation via gamma-aminobutyric acid (GABA) has been implicated (Kaelin-Lang et al. 2002; Woodin et al. 2003) as well as downstream mechanisms (Dan and Poo 2004).

Most of the literature investigating LTP and LDP involves animal models (Bliss and Lomo 1973; Woodin et al 2003), *in vitro* methodologies (Markram et al. 1997) and often focus on memory and the hippocampus (Bliss and Lomo 1973). As a result, it is important to exercise caution when proposing these mechanisms for the data collected from non-invasive and indirect studies in humans.

Nonetheless, plasticity in cortical circuits can be investigated by methods using TMS. For example, paired-pulse TMS is a technique that involves the application of two strategically timed TMS pulses to the cortex; the first pulse is a conditioning stimulus and the second is the test stimulus (Kujirai et al. 1993). Depending on the timing between these two pulses, either excitation or inhibition can be induced and as a result intracortical inhibition or intracortical facilitation can be investigated (Kaelin-Lang et al. 2002; Kujirai et al. 1993). Another technique used to study cortical circuits is afferent conditioning, in which the conditioning stimulus is from peripheral nerve electrical stimulation, and the test stimulus is from TMS over the cortex (Stefan et al. 2002); this technique enables the analysis of afferent facilitation or afferent inhibition (Mang et al. 2012; Tokimura et al. 2000). Studies investigating the influence of NMES on CS excitability found no changes in short-interval intracortical inhibition or intracortical facilitation with paired-pulse TMS (Kaelin-Lang et al. 2002), though with afferent conditioning found increases in afferent facilitation and a decrease in short-latency afferent inhibition (Mang et al. 2012). These findings suggest an enhancement of facilitatory pathways and a suppression of inhibitory pathways (Mang et al. 2012), and these changes have been attributed to LTP-like mechanisms (Stefan et al. 2002; 2006).

1.4 Transcranial Magnetic Stimulation for Measuring Changes in Corticospinal Excitability

Though identifying the mechanisms behind changes in CS excitability requires further research, there is widespread evidence that sensory inputs have lasting effects on CS excitability. In humans, CS excitability is often quantified by the amplitude of motor evoked potentials (MEPs) generated by delivering a single pulse of TMS over the motor cortex. The TMS coil consists of a wire through which rapidly changing electrical

current is passed (Terao and Ugawa 2002). This current produces a magnetic field beneath the coil that induces an eddy current in the cortex (Terao and Ugawa 2002). The induced current can evoke action potentials, excitatory postsynaptic potentials, or inhibitory postsynaptic potentials (Terao and Ugawa 2002). There are a variety of types of TMS coils including circular coils, figure-of-eight coils, and double-cone coils (Terao and Ugawa 2002). Each coil type will vary magnetic strength and focus (Terao and Ugawa 2002). The type of coil used for the research in this thesis is a figure-of-eight coil. With this type of coil, the largest responses to TMS from intrinsic hand muscles are generated when the current is applied in a posterior-anterior direction (Hallett 2007), as such, the coil is typically held at a 45° angle to sagittal midline (Chipchase et al. 2011; Hallett 2007; McKay et al. 2002). The application of TMS generates two types of responses; "direct-waves" (D-waves) that occur with direct stimulation of descending neurons in the CS pathway; and "indirect-waves" (I-waves) that occur when these same neurons are activated transsynaptically (Terao and Ugawa 2002). There are multiple I-waves and they appear at respectively longer latencies and with lower thresholds than D-waves (Terao and Ugawa 2002). As such, MEPs are predominantly generated transsynaptically (via I-waves) at lower TMS intensities and the contribution of directly activated CS fibers (via D-waves) increases at higher TMS intensities (Di Lazzaro et al. 1998).

When action potentials generated by TMS evoke a muscle contraction, it can be observed using electromyography as an MEP. However, due to spontaneous variability in primarily cortical (Ellaway et al. 1998) but also segmental motoneuronal excitability, there is random variability in the amplitude of MEPs from stimulus to stimulus (Kiers et al. 1993). Other variables such as the cognitive state of the participant, and even whether the participant is thinking of the target muscle contracting can impact MEP amplitudes (Gandevia and Rothwell 1987). The variability of MEPs can be reduced by increasing the TMS intensity (Kiers et al. 1993) to generate predominantly D-waves (Di Lazzaro et al. 1998). Variability can also be reduced by holding a background contraction in the target muscle to create a stable level of descending input and motoneuron excitability (Darling et al. 2006; Kiers et al. 1993). For this purpose,

investigators will record MEPs while participants hold a small background contraction (Knash et al. 2003), and refer to the lowest level of TMS intensity required to generate an MEP as "active MEP threshold" (AMT). In this way, changes in AMT are used to reflect changes in CS excitability. However, despite MEP variability at rest, many studies continue to collect MEPs while the subject is relaxed (Charlton et al. 2003; Khaslavskaia and Sinkjaer 2005; McKay et al. 2002; Ridding et al. 2000); in resting conditions, the lowest level of TMS intensity to generate an MEP is referred to as "resting MEP threshold" (RMT) which is also used as an indicator of CS excitability. For both AMT and RMT, a decrease in threshold indicates an increase in CS excitability. A limitation of measuring AMT instead of RMT is that the mechanisms responsible for the observed changes in CS excitability may not be the same between the active and resting conditions, though this has neither been supported nor refuted in the literature. Should this limitation exist, RMT may be a more appropriate measure because NMES is commonly used in neurorehabilitation for people with motor deficits (Chae et al. 2008; Chipchase et al. 2011; Liberson et al. 1961; Merletti et al. 1978) and volitional muscle control is not necessary to establish RMT.

CS excitability can also be quantified by collecting MEPs at TMS intensities above threshold levels. Often a percentage of threshold TMS intensity is delivered ([i.e. ~120% RMT] Mang et al. 2010, Khaslavskaia et al. 2002, Ridding et al. 2000), or a set increment of TMS intensity above threshold is used ([i.e. RMT + 15% maximum TMS output] McKay et al. 2002) to generate MEPs and observe changes in CS excitability. CS excitability can be assessed by comparing the amplitudes of maximal MEPs (i.e. MEP_{max}) as well. Stimulus/response curves, or recruitment curves, can also be constructed from experiments in which MEPs are recorded at TMS intensities ranging from below threshold to above maximum MEP amplitudes (Khaslavskaia et al. 2002; Ridding and Rothwell 1997). By constructing pre- and post-intervention MEP recruitment curves it is possible to compare the relative input required before and after an intervention to achieve a given response to the TMS. In this way changes in threshold, recruitment curve slope and MEP_{max} can be more accurately determined and compared. To reliably observe changes in MEP amplitudes while minimizing the impact of MEP variability on

results, multiple MEPs are collected at a given TMS intensity, and average MEP amplitudes are calculated (Ellaway et al. 1998).

1.5 Non-invasive Techniques for Influencing Corticospinal Excitability

After motor rehabilitation, the induced increases in CS excitability are correlated with lasting improvements in movement (Everaert et al. 2010). As such, there are a variety of stimulation techniques that have been developed to increase CS excitability. These experimental and clinical techniques are varied, and include electrical stimuli over peripheral nerves and muscles (i.e. NMES); electrical stimulation of the cortex through the intact skull (transcranial direct current stimulation [tDCS]); magnetic stimuli applied repetitively to the motor cortex (repetitive transcranial magnetic stimulation [rTMS]); and the application of paired peripheral electrical stimulation and cortical magnetic stimulation (PAS). These techniques are discussed below.

1.5.1 Neuromuscular Electrical Stimulation (NMES)

NMES is a rehabilitative technique commonly used to facilitate motor relearning, counteract muscle atrophy and produce functional movements for patients with spinal cord injury or stroke (Bergquist et al. 2011; Sheffler and Chae 2007). When NMES is applied over a peripheral nerve or muscle belly, and depolarizes underlying axons, generating action potentials in motor fibers that travel directly from the stimulus site toward the muscle (peripheral pathway), and also generates action potentials in sensory fibers that travel from the stimulus site to the spinal cord ([central pathway] Misiaszek 2003; Bergquist et al. 2011). The central and peripheral pathways activated by NMES are outlined in Figure 1-1 where orthodromic transmission along sensory and motor axons is displayed by solid arrows. In the peripheral pathway, action potentials will generate muscle activity that can be observed using electromyography as the “M-wave” (Bergquist et al. 2011). As well, an antidromic volley will be sent along the motor axon to the motoneuron. When the motoneuron “backfires”, action potentials are sent back along the motor fiber towards the muscle, and is observed using electromyography as an “F-wave” (Fisher 2007). Simultaneously, in the central pathway, the activated

sensory fibers synapse with interneurons and motoneurons in the spinal cord, thus sending a motor volley back towards the muscle; this will result in a waveform observed in electromyography as the “Hoffmann-reflex”, or the “H-reflex” (Misiaszek 2003; Bergquist et al. 2011). Additionally, the depolarized sensory fibers can send feedback from the stimulus site through the spinal cord to the brain (Bergquist et al. 2011) and this is what can alter CS excitability.

1.5.1.1 NMES Alters Corticospinal Excitability

The sensory volley induced by NMES and can lead to the reorganization of the human motor and sensory cortices (Pitcher et al. 2003; Ridding et al. 2001). Sensory input from NMES reduce afferent inhibition and increase CS excitability (Mang et al. 2012). Overtime, these changes are hypothesized to strengthen the CS pathway and lead to improvements in functional movements that outlast the NMES sessions (Everaert et al. 2010; Fraser et al. 2002). However, in order for NMES to increase CS excitability, both the motor and sensory fibers must be activated simultaneously (Knash et al. 2003). Sensory activation alone, in the absence of motor activity, has been shown to have no effect on CS excitability (Knash et al. 2003) or even decrease CS excitability in some cases (Chipchase et al. 2011). Sensory input is thought to be a “primer” for the motor cortex (Hoffman and Field-Fote 2007) and when paired with motor fiber activation can lead to increased CS excitability (Knash et al. 2003). This observation is likely due to the higher amplitudes of stimulation required to elicit motor responses; as the NMES amplitude is increased, motor fibers will be recruited, and also greater number of sensory fibers will be activated, and this larger sensory volley is required to increase CS excitability.

1.5.1.2 The Effect of NMES Parameters on Corticospinal Excitability

The magnitude of the sensory volley is a vital component in altering CS excitability following NMES. Because the parameters of NMES determine the magnitude of the sensory volley, the parameters of NMES thus determine the effect on CS excitability (Chipchase et al. 2011). NMES parameters that influence the sensory

volley include stimulus location (i.e. over a muscle belly or nerve trunk), pulse amplitude, pulse duration and pulse frequency (Bergquist et al. 2011). A schematic of pulse amplitude, duration and frequency is displayed in Figure 1-2. The location of stimulation will influence the recruitment of motor units and the relative contribution of central and peripheral pathways to the muscle contraction (Bergquist et al. 2012). The pulse amplitude and pulse duration will determine the number of axons activated.

Longer pulse durations preferentially activate sensory fibers over motor fibers (Lagerquist and Collins 2008; Panizza et al. 1989; Veale et al. 1973) and increase reflex contributions in muscle contractions (Lagerquist and Collins 2010). This effect is attributed to the lower rheobase (the minimum current required to elicit an action potential) and longer strength-duration time-constant (the relationship between the strength and duration of stimulus required to elicit an action potential) of sensory axons versus motor axons (Veale et al. 1973). These membrane properties have been hypothesized to be a result of sensory axons having a greater quantity of "threshold channels" (likely persistent sodium channels) that are not completely inactive at resting membrane potentials (Bostock and Rothwell 1997). Sensory axons are closer to threshold at rest, and thus may have more of these "threshold channels" or that they are more active in sensory axons than motor axons (Bostock and Rothwell 1997). Alternatively, inwardly rectifying currents activated by hyperpolarization are also implicated as responsible for the longer strength-duration time-constant of afferent fibers (Howells et al. 2012). Although it is clear that increasing pulse duration increases the sensory volley and H-reflex amplitude for soleus (Lagerquist and Collins 2008), it is unknown whether this effect is similar for tibialis anterior, and whether there is an effect on CS excitability. As such, Chapter 3 of this thesis investigates the effect of different pulse durations on H-reflex amplitudes and on increasing CS excitability for tibialis anterior.

The final NMES parameter that affects the sensory volley is pulse frequency. This parameter determines the firing rate of action potentials, and thus the temporal summation at the muscle and within the central nervous system (Sheffler and Chae 2007). The natural firing rates of motor fibers rarely exceed 30Hz (Macefield et al.

1993), and though motor fibers are capable of firing at higher frequencies with NMES, it is speculated that the application of higher frequencies may preferentially activate sensory fibers as they have higher intrinsic firing rates (Knash et al. 2003). The effect of specific NMES frequencies on altering excitability may differ between ascending pathways. For the corticobulbar pathway, frequencies of 1Hz and 5Hz increased excitability, while 10Hz and 20Hz decreased excitability (Fraser et al. 2002). Whereas in the CS pathway, it is reported that frequencies above 90Hz-100Hz increase excitability (Chipchase et al. 2011; Mang et al. 2010), while 10Hz, 50Hz and 200Hz did not affected CS excitability (Mang et al. 2010).

1.5.1.3 The Effect of NMES Protocols on Corticospinal Excitability

The NMES parameters used to increase CS excitability throughout the literature are not consistent, so a comparison of the effects of different parameters on changes to CS excitability is not currently possible (Bergquist et al. 2011; Chipchase et al. 2011). Often more than one parameter is manipulated at a time, creating different "protocols" of NMES. Two commonly used NMES protocols are somatosensory stimulation (SS) and functional electrical stimulation (FES). SS is designed to primarily activate sensory fibers to "prime" the CS pathway for training (Hoffman and Field-Fote 2007). Throughout the literature, the application of SS for the purpose of increasing CS excitability has involved stimulation amplitudes near or slightly above resting motor threshold, at frequencies below 30Hz and long pulse durations of ~1ms (Bergquist et al. 2011; Kaelin-Lang et al. 2002; McKay et al. 2002; Ridding et al. 2000). FES is used to evoke fused muscle contractions, and can be used to generate functional motor output and assist in performing movements such as ankle dorsiflexion for individuals with foot drop (Everaert et al. 2010; Sheffler and Chae 2007; Wieler et al. 1999) and grasping (Prochazka et al. 1997; Sheffler and Chae 2007) for people who have had a spinal cord injury or stroke. FES is typically delivered at a high stimulation amplitude, well above motor threshold, at frequencies of ~12-25Hz and pulse durations of 200μs (Sheffler and Chae 2007).

The experiments described in Chapter 2 of this thesis were designed to compare the effects of FES and SS on changes in CS excitability. Previously, FES and SS were found to increase CS excitability for a muscle of the hand from pre- to post-intervention, but the time-course of the changes in CS excitability was not investigated (Bergquist et al. 2011). Ridding and colleagues (2000) also demonstrated that SS increases CS excitability, but again did not investigate the time-course of changes in CS excitability. Further research is necessary to compare the effect of NMES protocols on the time-course of changes in CS excitability given that these changes appear to be nerve and/or muscle dependent (Mang et al. 2011). Chapter 2 addresses this gap in the literature, and the time-course of changes in CS excitability during the application of FES and SS over the median and ulnar nerves were investigated.

There is wide inter-study and inter-subject variability in how CS excitability is affected by NMES, which may mean that there will also be a difference in the associated benefits between individuals (Chipchase et al. 2011). NMES is reported to increase CS excitability by up to 100% (Khaslavskaia et al. 2002), and is typically delivered above motor threshold to generate increases in CS excitability (Chipchase et al. 2011). However, decreases in CS excitability by ~50% are also reported (Charlton et al. 2003; Murakami et al. 2007) and typically occur when NMES is delivered below motor threshold (Chipchase et al. 2011). Overall, the evidence in the literature is unclear as to which parameters or protocols of NMES influence CS excitability in either direction (Chipchase et al. 2011).

1.5.1.4 Identifying the Locus of NMES-induced Changes in Corticospinal Excitability

As the CS pathway is comprised of many components (i.e. the motor cortex, CS tract, spinal cord and alpha motor neuron) to identify the mechanism of change to CS excitability, monitoring the locus of change is necessary. To determine whether changes in excitability occur within the muscle (via motor discharge with NMES) one must consider M-wave data, as changes in the M-wave reflect changes in the peripheral pathway, from the stimulus site to the muscle fiber. By observing maximum M-wave amplitude (M_{max}) measurements, it may be possible to identify changes in excitability at

the level of the muscle (Knash et al. 2003). If present, increases in excitability at the level of the muscle would potentiate the amplitude of the MEP generated from TMS (Knash et al. 2003). Further, motoneuron excitability can be monitored by analyzing F-wave responses (Stefan et al. 2002). However, changes at the level of the muscle and motoneuron are not expected as changes in CS excitability are thought to occur at the level of the cortex (Kaelin-Lang et al 2002; Knash et al. 2003; Ridding et al. 2000).

To investigate whether changes in CS excitability occur at the level of the spinal cord, H-reflex analysis is a useful tool (Misiaszek 2003). When an H-reflex is evoked, the electrical volley activates sensory fibers directly, and bypasses the muscle spindle as well as the γ-motoneuron influence on muscle spindle sensitivity, as shown in the solid arrows in Figure 1-1 (Misiaszek 2003). As such, alterations in the amplitude of the H-reflex reflect changes in the excitability of the motoneurons or changes in the quantity of neurotransmitter released at the synaptic terminal, and not changes in muscle spindle sensitivity (Misiaszek 2003). The excitability of the motoneuron is a major influence on the amplitude of the H-reflex (Misiaszek 2003), and it is common to use the H-reflex as an indicator of spinal excitability though there are limitations to this assumption which include the influence of presynaptic inhibition and post activation depression on H-reflex amplitude (Misiaszek 2003; Zehr 2002).

Tools for analyzing the locus of change in CS excitability are not limited to analyzing M-waves and H-reflexes, or to deciphering muscle and spinal contributions. Differentiating between spinal and supraspinal influences on CS excitability can be evaluated through analyzing MEP recruitment curves in conjunction with M-wave, H-reflex and F-wave data. A steeper gain in the MEP recruitment curve accompanied by M-wave, H-reflex and F-wave data that do not reflect changes in excitability at lower segments of the CS pathway suggests that the potentiation of MEPs is due to supraspinal influences. As such, conclusions from these data help to decipher whether the changes in excitability are due to muscular, spinal or supraspinal influences (Knash et al. 2003; Mang et al. 2010; Misiaszek 2003; Ridding et al. 2000). Research indicates that the locus of change in CS excitability after NMES occurs at the cortex (Ridding et al. 2000). This was identified by comparing changes in MEPs after NMES to changes in

motoneuron excitability ([observed via F-wave amplitude and incidence] Ridding et al. 2000). In another study, changes in CS excitability were observed via MEPs generated from the level of the cortex, though responses generated from brainstem electrical stimulation were not larger after NMES (Kaelin-Lang et al. 2002). Thus, with these results combined, larger MEPs evoked from the cortex (Ridding et al. 2000) but not the brainstem (Kaelin-Lang et al. 2002) and stable motoneuron excitability (Ridding et al. 2000) after NMES suggest that the changes in CS excitability are attributed to changes at the level of the cortex. The evidence of increased amplitudes of MEPs generated from the cortex but not from the brainstem is also supported by Ridding and colleagues (2000), who also monitored F-wave amplitude and found no change. Identifying the locus of change is important to better understand how NMES influences CS excitability, and may lead to identifying NMES parameters that induce optimal neuroplasticity.

1.5.2 Repetitive Transcranial Magnetic Stimulation (rTMS)

Another technique that is used to alter CS excitability is rTMS which involves trains of TMS pulses delivered to the cortex (Hoogendam et al. 2010). rTMS has a wide range of applications from improving symptoms in patients with Parkinson's disease (Pascual-Leone et al. 1994), to treating depression (George et al. 1997), to regionally modulating the excitability of the motor cortex (Wassermann 1998). Changes in excitability of the motor cortex induced by rTMS are dependent on pulse frequency (Di Lazzaro et al. 2011); increases in CS excitability occur with high frequency (>1Hz) rTMS (Ridding and Rothwell 2007) and decreases in CS excitability occur with low frequency (< 1 Hz) rTMS (Muellbacher et al. 2000). The magnitude of changes induced by rTMS has been reported from a 40% decrease in MEP amplitude after inhibitory rTMS to a 40% increase after facilitatory rTMS (Maeda et al. 2000). As with other TMS techniques, there is substantial inter-individual variability in responses to rTMS, and the frequency of rTMS does not always induce predictable changes in CS excitability across all subjects (Maeda et al. 2000). Unfortunately, there are potential adverse side effects from using rTMS including the risk of pain and headaches (Wassermann 1998). Further limitations of using rTMS include that it is non-focal, clear safety guidelines are still being

established (Huang et al. 2005; Wu et al. 2012), there is risk of excessive coil heating and specialized equipment is required.

1.5.2.1 Theta Burst Stimulation

Theta burst stimulation is a type of rTMS where three bursts of high frequency (30-50Hz), low amplitude (80% AMT) TMS stimuli are applied to the motor cortex and repeated every 200ms ([i.e. at 5Hz] Huang et al. 2005; Wu et al. 2012). LTD occurs when theta bust stimulation is applied continuously for 40 seconds and motor cortex excitability decreases for up to 60 minutes (Huang et al. 2005; Zafar et al. 2008). LTP occurs when theta burst stimulation is applied in an intermittent pattern for 3 minutes, and motor cortex excitability increases for 15 minutes (Huang et al. 2005; Zafar et al. 2008) to an hour (Di Lazzaro et al. 2008). The magnitude of increases in MEP amplitudes has been reported at ~200% (Huang et al. 2005). The benefits of using theta burst stimulation over other types of rTMS is that a lower stimulus intensity can be utilized, a fewer number of pulses are required to induce changes, the duration of the application is much shorter, and the effects can last up to one hour (Wu et al. 2012; Zafar et al. 2008; Huang et al. 2005).

1.5.2.2 Paired Pulse TMS and Quadripulse Stimulation

Repetitive paired-pulse TMS is another type of rTMS where two TMS pulses are separated by a short inter stimulus interval and repeated every 5 seconds (Thickbroom et al. 2006). Thirty minutes of paired-pulse stimulation, with inter-stimulus intervals of 1.5ms, has been shown to increase MEP amplitude up to 400%, and this effect persists for 10 minutes post-treatment (Thickbroom et al. 2006). When the number of paired TMS pulses is increased to four pulses, it is referred to as “quadripulse stimulation” (Hamada et al. 2007). After thirty minutes of quadripulse stimulation, with an inter-stimulus interval of 1.5ms, MEP amplitudes have been reported to increase by 300% for up to 90 minutes after stimulation (Hamada et al. 2007). Longer inter-stimulus intervals of paired pulse stimulation (beyond 3ms) have been shown to have the opposite effect on MEP amplitudes (Khedr et al. 2004), as have longer inter-stimulus intervals of

quadripulse stimulation ([beyond 30ms] Hamada et al. 2008). A disadvantage to paired pulse and quadripulse stimulation is the high TMS intensity required and the long stimulation period (~30 minutes). An advantage to these methods is the large amplitude of effect and the long effect duration after the stimulation is over.

1.5.3 Paired Associative Stimulation (PAS)

PAS is the activation of the motor cortex by two nearly-simultaneous inputs; the first from the sensory volley generated by peripheral electrical stimulation and the second from TMS. PAS is typically delivered every 20 seconds (0.05Hz) over a conditioning period of approximately 30 minutes for a total of 90 paired stimuli (Di Lazzaro et al. 2008; Di Lazzaro et al. 2011; Huang et al. 2005; Stefan et al. 2000). Excitability of the sensorimotor cortex will increase if the TMS pulse is delivered slightly after (25ms) the latency required for the sensory input to reach the cortex (Di Lazzaro et al. 2009; Di Lazzaro et al. 2011). The opposite occurs when the TMS pulse is delivered slightly earlier (10ms) than the peripheral nerve stimulation sensory volley (Di Lazzaro et al. 2009; Di Lazzaro et al. 2011). Both cases are hypothesized to utilize a STDP-like mechanism (Bliss and Cooke 2011). The magnitude of effects from PAS is the greatest with an inter-stimulus interval of 25ms, and MEPs increase in amplitude by ~77% (Stefan et al. 2000). These effects persist for 30 minutes after the stimulation (Di Lazzaro et al. 2011; Stefan et al. 2000).

1.5.4 Transcranial Direct Current Stimulation (tDCS)

tDCS involves the application of low amplitude, continuous electric current to the brain (Nitsche and Paulus 2000). tDCS is not painful, and is described as a “mild itching under the electrodes” (Uy and Ridding 2003). Over the motor cortex in humans, anodal stimulation with tDCS induces excitation of the underlying cortex, while cathodal stimulation induces inhibition (Hallett 2007; Uy and Ridding 2003). MEP amplitudes increase up to ~40% after 5 minutes of anodal tDCS and return to baseline within 5 minutes post stimulation (Nitsche and Paulus 2000). The mechanism of change is attributed to LTP-like mechanisms (Uy and Ridding 2003). Further, tDCS has been

shown to enhance the effects of changes in CS excitability after 10 minutes of SS by 50% versus no changes in CS excitability when SS was applied alone (Uy and Ridding 2003). This effect persisted for 30 minutes after stimulation (Uy and Ridding 2003).

1.6 Thesis Outline

Neuroplasticity has the potential to be adaptive or maladaptive, resulting in improvements or decrements in the ability to control movement, respectively. Understanding how to induce beneficial changes in CS excitability is important to create efficient rehabilitative programs for improving motor control. The overarching goal of this thesis was to better understand how NMES parameters affect CS excitability. This is part of a larger body of work aiming to design rehabilitative programs that optimize beneficial neuroplasticity while simultaneously generating the cardiovascular and musculoskeletal benefits induced when NMES is used to produce movements. Although there are several different techniques to manipulate CS excitability, NMES is one of the more practical techniques for clinical use in part due to increased accessibility and safety. As such, the theme of this thesis was to investigate different protocols and parameters of NMES and how changing these variables influence CS excitability.

1.6.1 Chapter Two: A Comparison of Two Neuromuscular Electrical Stimulation Protocols on Corticospinal Excitability

The sensory volley evoked by NMES is linked to increasing CS excitability, and this may be helpful to counteract the maladaptive changes that occur after spinal cord injury or stroke (Bergquist et al. 2011; Liberson et al. 1961; Mang et al. 2010; Sheffler and Chae 2007). The goal of the experiments in Chapter 2 was to characterize the time-course of changes in CS excitability induced by two types of NMES, FES and SS, to determine if one protocol of NMES increases CS excitability sooner, and/or if one protocol of NMES increases CS excitability at a faster rate. It was hypothesized that FES would increase CS excitability sooner and at a faster rate than SS. These findings were intended to provide clearer insight for best maximizing the neuroplasticity induced by NMES. However, the results were not as expected; when FES and SS were applied in 5

minute intervals (for a total of 60 minutes) there was no significant change in CS excitability when measured as MEPs collected at 1.2x RMT. To determine why neither NMES protocol increased CS excitability at 1.2x RMT, additional data were collected from experiments where FES was delivered continuously for 60 minutes. It was hypothesized that FES would increase CS excitability to a greater extent when applied continuously as compared to when FES was applied in 5-minute intervals. The findings from this second experiment provided an indicator of whether the nature of the delivery of FES (i.e. with or without breaks) affects changes in CS excitability.

1.6.2 Chapter Three: Assessing the Influence of Changing Pulse Duration on Corticospinal Excitability

Though it has been shown that manipulating pulse durations of NMES changes the sensory volley at the level of the spinal cord (Lagerquist and Collins 2008; Lin et al. 2002; Panizza et al. 1989), there is a gap in the literature regarding whether pulse duration influences CS excitability. In experiments described in Chapter 3, three different pulse durations of NMES were applied over the CP nerve to investigate whether manipulating pulse duration affects CS excitability. Because NMES-induced increases in CS excitability are initiated by the sensory volley (Knash et al. 2003; Ridding et al. 2000), it may be advantageous to apply NMES with longer pulse durations to preferentially activate sensory fibers; thus maximizing the sensory volley and increasing the potential for changes to CS excitability. The experiments in this chapter were designed to determine whether longer pulse durations generate larger H-reflexes in tibialis anterior, and also whether there is an effect of pulse duration on CS excitability for this muscle. It was hypothesized that longer pulse durations would increase CS excitability and H-reflex amplitudes more than shorter pulse durations. The findings from this experiment provided insight into which pulse durations of NMES should be used in rehabilitation to best influence the sensory volley with the goal of potentiating beneficial neuroplasticity.

1.6.3 General Discussion

The final chapter of this thesis reviews the findings from the two research chapters, and discusses the commonalities of the findings, linking them together. Following this is a discussion of the practicalities of using these findings in "real-world" rehabilitative settings, and whether there are better techniques available to influence CS excitability. The limitations of the research herein are discussed as well as future directions and closing remarks.

1.7 Appendix: Figures

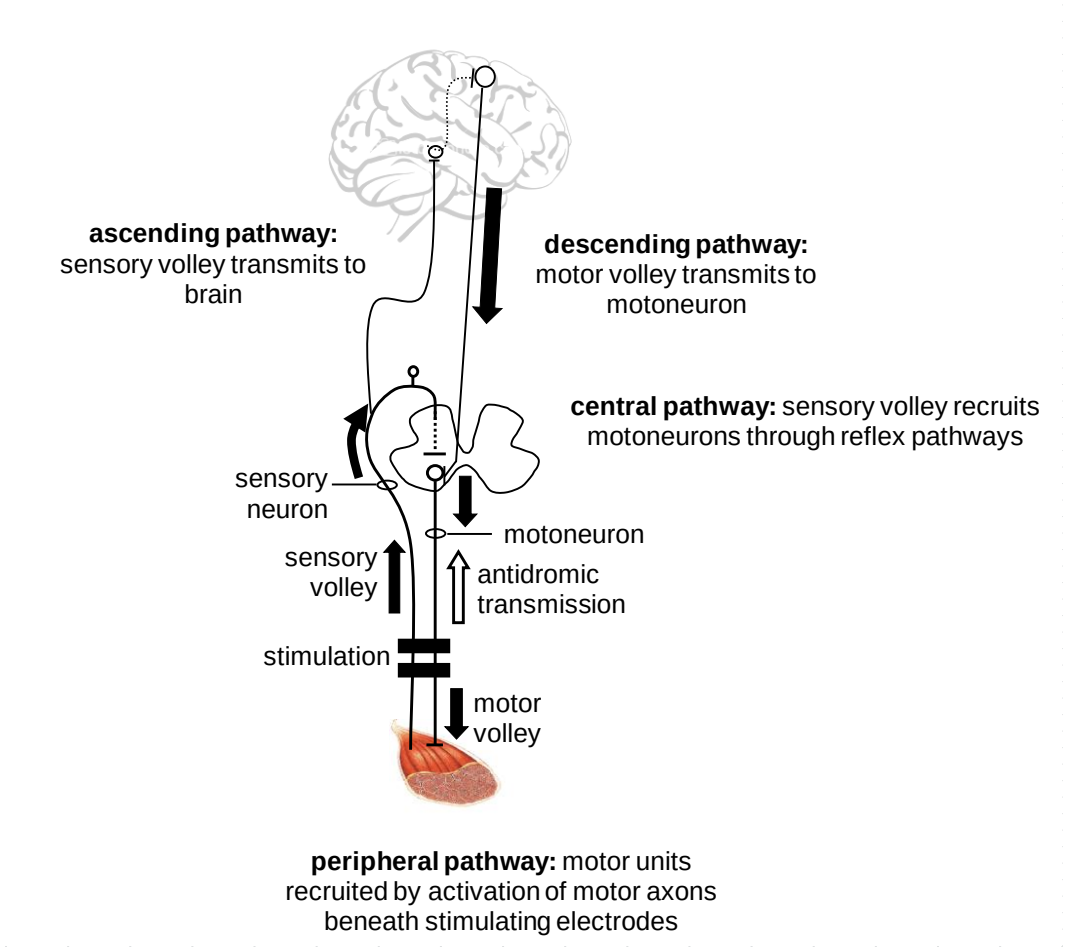

Figure 1-1. Schematic of the central and peripheral pathways activated via the application of NMES over a mixed peripheral nerve, as well as the ascending and descending pathways (adapted from Bergquist et al. 2011 and Collins 2007).

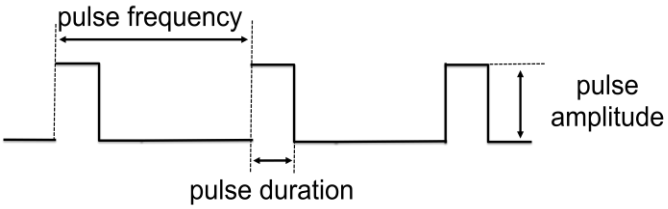

Figure 1-2. Schematic of different neuromuscular electrical stimulation parameters.

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CHAPTER 2: Functional electrical stimulation increases maximal motor evoked potentials while somatosensory stimulation does not.

2.1 Introduction

Damage to the central nervous system (CNS), such as spinal cord injury or stroke, can result in maladaptive neuromuscular changes and movement impairments. Neuromuscular electrical stimulation (NMES) is a commonly used technique to counteract these maladaptive changes and facilitate movement (Chae et al. 2008; Chipchase et al. 2011b; Liberson et al. 1961; Merletti et al. 1978). NMES involves the application of an electrical current over a muscle belly or nerve trunk to depolarize the axons beneath the stimulating electrodes. This results in action potentials that travel along central and peripheral pathways (Bergquist et al. 2011). The central pathway includes transmission of the afferent volley along the ascending pathways towards the brain. These ascending connections provide a route for the NMES input to reach supraspinal circuits that control movement (Bergquist et al. 2011). There is a growing body of evidence indicating that the afferent volley induced by NMES increases corticospinal (CS) excitability (Chipchase et al. 2011b; Mang et al. 2010; Ridding et al. 2000) and this can facilitate motor relearning and rehabilitation (Sheffler and Chae 2007). The experiments described in this chapter were designed to compare the effects of two commonly used NMES protocols on CS excitability.

Prolonged application of NMES has different effects on CS excitability depending on the parameters of NMES applied and on the individuals studied (Charlton et al. 2003; Chipchase et al. 2011a). NMES parameters include pulse amplitude, duration and frequency. The characteristics of these parameters affect the magnitude of the afferent volley (Bergquist et al. 2011; Mang et al. 2010). Currently, literature outlining the optimal NMES parameters for increasing CS excitability is limited, and the parameters used across studies varies widely; thus the optimal NMES parameters to increase CS excitability have not been identified (Chipchase et al. 2011b).

Presently we compare two types of NMES that are commonly used in rehabilitative settings and in research, known as functional electrical stimulation ([FES] Sheffler and Chae 2007; Wieler et al. 1999) and somatosensory stimulation ([SS]

Hoffman and Field-Fote 2007). SS is primarily used to send sensory input to the CNS, and thus "prime" the CNS for training (Hoffman and Field-Fote 2007). This "priming" has been shown to increase CS excitability and enhance the effects of training in patients with chronic stroke (Celnik et al. 2007; Ridding et al. 2000). During SS, stimulation is delivered at amplitudes near resting motor threshold, at relatively low frequency (below 30 Hz) and long pulse duration ([1ms] Hoffman and Field-Fote 2007; Kaelin-Lang et al. 2002; McKay et al. 2002; Ridding et al. 2000). FES, on the other hand, is delivered at higher amplitudes and frequencies than SS (i.e. at 25-200Hz) to generate fused muscle contractions (Khaslavskaia et al. 2002; Knash et al. 2003; Mang et al. 2011) to produce functional movements in people recovering from spinal cord injury or stroke (Everaert et al. 2010; Liberson et al. 1961). Following single (Fraser et al. 2002) and repeated (Everaert et al. 2010) NMES sessions there are functional improvements that outlast the stimulation.

Research indicates that there may be a "ceiling" effect for the extent to which NMES can increase CS excitability (Bergquist et al. 2011; Mang et al. 2010; McKay et al. 2002). This is indicated by three separate studies. In one study, 40 minutes of FES over the common peroneal nerve increased CS excitability to a plateau after 24 minutes (Mang et al. 2010), while 2 hours of SS over the radial and ulnar nerves increased CS excitability to a plateau after 45 minutes (McKay et al. 2002) in a second study. To directly compare the NMES-induced increases in CS excitability between FES and SS, these two types of NMES were applied over the median nerve in a third study; it was found that the changes in CS excitability were not different after 40 minutes of FES and 2 hours of SS (Bergquist et al. 2011). Thus, with these three separate studies combined, it is indicated that a "ceiling" effect exists for the extent to which NMES can increase CS excitability (Bergquist et al. 2011). However, a direct comparison in which CS excitability is monitored *during* FES and SS over the median and ulnar nerves has not previously been done. This type of time-course comparison of changes in CS excitability may be valuable for rehabilitative purposes should one type of NMES induce increases in CS excitability earlier or at a faster rate than the other. If so, this faster-acting NMES type may be better to use in rehabilitation. As such, the present study was designed to

compare the time-course of changes in CS excitability throughout the application of FES and SS. Overall, when compared to an equal duration of SS, FES generates a larger afferent volley due to the higher amplitude and frequency of stimulation, and greater overall number of pulses delivered. As such, the first set of experiments of the present study were conducted to test the hypothesis that FES would increase CS excitability sooner and CS excitability would reach a maximal level (or, a ceiling level) faster than with SS. Upon completing these experiments in 10 participants, we found changes in CS excitability in only one measure (maximum motor evoked potential [MEP_{max}]) and only after FES. SS had no effect on CS excitability. Measures of CS excitability over the time-course of NMES (measured as motor evoked potentials [MEPs] evoked at 1.2x resting MEP threshold [RMT]) did not change during or after FES or SS.

In the original FES versus SS experiments described above, in which 40 minutes of FES and 2 hours of SS increased CS excitability (Bergquist et al. 2011), there were no measures of CS excitability *during* the NMES protocols, and thus there were no breaks in the NMES to make these measurements. Breaks in the NMES to measured CS excitability has previously been done and these studies have shown significant increases in CS excitability (Khaslavskaia et al. 2002; McKay et al. 2002), but the breaks were less frequent and less lengthy than in the experiments herein. However, in the experiments in this thesis, when NMES was delivered with lengthy and frequent breaks, changes in CS excitability were only observed in FES, and these changes were *not* observed from the measure of CS excitability that has been previously used throughout the literature. In these thesis experiments, after FES, MEP_{max} increased but not MEPs at 1.2x RMT as in other experiments. Because the effects of NMES are cumulative (Mang et al. 2010; McKay et al. 2002) and must "build-up" over time, we hypothesized that the lack of changes in CS excitability (as quantified by MEPs generated at 1.2x RMT) were due to the frequent and lengthy breaks in NMES during the FES versus SS time-course experiments. To investigate this hypothesis, an additional set of experiments were conducted in which FES was delivered without breaks and the data were compared to the time-course FES experiments (i.e. FES with breaks).

The goal of the first experiment was to identify which NMES parameters most effectively increase CS excitability, and the goal of the second experiment was to determine whether breaks in the application of NMES influence the effects on CS excitability. Combined, the results from these experiments may provide insight for the optimal parameters to use with individuals who are undergoing neurorehabilitation with NMES.

2.2 Methods

Six men and four women ranging in age from 19 to 49 with no known neurological disorders participated in this study. The procedures were approved by the Human Research Ethics Board at the University of Alberta and all subjects gave written informed consent prior to testing. Individuals who had not experienced either NMES or transcranial magnetic stimulation (TMS) previously, attended an additional ~30 minute orientation session several days prior to the initiation of the experiment. For a given participant, each session was delivered at the same time of day to control for diurnal changes in CS excitability (Tamm et al. 2009) and was separated by at least 48 hours to avoid interaction effects. During the experimental sessions, participants were reclined in a chair, with their shoulder, elbow, and wrist joints at approximately 15°, 120° and 180°, respectively. The hand was taped around a support to enable the participants to remain relaxed while maintaining a position similar to that of grasping a can. Participants were instructed to avoid caffeine, alcohol and strenuous exercise within 12 hours before the session.

2.2.1 Neuromuscular Electrical Stimulation

During each session, NMES was applied through one pair of bipolar electrodes over the median and ulnar nerves at the wrist to simultaneously activate the abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles, with the cathode located distal to the anode. FES was delivered in a 20s-on-20s-off pattern at 100Hz at an intensity that elicited an M-wave that was 10-15% of maximal (M_{max}). SS was delivered in a 0.5s-on-0.5s-off pattern at 10Hz, at the lowest intensity at which an M-wave could

be consistently identified (i.e. at M-threshold), defined here as 3-5% M_{max}. The NMES was applied with 1ms pulse duration using round (3.2cm diameter) neurostimulation electrodes (Axelgaard Manufacturing Co., Ltd.). Participants were instructed to remain relaxed during the FES and SS.

2.2.1.1 FES versus SS Time-course Experiments

Each person participated in 2 separate ~3 hour FES versus SS time-course experimental sessions. The order of the NMES sessions was pseudo-randomized. Breaks in the stimulation occurred every 5 minutes to collect CS excitability measures. These 5-minute-intervals of NMES were repeated 12 times for a total of 60 minutes of NMES. This stimulation protocol is displayed in Figure 2-1. Every second break of NMES, three M_{max} measurements from APB were collected to monitor recording site quality. Additionally, M_{max} was measured from APB and FDI pre- and post-NMES.

2.2.1.2 FES with Breaks versus FES without Breaks Experiments

Following the FES versus SS time-course experiments, the same ten participants returned for an FES session without breaks in the stimulation. These experiments occurred 3-7 months following the FES versus SS time-course experiments. The FES was delivered in a 20s-on-20s-off pattern for a total of 60 minutes. M_{max} was measured from APB and FDI before and after the FES. Three of the ten participants withdrew due to discomfort during the stimulation. As a result, data from five men and two women, ranging in age from 19 to 49, were included in this portion of the study. The CS excitability data from FES delivered without breaks were compared to the pre-FES and post-FES data from the FES versus SS time-course experiments.

2.2.2 Electromyography (EMG)

EMG was measured from APB, FDI, and extensor carpi ulnaris (ECU) of the right forearm and hand (see Figure 2-2) using disposable bipolar (2.25cm^2) surface recording electrodes (Vermed Medical, Vermont). Based on whichever configuration produced the least amount of noise in the EMG signal, the ground electrode was placed on the

styloid process of the ulna or over the first metacarpophalangeal joint. The skin over APB, FDI and ECU was cleaned with alcohol and allowed to dry before applying the electrodes over the muscle belly. EMG signals were amplified (500x) and band-pass filtered ([10- 1000 Hz] NeuroLog System; Digitimer, Hertfordshire, England). Data were sampled at 5000 Hz. To monitor recording site quality, M_{max} was measured after every second interval of NMES for a total of 5 times during the FES versus SS time-course experiments. This protocol is demonstrated schematically in Figure 2-1. For the FES without breaks experiments, M_{max} was measured only before and after the FES as shown in Figure 2-3. In all experiments, if the M_{max} amplitude changed more than 20%, the recording site was cleaned with alcohol, allowed to dry, and new electrodes were used.

2.2.3 Transcranial Magnetic Stimulation

MEPs were evoked using TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) applied with a figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota) over the "hot-spot" for APB. The hot-spot was identified by locating the position over the scalp which, at a constant TMS intensity, elicited the largest MEP from the APB. This location was then recorded using a BrainSight image-guided stimulation system (Rogue Research, Montreal, Quebec). The TMS coil was positioned at a 45° angle relative to the predicted orientation of the central sulcus with the handle pointing backwards. APB RMT was assessed before and after each NMES protocol. RMT was defined as the lowest TMS intensity that elicited an MEP of ~50mV in at least 4 of 8 consecutive trials. With the TMS over the APB hot-spot, MEP recruitment curves (MEP-RCs) were constructed both pre- and post-NMES intervention from data recorded from APB, FDI, and ECU simultaneously. With the subject relaxed, two sets of 5 TMS pulses were delivered 6-8s apart at 7 different TMS intensities in a random order ranging from ~0.6x RMT to ~1.3x the TMS intensity that evoked MEP_{max} for a total of 70 measurements. The TMS intensities in the MEP-RCs were determined by identifying the TMS intensity that evoked RMT and MEP_{max}, then allotting five equally spaced increments of TMS intensities around these two points. Thus the MEP-RCs consisted of MEPs evoked at TMS intensities 1 increment below RMT, at RMT, 3 intensities between

RMT and MEP_{max}, 1 increment at MEP_{max}, and 1 above $MEP_{max.}$ New RMT and MEP_{max} TMS intensity values were determined post-NMES intervention, and were used for the construction and collection of the post-NMES MEP-RC.

To assess time-course and pre- and post-NMES CS excitability, TMS pulses were delivered over the APB hot-spot at 1.2x RMT, with 6-8s between pulses. For the FES versus SS time-course experiments, CS excitability was assessed by delivering 20 TMS pulses immediately before and after the NMES, in addition to 10 TMS pulses every 5 minutes during the NMES session as shown schematically in Figure 2-1. For the FES with breaks versus FES without breaks experiments, changes in CS excitability were assessed by comparing the amplitude of 20 MEPs collected both before and after 60 minutes of FES without breaks, as shown in Figure 2-3.

2.3 Data Analyses

All analyses were conducted on group data. MEPs were measured peak-to-peak, and the MEPs from APB and FDI were normalized to the M_{max} obtained closest in time. The MEPs from ECU were not normalized, as M_{max} was not collected from that muscle. To ensure participants were relaxed, if there was EMG activity greater than 2 standard deviations from baseline in the 1s prior to each MEP, that MEP was removed from the analysis. "Baseline" was defined as the EMG activity in the 1s prior to the first sweep in the file. Of the 9840 MEPs recorded from the subjects, 57 MEPs from APB, 96 from FDI, and 30 from ECU were removed based on this criterion. This is a total of 183 rejected MEPs, which represents 1.7% of all MEPs from APB rejected, 2.9% of all MEPs from FDI, and 0.9% of all MEPs from ECU.

Changes in MEP amplitude from pre- to post-NMES are reported as absolute changes or relative changes as outlined in calculation 2-1 and 2-2 below. MEP_{max} was obtained only from APB, and thus will not be reported from FDI or ECU.

Calculation 2-1. Absolute change.

Change in MEP Amplitude = Normalized Post Amplitude – Normalized Pre Amplitude

Calculation 2-2. Relative change.

$$\text{Change in MEP Amplitude} = \left(\frac{\text{Normalized Post Amplitude}}{\text{Normalized Pre Amplitude}} - 1\right)*100$$

For all statistical analyses, repeated measures analysis of variance tests (RM-ANOVAs) were used, with NMES protocol and Time as factors. Because we were only interested in a main effect of time or an interaction between time and protocol, main effects of NMES protocol are not reported herein. All descriptive statistics are represented as the mean $\pm$ standard error. In the figures, a single asterisks indicates $p < 0.05$.

2.3.1 FES versus SS Time-course Experiments

Changes in CS excitability were assessed by comparing the group averages of the pre- and post-NMES MEPs (at 1.2x RMT, n=20) as well as each set of time-course MEPs (at 1.2x RMT, n=10), and pre- and post-NMES MEP_{max} and RMT. Two-way RM-ANOVAs were run on these data. The two way RM-ANOVA run on M_{max} from APB had factors of "NMES protocol" (2 levels: FES and SS) and "Time" (7 levels: pre-NMES, 5 time-course measures, and post-NMES). Separate two-way RM-ANOVAs used to compare the time-course of changes in MEP amplitudes (at 1.2x RMT) from APB, FDI, and ECU between FES and SS protocols had factors of "NMES protocol" (2 levels: FES and SS) and "Time" (13 levels: pre-NMES, post-NMES, and 11 intervals during the time-course). The two-way RM-ANOVAs for MEP_{max} and RMT had factors of "NMES protocol" (2 levels: FES and SS) and "Time" (2 levels: pre-NMES and post-NMES) and was run for measurements from APB only. The significance level was set at $p<0.05$. When the RM-ANOVA analysis identified a significant difference, a Fisher's least significant difference (LSD) *post-hoc* test was run, when appropriate.

2.3.2 FES with Breaks versus FES without Breaks Experiments

Changes in CS excitability were assessed by comparing the group averages of the pre- and post-NMES MEPs (at 1.2x RMT; n=20) collected immediately before and after the FES sessions, MEP_{max} and RMT. The data from the FES versus SS time-course experiments (i.e. FES delivered with breaks) were compared with this FES without breaks data. Separate two-way RM-ANOVA's were run on the data: for measuring changes in M_{max} (in APB), MEPs generated at 1.2x RMT (in APB, FDI, and ECU), MEP_{max} (in

APB) and RMT (in APB) from pre- to post-FES with factors of "NMES protocol" (2 levels: "FES without breaks" and "FES with breaks") and "Time" (2 levels: pre- and post-FES). The significance level was set at $p<0.05$. No *post-hoc* tests were necessary.

2.4 Results

2.4.1 M_{max}

For the FES versus SS time-course experiments, APB M_{max} did not change significantly throughout or following FES or SS. There was no main effect of Time [$F_{(6,42)}=0.961$, $p=0.462$] and no interaction between NMES protocol and Time [$F_{(6,42)}=0.484$, $p=0.816$]. Collapsed across time, the average M_{max} amplitude was 9.4 $\pm$ 1.3mV for FES and 10.4 $\pm$ 0.7mV for SS.

For the FES with breaks versus FES without breaks experiments, APB M_{max} also did not change significantly throughout, or following either FES condition. There was no main effect of Time [$F_{(1,6)}=0.091$, $p=0.773$] and no interaction between NMES protocol and Time [$F_{(1,6)}=1.43$, $p=0.277$].

2.4.2 MEPs measured at 1.2x RMT before, during and after NMES

In the FES versus SS time-course experiments, neither FES nor SS altered the amplitude of MEPs generated at 1.2x RMT in any of the muscles presently investigated. For MEPs recorded from APB there was no main effect of Time [$F_{(12,96)}=0.858$, $p=0.591$] and no interaction between NMES protocol and Time [$F_{(12,96)}=1.723$, $p=0.074$]. MEPs measured from APB throughout the FES and SS sessions are shown in Figure 2-4 where the normalized MEP amplitudes are expressed as a ratio of the pre-NMES value. MEP amplitudes from APB recorded pre- and post-NMES are shown in Table 2-1, in the "Time-course" columns. Also included in this table for comparison are the data from previous similar experiments done in our laboratory (Bergquist et al. 2011) in which CS excitability was only measured pre- and post-NMES, in the "Original Data" columns. All data in Table 2-1 are presented as both an absolute change (see Calculation 2-1) and a relative change (see Calculation 2-2). For FDI there was no main effect of Time [$F_{(12,96)}=1.584$, $p=0.112$] and no interaction between NMES protocol and Time

[$F_{(12,96)}$=0.805, p=0.645]. For ECU there was no main effect of Time [$F_{(12,96)}$=1.57, p=0.113] and no interaction between NMES protocol and Time [$F_{(12,96)}$=0.942, p=0.509].

Further, from the FES versus SS time-course experiments, a post-hoc achieved power analysis for a RM-ANOVA revealed that for APB, with the given sample size (n=10), the FES versus SS time-course study was powered at 99% for identifying changes in the interaction between NMES protocol and Time. This study was powered at 84% for identifying changes in the factor Time, and was underpowered at 60% for identifying changes in the factor NMES protocol.

In the FES with breaks versus FES without breaks experiments, MEP amplitudes evoked at 1.2x RMT were not altered in any of the muscles presently investigated. For MEPs recorded from APB there was no main effect of Time [$F_{(1,6)}$=1.307, p=0.97] and no interaction between NMES protocol and Time [$F_{(1,6)}$=0.032, p=0.864]. For FDI there was no main effect of Time [$F_{(1,6)}$=1.065, p=0.642] and no interaction between NMES protocol and Time [$F_{(1,6)}$=0.14, p=0.721]. For ECU there was no main effect of Time [$F_{(1,6)}$=0.017, p=0.902] and no interaction between NMES protocol and Time [$F_{(1,6)}$=0.466, p=0.520]. Group percent changes in MEP amplitudes from pre- to post-FES with and without breaks are shown in Figure 2-8 where positive numbers indicate increases in CS excitability. An individual participant's MEP traces before and after both protocols of FES are shown in Figure 2-9.

2.4.3 MEP_{max}

In the FES versus SS time-course experiments MEP_{max} for APB increased significantly following 60 minutes of FES, but not SS. There was a significant interaction between NMES protocol and Time [$F_{(1,9)}$=6.34, p=0.033] and no main effect of Time [$F_{(1,9)}$=1.771, p=0.216]. The *post-hoc* test revealed that MEP_{max} increased from pre- to post-FES by 0.7 $\pm$ 0.3mV ($p<0.05$), but did not change from pre- to post-SS (p=0.66). MEP_{max} values are displayed in Figure 2-5 panel A.

In the FES with breaks versus FES without breaks experiments MEP_{max} for APB increased significantly following 60 minutes of both types of FES; there was a main effect of Time [$F_{(1,6)}$=6.72, p=0.041] and no interaction between NMES protocol and Time [$F_{(1,6)}$=0.524, p=0.497]. In panel B of Figure 2-5 the change in raw MEP_{max}

amplitude is shown. When collapsed across FES protocol (i.e. FES with and without breaks), MEP_{max} increased by 0.3 $\pm$ 0.1mV.

2.4.4 RMT

In the FES versus SS time-course experiments RMT for APB did not change significantly following either FES or SS. These data are shown in Figure 2-6 panel A; in which an increase in CS excitability would be represented by a decrease in RMT, since a lower TMS intensity would be required to achieve RMT. There was no main effect of Time [$F_{(1,9)}$=0.192, p=0.672] and no interaction between NMES protocol and Time [$F_{(1,9)}$=0.451, p=0.519]. The change in RMTs for each participant from pre- to post-NMES can be seen in Figure 2-7, in which a negative number represents a decrease in RMT from pre- to post-NMES and thus an increase in CS excitability.

In the FES with breaks versus FES without breaks experiments, neither type of FES altered RMT (measured from APB only). There was no main effect of Time [$F_{(1,6)}$=0.229, p=0.649] and no interaction between NMES protocol and Time [$F_{(1,6)}$=0.488, p=0.511]. The RMTs assessed pre- and post-FES are shown in Figure 2-6 panel B.

2.5 Discussion

The purpose of these experiments was to determine the time-course of changes in CS excitability during the application of FES and SS. There were no changes in CS excitability assessed as the amplitude of MEPs generated at 1.2x RMT during or after any protocol of NMES investigated herein. That includes FES and SS delivered with breaks, as well as FES delivered without breaks. Because there were no changes in MEPs generated at 1.2x RMT, the results do not provide any insight into the time-course of changes in CS excitability. However, CS excitability assessed as MEP_{max} did increase, but only after FES (with and without breaks). SS did not induce any changes in MEP_{max}. MEP_{max} was only measured before and after the application of NMES, and as a result we were only able to observe changes in CS excitability from pre- to post-NMES. Overall, these experiments revealed that FES increased CS excitability, while SS did not.

The literature indicates that CS excitability increases to a plateau after 24 minutes of FES (Mang et al. 2010) and 45 minutes of SS (McKay et al. 2002). Therefore 60 minutes of each NMES type should have theoretically been enough time to observe increases in CS excitability when measured as MEPs generated at 1.2x RMT. However, in the time-course experiments herein, a break in the stimulation occurred after every 5-minute-interval of NMES to collect CS excitability measures with a high temporal resolution. Previously for time-course measures of CS excitability, the minimum amount of time between breaks has been 10 minutes (Knash et al. 2003). It should be noted that Mang et al. (2010) measured a 2-minute time-course resolution; however breaks in the NMES were not required because MEPs were measured during the "20s off" period of NMES. As it is well documented that the effects of NMES in a single session are cumulative (Khaslavskaia et al. 2002; Knash et al. 2003; Mang et al. 2010; McKay et al 2002) it was predicted that in the "time-course" experiments herein, the frequent and lengthy breaks "washed out" the effects in CS excitability, preventing the accumulation of CS plasticity. To test whether the breaks in the stimulation impeded changes in CS excitability, FES was delivered without breaks with the same subjects from the FES versus SS time-course experiments. These experiments also failed to evoke any changes in CS excitability at 1.2x RMT.

However, after both FES with and without breaks, MEP_{max} increased, and the increase was not different between FES protocols. In contrast, MEP_{max} did not change as a result of SS. This indicates that the larger afferent volley generated by FES had a larger effect on CS excitability than SS, regardless of breaks in the FES. Selective increases in high threshold MEPs (i.e. MEP_{max}) have been observed after NMES (Khaslavskaia et al. 2002), with activation of cutaneous afferents (Knash et al. 2003) and after training (Poh et al. 2013).

This observation of increased MEPs generated at high (i.e. MEP_{max}) but not low (i.e. 1.2x RMT and RMT) TMS intensities may be explained in two ways; either high threshold CS pathways increased in excitability while low threshold pathways did not, or the representation of the target muscle on the motor cortex increased (Ridding and Rothwell 1997). If the cortical map representation increased, MEP_{max} would increase

because larger cortical regions are activated at higher intensities of TMS. This does not necessarily indicate a change in excitability of a given CS neuron, as analyses of MEPs evoked over a single location alone cannot always distinguish between changes in excitability versus changes in cortical organization (Ridding and Rothwell 1997). In order to differentiate between these possibilities, measuring MEPs from several cortical locations to construct a "map" of the CS excitability would be appropriate (Ridding and Rothwell 1997).

It is also possible that the intraindividual variability in MEPs evoked at 1.2x RMT may have prevented any changes from being identified at lower TMS intensities. MEPs are less variable at higher TMS intensities (Kiers et al. 1993), such as those that generate MEP_{max}, because higher TMS intensities may preferentially generate MEPs directly, whereas lower intensities predominantly generate MEPs indirectly (Di Lazzaro et al. 1998). Indirect activation of descending pathways is more influenced by the excitability of the surrounding neurons, so the natural fluctuations in cortical excitability (Ellaway et al. 1998) could increase the intraindividual variability in low threshold MEPs. There are other intraindividual factors that can also influence the amplitude of MEPs; in a single session simply thinking of the target muscle can change MEP amplitude (Gandevia and Rothwell 1987), and between sessions the phase of the menstrual cycle for women (Hattemer et al. 2007) or time of day (Tamm et al. 2009) can influence MEP amplitudes.

Interindividual and inter-sample variability may also be a factor for the results herein, in which there was no change in MEPs generated at 1.2x RMT. Previous work from our laboratory demonstrated that 40 minutes of FES delivered without breaks increased MEPs generated at 1.2x RMT (Bergquist et al. 2011), though the results herein from 60 minutes of FES delivered without breaks did not support this finding. A plausible explanation for incongruent results may be the different people tested and thus different interindividual, and inter-sample variability (i.e. responder versus non-responders). Table 2-1 compares the inter-sample variability in the original data ([labeled as "Original Data" in the table] Bergquist et al. 2011) to the FES versus SS time-course experiments (labeled as "Time-course" in the table). Here, large inter-sample variability in the response to FES can be observed through this comparison. This inter-

sample variability has been documented in previous studies of this nature and is common in TMS studies (Chipchase et al. 2011b; Khaslavskaia et al. 2002; Mang et al. 2010). Interindividual variability may be attributed to a common polymorphism in brain derived neurotrophic factor ([i.e. the Val66Met allele] Cheeran et al. 2008; Fritsch et al. 2010). This genotype results in a significant reduction in responses to interventions that typically influence CS excitability (Cheeran et al. 2008). As none of the participants in the experiments herein have been genotyped, this gene may be a factor in the interindividual variability of responses, and inability to replicate previous results.

Finally, differences in methodology between the present experiments and the previous experiment from our lab (Bergquist et al. 2011), beyond the inclusion of breaks in the NMES, may have led to the discordant results. The first altered variable included slightly different locations of stimulation site, in which the NMES herein was delivered over both median and ulnar nerves instead of more selectively over the median nerve. As stimulating different muscles can result in different changes in CS excitability (Chipchase et al. 2011b; Mang et al.2011), this may have led to the incongruent results. The second altered variable was the configuration of the stimulating electrodes in which, herein, the cathode was placed distal to the anode, although electrode configuration was not specified in the original experiments (Bergquist et al. 2011). A distal cathode may have led to an "anodal block" whereby hyperpolarization of the nerve beneath the anode impedes action potentials from travelling past it (Dreyer et al. 1993), thus preventing the sensory stimuli from reaching the CNS. Whether or not anodal block is a factor during surface stimulation in humans is controversial (Dreyer et al. 1993; Wee et al. 2000), and it is suggested that the current amplitude required to elicit an anodal block would exceed a subject's tolerance threshold (Dreyer et al. 1993). As such, although anodal block cannot be excluded as a possibility from the present experiment, it is unlikely that an anodal block is a concern in human studies (Dreyer et al. 1993).

Neither SS nor FES altered the amplitude of MEPs generated at 1.2x RMT that were used to assess the time-course of changes in CS excitability during the 2 protocols. Thus, whether the time-course of change in CS excitability during the application of FES

is different from SS remains unidentified. However, FES with and without breaks increased MEP_{max} while SS did not. Thus CS excitability increased after FES but not after SS. This supports the notion that FES generates a larger afferent volley than SS, hence having a greater effect on CS excitability. FES delivered with and without breaks did not differ in their influence on CS excitability. This suggests that the breaks in NMES were not the reason for the lack of changes in CS excitability at lower TMS intensities (i.e. 1.2x RMT), and also that incorporating breaks in FES does not impact the extent to which high threshold CS pathways are influenced by the stimulation. An increase in MEP_{max} indicates that only the high threshold CS pathways were influenced by FES or that the cortical representation of the target muscle increased. Overall, the optimal NMES parameters to influence CS excitability to maximize the benefit of NMES rehabilitation procedures have yet to be determined; however, the present data suggest that NMES parameters that generate a larger sensory volley (i.e. FES) increase CS excitability more. Once optimal NMES parameters are identified for best strengthening the CS pathways, this knowledge may aid in creating more beneficial rehabilitation protocols for people recovering from spinal cord injury or stroke. Presently we show that 60 minutes of FES increases CS excitability while 60 minutes of SS does not.

2.6 Appendix: Tables and Figures

Table 2-1. Absolute and relative changes in APB MEP amplitude generated at 1.2x RMT from the FES versus SS time-course experiment described in this thesis ("Time-course" columns), as well as from FES versus SS experiment data previously collected from our laboratory (["Original Data" columns] Bergquist et al. 2011).

Method of reporting Change	FES		SS	
	Original Data	Time-course	Original Data	Time-course
Absolute change in MEP amplitude (%M_{max} ± SD)	4.2± 6.3	-2.8± 7.9	5.2± 13.7	0.19± 2.7
Range (%M_{max})	20.1 to -4.7%	5.5 to -21.3%	51.4 to -6.3%	4.1 to -3.4%
Relative change in MEP amplitude (%change ± SD)	65.5 ± 99.3%	3.2 ± 74.6%	48.6 ± 88.2%	19.9 ± 57.6%
Range (%change)	284.5 to -64.4%	189.6 to -74.6%	283.3 to -63.9%	105.9 to -45.6%

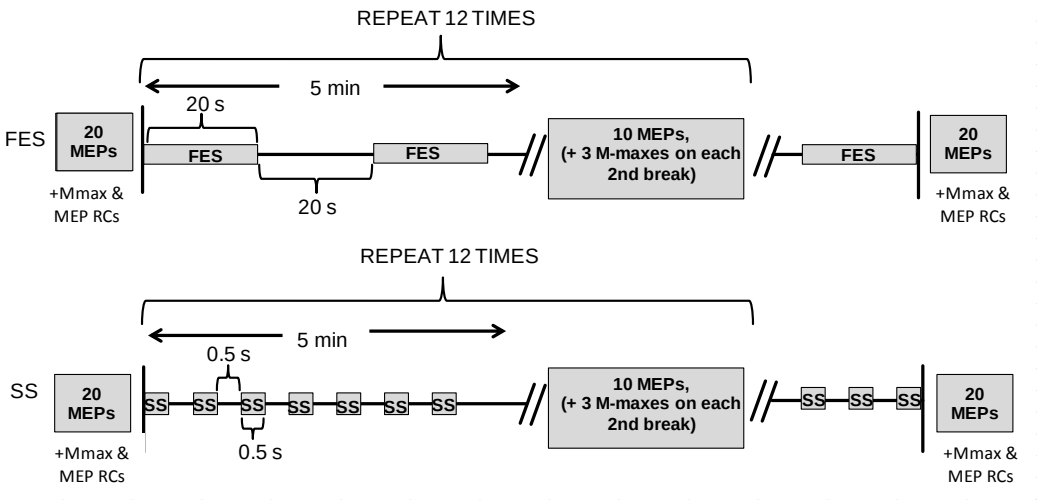

Figure 2-1. A schematic of the protocols used for the FES versus SS time-course experiments. Each grey box represents a procedural step and is followed by a black line which represents a rest period. The top panel shows the FES time-course protocol and the bottom panel shows the SS time-course protocol.

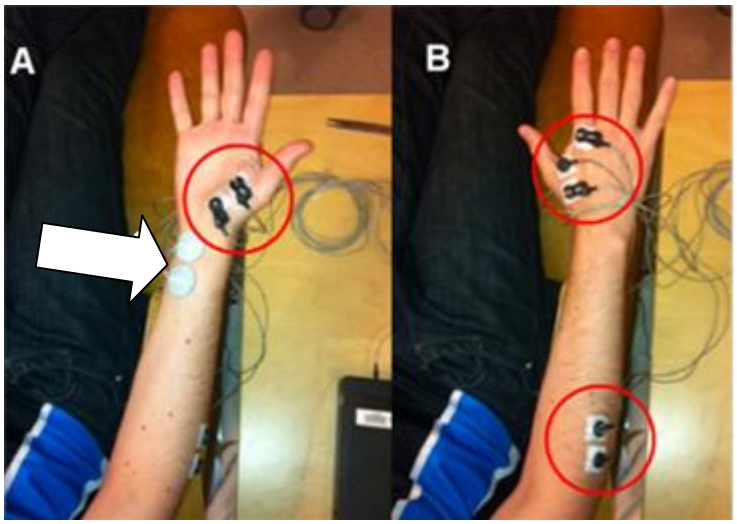

Figure 2-2. Placement for EMG recording electrodes (circles) and NMES stimulating electrodes (arrow) on the right forearm, wrist and hand. Panel A depicts the ventral side of the limb; panel B depicts the dorsal side.

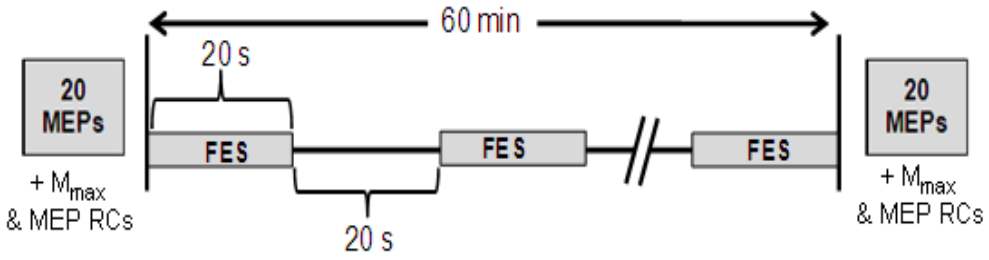

Figure 2-3. A schematic of the protocol used for the FES without breaks experiment. Each grey box represents a procedural step and is followed by a black line which represents a rest period.

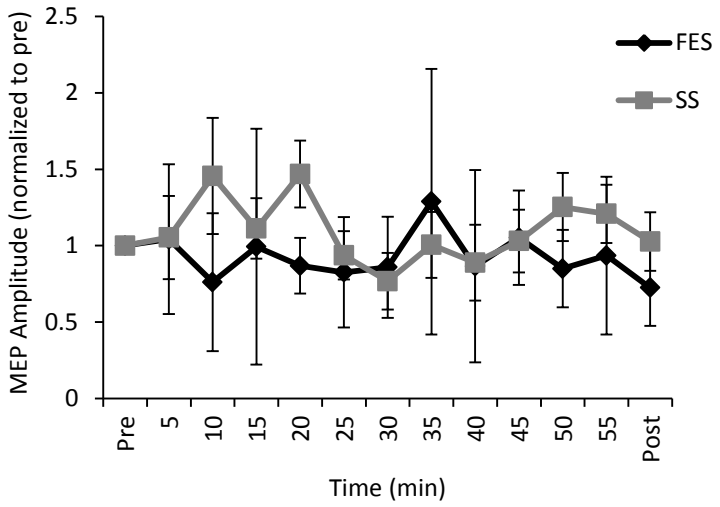

Figure 2-4. Changes in normalized MEP amplitudes from APB before, during and after the FES versus SS time-course experiments averaged across the group (n=10), expressed as a ratio of the pre-value. (Error bars = 1SE)

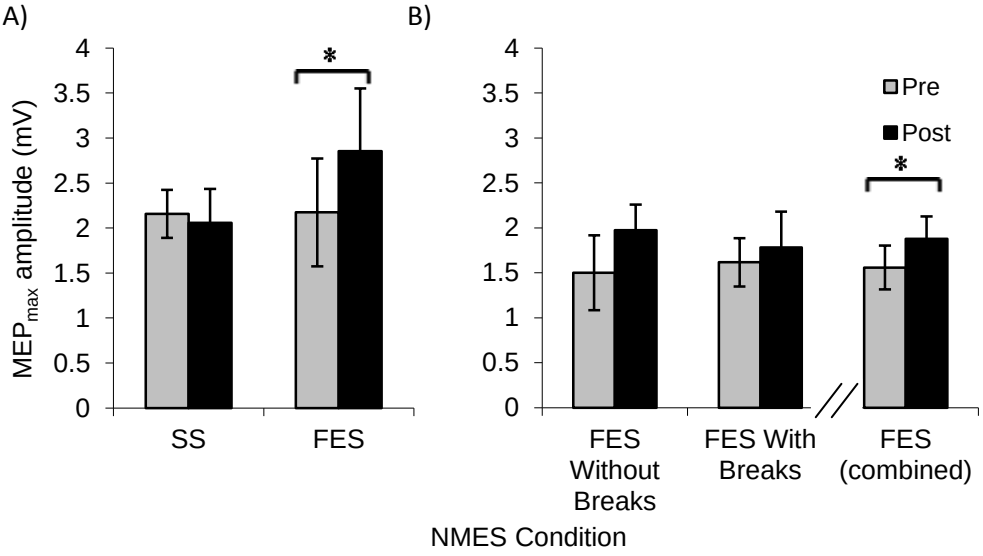

Figure 2-5. Group changes in MEP_{max} from APB before (light fill) and after (dark fill) NMES (error bars = 1 SE). Panel A depicts changes from pre- to post- FES and SS delivered with breaks (n=10, FES versus SS time-course experiments). Panel B depicts changes from pre- to post-FES (n=7) delivered with and without breaks. Note that three participants were excluded from the FES without breaks experiments and thus have been removed from the data shown in Panel B, "FES With Breaks". Asterisks indicate $p < 0.05$.

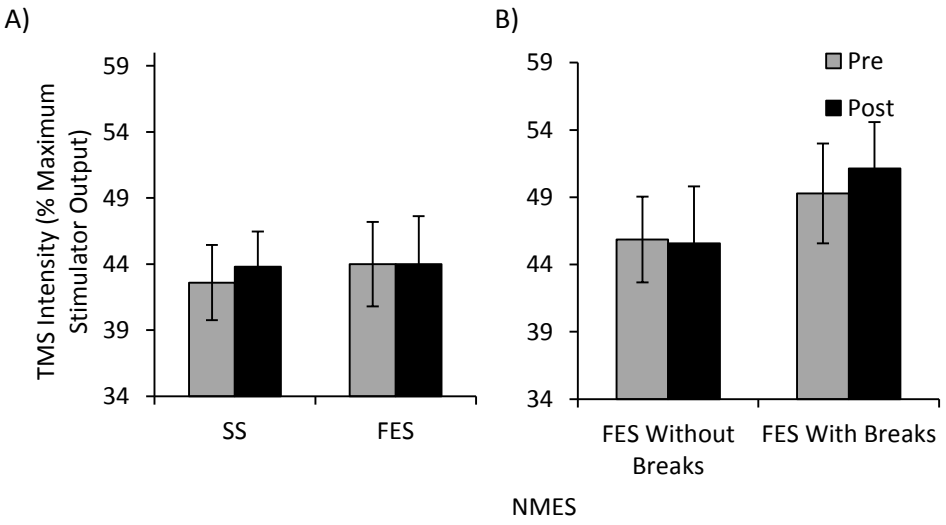

Figure 2-6. Group APB RMT changes from pre- (light fill) to post- (dark fill) NMES (error bars = 1 SE). Panel A depicts changes from pre- to post- FES and SS delivered with breaks (n=10, FES versus SS time-course experiments). Panel B depicts changes from pre- to post-FES delivered with and without breaks (n=7).

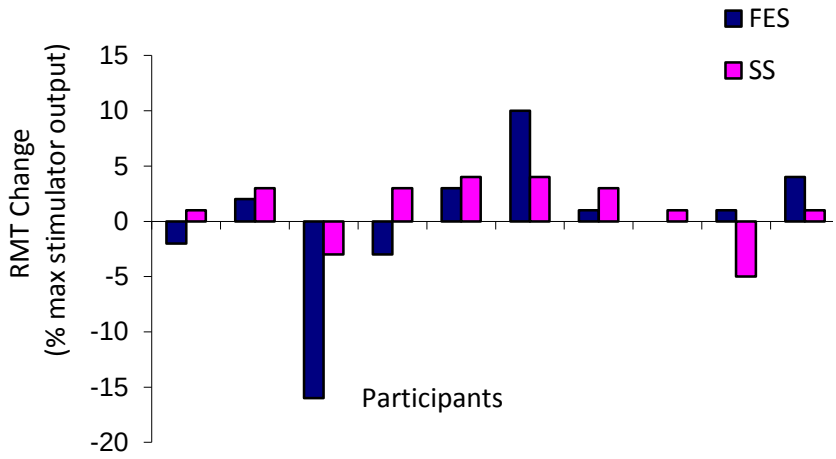

Figure 2-7. Individual APB RMT changes from pre- to post- FES and SS delivered with breaks, for each participant (n=10) in the FES versus SS time-course experiments.

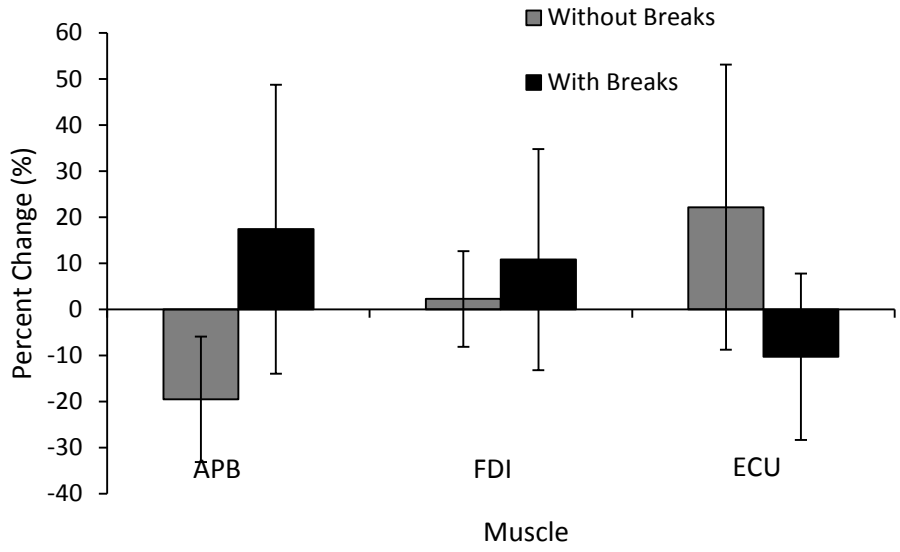

Figure 2-8. Group (n=7) percent change in MEP amplitude from APB generated at 1.2x RMT from pre- to post-FES delivered with (light fill) and without (dark fill) breaks (error bars = 1 SE).

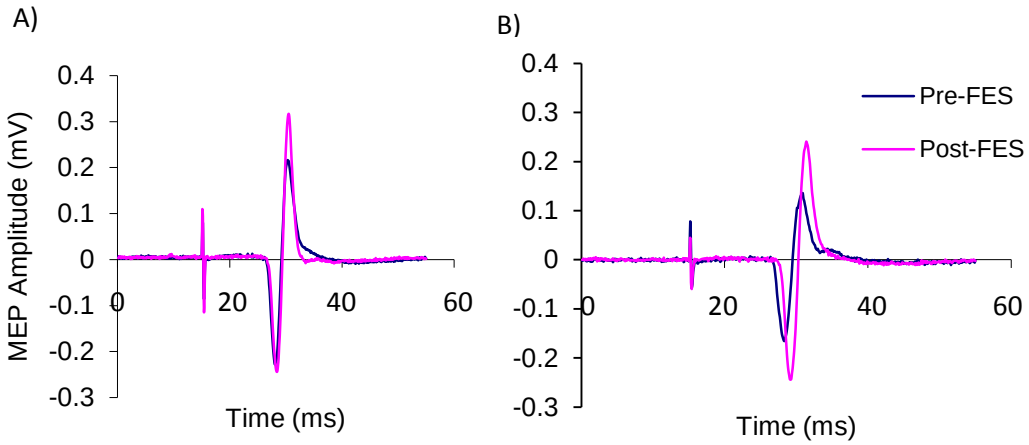

Figure 2-9. Individual MEP traces from APB generated at 1.2x RMT both pre- and post-FES delivered with and without breaks. Panel A data from FES delivered without breaks, and panel B shows data from FES delivered with breaks.

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CHAPTER 3: The pulse duration of electrical stimulation influences H-reflex amplitudes but not corticospinal excitability for tibialis anterior.

3.1 Introduction

Neuromuscular electrical stimulation (NMES) involves the application of an electrical current over a muscle belly or nerve trunk to "prime" the central nervous system (CNS) for training (Hoffman and Field-Fote 2007) or to restore movement and reduce muscle atrophy in people who have had a spinal cord injury or stroke (Sheffler and Chae 2007). At sufficient stimulation amplitudes NMES activates both efferent and afferent fibers, resulting in muscle contractions and the transmission of an afferent volley to the CNS, respectively. Repetitive stimulation of a peripheral nerve, as occurs during NMES, produces an afferent volley that can alter transmission along spinal (Clair et al. 2011; Crone and Nielsen 1989; Lagerquist and Collins 2008; Lin et al. 2002; Lloyd 1949; Panizza et al. 1989) and corticospinal ([CS] Charlton et al. 2003; Mang et al. 2010) pathways. At the level of the brain, the electrically-evoked afferent volley can increase CS excitability (Ridding et al. 2000; Chipchase et al. 2011b) which, over time, can strengthen CS pathways and result in lasting improvements in movement (Everaert 2010). To capitalize on this effect for patients undergoing NMES rehabilitation, it is important to understand how to maximize the influence of NMES on CS excitability. One way to do this is to manipulate the parameters of NMES, which include pulse amplitude, duration and frequency, to increase the magnitude of the NMES-induced afferent volley. By increasing the magnitude of the afferent volley, the effect NMES has on CS excitability is also increased (Chipchase et al. 2011b;Mang et al. 2010). Despite this evidence that altering NMES parameters can alter CS excitability, the specific effect on CS excitability of each parameter is not well defined. The present study was designed to investigate the effect of NMES pulse duration on CS excitability.

Relatively long pulse durations preferentially recruit afferent fibers over efferent fibers in the tibial (Lagerquist and Collins 2008; Lin et al. 2002; Panizza et al. 1992), ulnar (Bostock and Rothwell 1997; Panizza et al. 1992; Veale et al. 1973), median (Lin et al. 2002; Panizza et al. 1992; Panizza et al. 1998) and radial (Panizza et al. 1998) nerves. For example, longer pulse durations generate larger H-reflexes for a given sized M-wave

compared to shorter pulse durations (Lagerquist and Collins 2008; Panizza et al. 1989). Therefore, relative to recruiting efferent axons, longer pulse durations recruit more afferent fibers, produce a larger afferent volley and generate larger H-reflexes (Lagerquist and Collins 2008; Panizza et al. 1989). The preferential recruitment of afferent fibers with longer pulse durations is the result of afferent fibers having a lower rheobase and longer strength-duration time constant (τ_{SD}) compared to efferent axons (Burke et al. 2001; Veale et al. 1973). This effect of pulse duration on the afferent volley, as assessed by the amplitude of H-reflexes, holds true when electrical stimulation is delivered at very low frequencies to generate recruitment curves (Lagerquist and Collins 2008; Panizza et al. 1989), and at higher frequencies, as during NMES (Lagerquist and Collins 2010).

Whether NMES delivered using longer pulse durations increases CS excitability more than shorter pulses has not been tested. As such, the first set of experiments in the present study were designed to test the hypothesis that NMES delivered over the common peroneal (CP) nerve using relatively long pulse durations (200μs and 1000μs) increases CS excitability more than NMES with shorter pulse durations (50μs). To test this, NMES was delivered for 30 minutes at each of the three pulse durations in the same participants on separate days. CS excitability was assessed by comparing the amplitudes of motor evoked potentials (MEPs) pre- and post-NMES. This hypothesis, however, was not supported, and the effect of NMES on CS excitability was not different between pulse durations. This hypothesis had been formed on the understanding that relatively longer pulse durations generate a larger afferent volley, however this assumption had not been evaluated in the first set of experiments. Thus, we conducted a second series of experiments to determine whether the pulse durations used in the initial experiments had the anticipated differential effect on the electrically-evoked afferent volley. As such, M-wave and H-reflex recruitment curves (MH-RCs) were collected from tibialis anterior (TA). Based on previous research for other muscles (Lagerquist and Collins 2008; Panizza et al. 1989; Panizza et al. 1992), we hypothesized that in TA, compared to shorter pulse durations, longer pulse durations would generate larger maximal H-reflexes (H_{max}) and larger H-reflexes at a given sized M-wave.

Specifically, we predicted the H_{max} to maximal M-wave (M_{max}) ratio (H_{max}:M_{max}) would be larger when generated by longer pulse durations (200μs and 1000μs) than shorter pulse durations (50μs), therefore indicating an increase in H_{max}. Further, we predicted that when generated by longer pulse durations (200μs and 1000μs), the M-wave when the H-reflex was maximal (M_{Hmax}) would decrease, that the H-reflex at ~6% M_{max} ($H_{6\%Mmax}$) would increase. These latter predictions are consistent with a leftward shift in the MH-RC generated by longer pulses. Additionally, we predicted that the M_{max} would not change between pulse durations as the stimulus location was not different, and thus the population of activated efferent fibers should remain constant with maximal recruitment. Together, these experiments are part of a larger body of work designed to identify NMES parameters that maximize the beneficial effects on CNS circuits (Chipchase et al. 2011b; Mang et al. 2010) by increasing CS excitability, while simultaneously producing muscle contractions.

3.2 Methods

Nine men and eleven women ranging in age from 18 to 50 with no known neurological disorders participated in this study. The procedures were approved by the Research Ethics Board at the University of Alberta, and all subjects gave written informed consent prior to testing. For participants who had not previously experienced either NMES or transcranial magnetic stimulation (TMS), a brief orientation session was completed several days prior to the data collection sessions. For all sessions, the participants were seated in a Biodex dynamometer chair (System 3, Biodex Medical Systems, Shirley NY) with the right knee at 100° and the ankle at 90°, and their right foot secured to a footplate. Participants were instructed to avoid caffeine and alcohol for 12 hours prior to the experiment, and to avoid strenuous exercise for 6 hours prior.

3.2.1 Electromyography (EMG)

EMG was measured from TA of the right leg, as shown in Figure 3-1, using disposable bipolar (2.25 cm^2) surface recording electrodes (Vermed Medical, Vermont). The ground electrode was placed over the patella, or over the tibia, depending on

whichever configuration elicited the least amount of noise in the EMG signal. The skin over TA was cleaned with alcohol and allowed to dry before applying the electrodes over the muscle belly. EMG signals were amplified (500x) and band-pass filtered (10-1000 Hz; NeuroLog System; Digitimer, Hertfordshire, England). Data were sampled at 5000 Hz, except for during the MH-RCs in which the data were sampled at 50 000Hz to provide enough resolution to capture the current spike at short pulse durations. For visual feedback, the EMG channel for maximum voluntary contractions (MVCs) was rectified, amplified (10x) and low-pass filtered (0.3 Hz).

3.2.2 Neuromuscular Electrical Stimulation

Fifteen of the twenty participants took part in the experiments to evaluate the effect of pulse duration on CS excitability. These participants completed three ~2 hour sessions, at least 48 hours apart, where NMES was applied at each of the three pulse durations investigated herein and CS excitability was measured. For a given participant, these sessions were held at the same time of day to control for diurnal changes in CS excitability (Tamm et al. 2009).

NMES was delivered for 30 minutes (100Hz, 20s-on-20s off) over the CP nerve to evoke an M-wave in TA that was 10-15%M_{max}. The NMES was applied using round (3.2 cm) neurostimulation electrodes (Axelgaard Manufacturing Co., Ltd.) arranged in a bipolar configuration, with the cathode proximal. The NMES was delivered while the subject was at rest using three pulse durations (50µs, 200µs and 1000µs) on different days, delivered in a randomized order.

M_{max} was used to monitor EMG recording site consistency; if the amplitude of M_{max} changed more than 20%, the recording site was cleaned with alcohol, allowed to dry, and the electrodes were replaced. M_{max} (n=3 repetitions) was also collected before and after the NMES. Additionally, after the first 15 minute of NMES, the stimulation was paused to measure M_{max} (n=3 repetitions). At this time, if necessary, current amplitude was adjusted to maintain an M-wave 10-15% M_{max}.

3.2.3 Transcranial Magnetic Stimulation (TMS)

To assess CS excitability, TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) was applied with a figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota) to generate MEPs in TA. All MEPs were generated while the subject was at rest. The TMS "hot-spot" for TA was identified by locating the position over the scalp where, at a constant TMS intensity, the largest MEP was generated from TA. This location was recorded using a BrainSight image-guided stimulation system (Rogue Research, Montreal Quebec). The TMS coil was positioned at a 45°angle relative to the predicted orientation of the central sulcus with the handle pointing backwards.

Pre- and post-NMES CS excitability measures consisted of 20 MEPs collected immediately before and after the NMES session. The stimulation was delivered over the TA "hotspot" at 1.2x resting MEP threshold (RMT), with 6-8s between pulses. RMT was identified as the lowest TMS intensity that elicited a MEP amplitude of at least ~50mV in at least 4 of 8 consecutive trials.

3.2.4 M-wave and H-reflex Recruitment Curves

Following the NMES sessions, all twenty participants completed a single ~30 minute session to collect MH-RCs at each of the three pulse durations investigated herein. The order of testing each pulse duration was randomized between the participants. For each MH-RC, forty single pulses of stimulation were delivered over the CP nerve, with 8-10s between pulses. The stimulation amplitude was varied randomly from below M-wave and H-reflex thresholds to ~1.1x M_{max}. Prior to collecting data for the MH-RCs, the subjects performed an MVC. During the data collection, the subjects held a background contraction of ~5% EMG MVC, and matched this contraction throughout the duration of MH-RCs using the aid of visual feedback. The subjects were instructed to relax between pulses of stimulation and were prompted to resume the contraction prior to each stimulation pulse.

3.3 Data Analyses

All analyses were conducted on group data, and descriptive statistics are reported as mean ± standard error. In the figures a single asterisk indicates $p<0.05$, and a double asterisk indicates $p<0.01$.

3.3.1 Corticospinal Excitability (i.e. MEPs)

Changes in CS excitability were assessed by comparing the mean amplitude of 20 MEPs generated at 1.2x RMT collected immediately before and after the NMES session. MEPs were measured peak-to-peak, and normalized to the M_{max} obtained closest in time. To ensure that inadvertent background muscle contractions did not influence MEP analyses, MEPs were removed from analysis if there was EMG activity greater than 2 standard deviations from baseline in the 1s prior to each MEP. "Baseline" was defined as the EMG activity in the 1s prior to the first sweep in the file. Of the 1800 MEPs recorded from the participants (120 MEPs per participant), 9 were removed based on this criterion, which represents 0.5% of the total MEPs.

A two-way repeated measures analyses of variance (RM-ANOVA) test was used to test for differences in MEP amplitude with Time (2 levels: pre, post) and Pulse Duration (3 levels: 50µs, 200µs and 1000µs) as factors. Another RM-ANOVA was also used to test for differences in M_{max} amplitudes with Time (3 levels: pre, mid and post) and Pulse Duration (3 levels: 50µs, 200µs and 1000µs) as factors.

3.3.2 Afferent Volley (i.e. MH-RCs)

MH-RC data from individual participants were included for analysis based on the following criteria for the presence of an H-reflex. The H-reflex epoch was selected surrounding a ~30ms latency, and if the average peak-to-peak amplitude in the 20 sweeps of lowest current was greater than 2%M_{max} in the EMG signal in at least one MH-RC from that participant, the data were included for analysis.

Four outcome measures were analyzed from the MH-RCs:

1. H_{max}:M_{max} ratio; the average of the three largest H-reflexes was expressed as a percentage of the single largest M-wave.

2. M_{Hmax} amplitude; the average M-wave amplitude corresponding with the three largest H-reflexes (i.e. M-wave amplitude at H_{max}).
3. $H_{6\%Mmax}$ amplitude; the average H-reflex amplitude when the M-wave was ~6%M_{max}, specifically when the M-wave was between 2-9% M_{max}.
4. M_{max}; the single largest M-wave amplitude.

For all of these MH-RC outcome measures, separate one-way RM-ANOVAs were run with Pulse Duration as the only factor (3 levels: 50µs, 200µs and 1000µs) and the significance level was set at $p<0.05$. When the RM-ANOVA analysis identified a significant difference, Fisher's least significant difference (LSD) *post-hoc* tests were run, when appropriate.

3.4 Results

3.4.1 Corticospinal Excitability (i.e. MEPs)

Fifteen subjects participated in the CS excitability portion of the experiments.

3.4.1.1 M_{max}

Across the group of 15 participants, M_{max} was not different between pulse durations and did not change over time for any pulse duration studied herein. There was no main effect of Pulse Duration [$F_{(2,28)}=0.752$, $p=0.467$], no main effect of Time [$F_{(2,28)}=1.695$, $p=0.202$], and no interaction between Pulse Duration and Time [$F_{(4,56)}=1.165$, $p=0.336$]. Collapsed across time, the group averages of M_{max} for pulse durations of 50µs, 200µs and 1000µS were 6.6 $\pm$ 1.1mV, 5.5 $\pm$ 0.6 mV and 7.1 $\pm$ 1.1 mV, respectively.

3.4.1.2 MEP Amplitude Changes from Pre- to Post-NMES

MEPs evoked at 1.2x RMT increased following NMES, but this increase did not depend on pulse duration. There was a main effect of Time [$F_{(1,14)}= 7.29$, $p=0.017$], no main effect of Pulse Duration [$F_{(2,28)}=1.169$, $p=0.326$] and no interaction between Pulse Duration and Time [$F_{(2,28)}=2.19$, $p=0.131$]. Thus, when the data were collapsed across pulse durations, CS excitability significantly increased by 2.1 $\pm$ 0.6%M_{max}. Panel A of Figure 3-2 shows the mean increases for the group for each pulse duration on the left,

and the main effect of time on the right. Panel B of Figure 3-2 shows data for each participant pre- and post-NMES at each of the three pulse durations. This figure shows that the majority of the participants had increases in CS excitability from pre- to post-NMES.

3.4.2 Afferent Volley (i.e. MH-RCs)

MH-RC data from thirteen subjects were included based on the criteria for the presence of H-reflexes in the EMG signal.

3.4.2.1 H_{max}:M_{max} ratio

For the H_{max}:M_{max} ratio, the RM-ANOVA revealed a main effect of Pulse Duration [$F_{(2,24)}$=6.922, p=0.004]. The *post-hoc* test revealed that the H_{max}:M_{max} ratio was larger with 200µs ($p<0.05$) and 1000µs ($p<0.01$) as compared to 50µs. The H_{max}:M_{max} ratios can be seen in Figure 3-3 where data were averaged across the group (n=13).

3.4.2.2 M_{Hmax}

There was no difference in M-wave amplitudes when the H-reflex was maximal (M_{Hmax}) between the different pulse durations, as there was no main effect of pulse duration [$F_{(2,24)}$=0.737, p=0.49]. Collapsed across pulse durations the average M_{Hmax} was 26.0 $\pm$ 2.2%M_{max}.

3.4.2.3 $H_{6\%Mmax}$

There was no difference in $H_{6\%Mmax}$ amplitudes between the different pulse durations, as there was no main effect of pulse duration [$F_{(2,24)}$=1.595, p=0.22]. The average H-reflex amplitude for 50µs, 200µs and 1000µs can be seen in Figure 3-4. Collapsed across pulse duration, the average H-reflex amplitude at ~6% M_{max} was 4.7 $\pm$ 0.3% M_{max}. An average of 9.4 $\pm$ 0.7 H-reflexes per participant for each pulse duration were included in this portion of the data analysis.

3.4.2.4 M_{max}

M_{max} did not change significantly between pulse durations. There was no main effect of Pulse Duration [$F_{(2,24)}$=2.067, p=0.148]. Collapsed across pulse durations, the average M_{max} amplitude was 6.9 $\pm$ 0.3mV.

3.5 Discussion

NMES applied for half an hour over the CP nerve increased CS excitability for TA by ~50%, but there was no difference in the magnitude of change between the three NMES pulse durations. Thus, to investigate whether pulse duration affects the afferent volley for TA, a second series of experiments was conducted in which MH-RCs were constructed using the three pulse durations from the initial experiments. We found that there was an effect of pulse duration on H-reflex amplitudes, and thus on the afferent volley. Longer pulses (i.e. 1000µs, 200µs) applied over the CP nerve evoked ~20% larger H_{max}:M_{max} ratios in TA than shorter pulse durations (i.e. 50µs), although no leftward shift in the MH-RCs were observed. Combined with the CS excitability observations, we suggest that although there is an effect of pulse duration on the afferent volley when measured at the level of the spinal cord (via H-reflexes), there is no effect of pulse duration at the level of the CS pathway. As a result, for NMES rehabilitation, manipulating pulse duration does not have an effect on increasing CS excitability, and thus relatively short or long pulse durations are equally effective for this purpose.

NMES rehabilitation generates lasting functional improvements in both strength (Conforto et al. 2002; Powell et al. 1999) and skill (Fraser et al. 2002), which are attributed to the accompanying increases in cortical excitability (Everaert et al. 2010; Fraser et al. 2002). Accordingly, it is important to identify which parameters of NMES best increase CS excitability. Given that CS excitability is altered by changing NMES pulse amplitude (Chipchase et al. 2011b) and frequency (Mang et al. 2010), and these changes in CS excitability occur as a result of the afferent volley (Knash et al. 2003; Ridding et al. 2000), we predicted that a larger afferent volley to the CNS would increase CS excitability to a greater extent. Longer pulse durations of NMES increase afferent fiber recruitment measured at the level of the spinal cord (Lagerquist et al. 2008; Lin et

al. 2002; Panizza et al. 1992); however whether there is an effect of pulse duration on CS excitability had not been previously studied. As such, we measured CS excitability before and after 30 minutes of NMES delivered using three different pulse durations, but found no differential effect of pulse duration at the level of CS pathway. This result was not expected, as it is well established that longer pulse durations generate a larger afferent volley in nerves other than the CP nerve (Lagerquist et al. 2008; Lin et al. 2002; Panizza et al. 1992). If the larger pulse duration increased the afferent volley as anticipated, there was either no effect on CS excitability or our outcome measure was not sensitive enough to detect a change in CS excitability. Alternatively, it may have been that pulse duration did not affect the afferent volley, as this effect had not been demonstrated in the CP nerve.

To determine whether there was an effect of pulse duration on the afferent volley for the CP nerve, further experiments were conducted in which MH-RCs were collected from TA. In these experiments the H-reflex was used as an indirect measure of the magnitude of the afferent volley. Consistent with research done with other nerves (Lagerquist and Collins 2008; Panizza et al. 1989; Panizza et al. 1992), in which longer pulse durations evoked larger H-reflexes, we found an effect of pulse duration on TA H-reflexes. Specifically, we found H_{max}:M_{max} ratios were ~20% larger when evoked with 1000µs and 200µs pulse durations than with 50µs pulse durations. Throughout the literature, the effect of pulse duration on maximal H-reflex amplitude is varied. Panizza et al. (1989) showed an increase in H_{max} from the median and tibial nerves. However, Lagerquist and Collins (2008) did not observe an increase in soleus H_{max} with longer pulse durations. In the present experiments, the other two measures of the magnitude of the afferent volley (i.e. M_{Hmax} and $H_{6\%Mmax}$) did not significantly change, and thus did not reflect a leftward shift in the MH-RC, contrary to our predictions. This result does not support previous research; Panizza et al. (1989) and Lagerquist and Collins (2008) reported a leftward shifts in MH-RCs generated by longer pulse durations. It must be noted that these two previous studies did not investigate the CP nerve, and thus the difference in results may be due to the different nerves studied. In the experiments herein, the increase in H_{max} without concomitant leftward shift in the MH-RC may be

attributed to the small H-reflexes in TA as compared to soleus (Brooke et al. 1997); it is possible that a leftward shift in the MH-RC was present, but was not identified due to the small magnitude of change that would occur in the already small H-reflex amplitudes. Figure 3-4 depicts the amplitude of the H-reflex when the M-wave was approximately 6%M_{max}, and although no statistical significance was observed (p=0.22), there appears to be a small trend of larger H-reflexes generated by longer pulse durations (i.e. 200µs and 1000µs as compared to 50µs). Perhaps with a larger sample size this effect would be more apparent, though we cannot build any conclusions from this data as it is, and this insignificant "trend" is a speculation. Despite the conflicting evidence, increases in H-reflex amplitude reflect the generation of a larger afferent volley with longer pulse durations, whether it is increases in H-reflex amplitudes at a given sized M-wave or in H_{max}. As such, based on the H_{max}:M_{max} data herein, we concluded that a larger afferent volley was generated when longer pulse durations were delivered over the CP nerve.

The effect of pulse duration on the afferent volley is attributed to differences in the membrane properties of efferent and afferent axons (Panizza et al. 1992). When electrical stimulation is applied over a mixed nerve, the proportion of afferent and efferent fibers recruited will depend on the pulse duration used (Panizza et al. 1992; Veale et al. 1973). Compared to efferent fibers, afferent axons exhibit a lower rheobase and longer τ_{SD} (Veale et al. 1973), both of which are related to afferent axons exhibiting a higher resting membrane potential, which itself occurs as a result of several potential, though unconfirmed, factors (Howells et al. 2012). These factors have been hypothesized to be differences in voltage-dependent ion channels between the two fibers (Bostock and Rothwell 1997) and/or differences in inward rectifying currents (Howells et al. 2012). As compared to efferent fibers, afferent fibers may have a greater number of persistent and/or active "threshold channels" (specifically, persistent sodium channels), which are active below resting membrane potentials (Bostock and Rothwell 1997) and are responsible for depolarizing the axonal nodes (Burke et al. 2001). Additionally, or alternatively, there may be a decrease in slow potassium conductance, and/or an overall increase in the I_h current, an inward rectifying current activated by

hyperpolarization (Howells et al. 2012). These possible factors would be responsible for the lower threshold of afferent axons (Howells et al. 2012), and although the exact mechanisms behind the afferent fiber's lower rheobase and longer τ_{SD} is unconfirmed, these membrane properties result in the preferential recruitment of afferent axons at longer pulse durations (Panizza et al. 1992).

The results indicated that longer pulse durations increase the magnitude of the afferent volley for TA, however the increases in CS excitability did not show an effect of pulse duration. As such, two explanations remain; either the differences in the afferent volley produced by the different pulse durations was not large enough to have an effect on CS excitability, or, there was an effect of pulse duration but the outcome measure herein (i.e. MEPs generated at 1.2xRMT) was not sensitive enough to measure the effect. The former explanation may be related to the circuitry of the H-reflex being far simpler than that in the CS pathway; should longer pulse durations not have an effect CS excitability, it may be that the differential influence on the afferent volley (as observed at the level of the spinal cord) is not large enough to influence CS excitability due to the multiple levels and modulatory inputs in the CS pathway. As compared to the H-reflex, the pathway between the muscle and the cortex has many more connections and thus more opportunity for the afferent volley to be modified before it is consolidated at the level of the somatosensory cortex. It may be that the gating and modifications along this lengthy pathway (i.e. by the thalamus, for example) may reduce any differences in afferent volley magnitude generated by different pulse durations. In addition to the multiple possibilities for afferent volley modification in the ascending pathway, there is ample opportunity for inputs to influence the MEP while the efferent volley descends the CS pathway from the motor cortex to the muscle. Finally, the latter explanation is related to the limitations of using TMS for these types of studies; activation of descending pathways by TMS is not very focal (Burke and Pierrot-Deseilligny 2009) and MEPs are also quite variable (Ellaway et al. 1998; Kiers et al. 1993). Because TMS is not focal, there is a risk of unintentionally activating more distant cortical areas which may activate inhibitory or excitatory intracortical input and ultimately skew the CS

excitability observations. Further, the variability of MEPs may result in the inability to identify significance with small sample sizes.

In conclusion, relatively long pulse durations increased H-reflex amplitudes for TA, but different pulse durations did not have differential effects on CS excitability. We interpret the H-reflex data to indicate that a larger afferent volley was generated with the longer pulse durations. Although this effect was apparent at the level of the spinal cord (observed via H-reflexes), it did not influence CS excitability measure at the level of the CS pathway (observed via MEPs). Because functional improvements associated with NMES in rehabilitative settings have been attributed to the NMES-induced increases in CS excitability (Everaert et al. 2010), it is important to identify the parameters of NMES that will generate the greatest increase in CS excitability, and thus provide optimal benefit for patients. Our results indicated that for NMES delivered to the CP nerve, longer pulse durations were not different from shorter pulse durations for increasing CS excitability, and thus increasing pulse duration is not an effective strategy for increasing CS excitability for rehabilitation.

3.6 Appendix: Figures

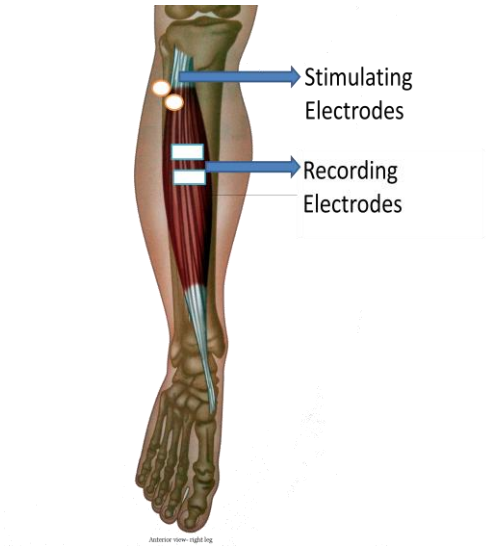
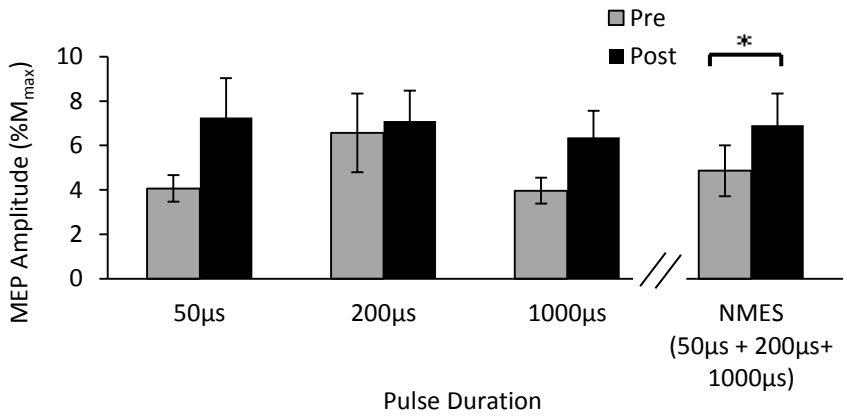


Figure 3-1. Placement for EMG recording electrodes (rectangles) over TA and NMES stimulating electrodes (circles) over the CP nerve on the right leg.

A)



B)

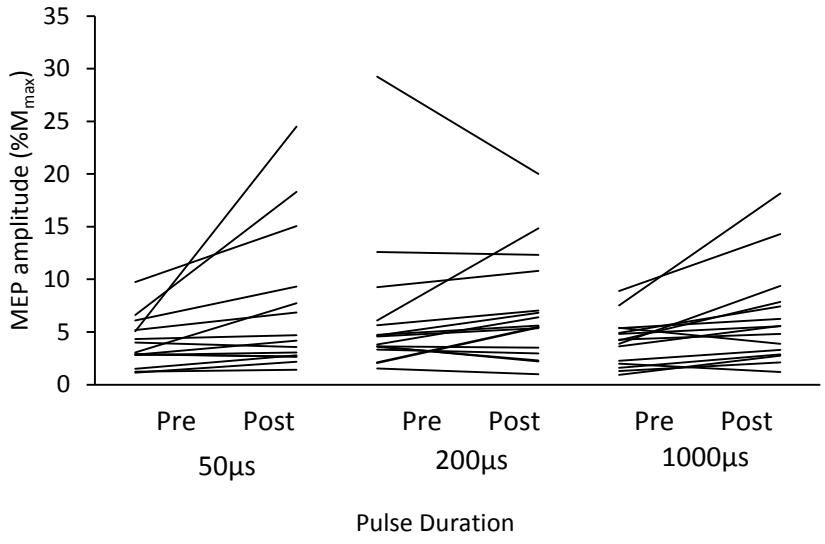


Figure 3-2. MEP amplitudes from TA pre- and post-NMES delivered with 50µs, 200µs, and 1000µs pulse durations. Panel A shows data averaged across the group (n=15, error bars = 1 SE, asterisks indicate $p<0.05$), and panel B shows individual responses.

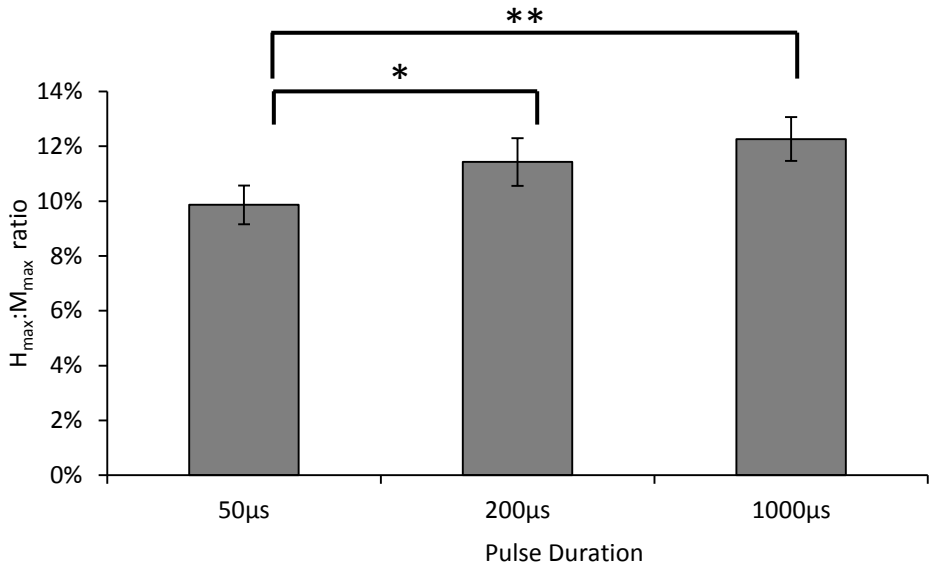


Figure 3-3. H_{max}:M_{max} ratio from TA evoked by singles pulses of NMES with 50µs, 200µs, and 1000µs pulse durations. The ratio is composed of the three largest H-reflex amplitudes and the single largest M-wave amplitude, expressed as a percentage (n=13, error bars = 1 SE, double asterisks indicate $p<0.01$, single asterisks indicate $p<0.05$).

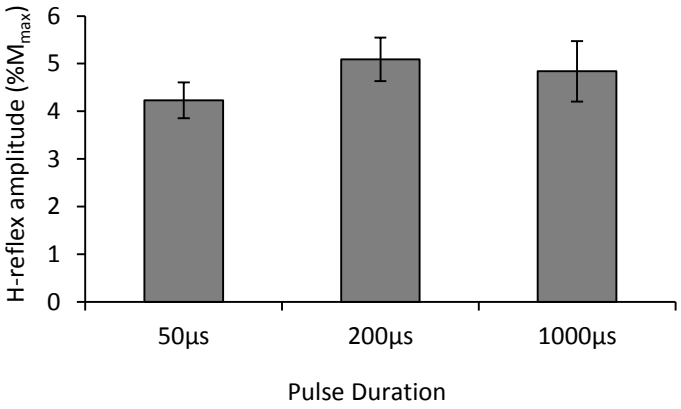


Figure 3-4. Average H-reflex amplitudes from TA when the M-wave was ~6% M_{max} (i.e. 2-9%M_{max}) evoked by single pulses of NMES with 50µs, 200µs, and 1000µs pulse durations. The value is composed of the three largest H-reflex amplitudes (error bars = 1 SE).

3.7 References

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CHAPTER 4: General Discussion

The theme of this thesis was to investigate how changing the parameters of neuromuscular electrical stimulation (NMES) affects corticospinal (CS) excitability. Because NMES induces lasting improvements in function that are related to increased CS excitability (Everaert et al. 2010), to maximize this benefit, NMES should be delivered with parameters that optimally increase CS excitability.

First in this General Discussion, a review of the results from the two thesis research chapters will be discussed, followed by an overview of common themes and findings that connect these experiments together. Secondly, a discussion of optimal techniques for increasing CS excitability in rehabilitation will be presented, including a critical appraisal of whether NMES is an appropriate technique for this purpose. Thirdly, limitations of the current work, future directions and closing remarks will be presented.

4.1 Functional Electrical Stimulation (FES), but not Somatosensory Stimulation (SS) Increased Corticospinal Excitability

In Chapter 2, FES and SS were applied over the median and ulnar nerves at the wrist for 60 minutes each, on separate days. The time-course of change in CS excitability was measured during the NMES, in which breaks occurred every 5 minutes to enable these measurements. It was hypothesized that, during the stimulation, FES would increase CS excitability earlier and at a faster rate than SS. This hypothesis was based on the premise that FES generates a larger afferent volley, in terms of a greater amplitude, frequency and overall number of pulses delivered. Additionally, based on previous literature outlining a "ceiling" effect in the extent to which NMES can influence CS excitability (Bergquist et al. 2011), it was hypothesized that both types of NMES would increase CS excitability to the same extent. The results were not as expected; the measures of CS excitability (i.e. motor evoked potentials [MEPs] generated at 1.2x resting MEP threshold [RMT]) did not change throughout either stimulation protocol, and as a result, no conclusions about the time-course of changes during FES or SS could be made. However, maximum MEP amplitude (MEP_{max}) did change, but following FES only.

As the results were not as expected, additional experiments were performed. Based on previous research that indicate the effect of NMES is cumulative (Knash et al. 2003; McKay et al. 2002) and must "build-up" over time, it was speculated that the breaks in the NMES were the cause for the lack of change in CS excitability as quantified by MEPs generated at 1.2x RMT. As such, FES was delivered continuously, without breaks, in the same participants and the data were compared to the earlier time-course FES experiment data (i.e. FES with breaks). It was hypothesized that the FES without breaks would increase CS excitability more than FES with breaks. The results showed that again, MEPs at 1.2x RMT were not altered and MEP_{max} increased, however the increase in MEP_{max} was not different between FES delivered with or without breaks. As such, regardless of breaks, FES over the median and ulnar nerves increased MEPs generated at high transcranial magnetic stimulation (TMS) intensities (i.e. MEP_{max}) but not those at low TMS intensities (i.e. MEPs generated at 1.2x RMT).

Andrews and colleagues (2013) investigated the effects of the duration of NMES application on CS excitability. In this study they identified increases in CS excitability after NMES sessions of 20 and 40 minutes, however they failed to identify any changes in CS excitability after a 60 minute NMES session (Andrews et al. 2013). Based on this evidence, it is clear that the duration of application of NMES must be considered as a factor for influencing changes in CS excitability in addition to the parameters of the stimulation themselves. Andrews (2013) hypothesized that a homeostatic mechanism may be responsible for preventing a continual build-up of CS excitability between 40-60 minutes of NMES. Given that the experiments in Chapter 2 were investigating changes in CS excitability during and after 60 minutes of NMES, it may be that the lack of changes in MEP amplitudes generated at 1.2x RMT was due to that length of NMES duration.

An increase in MEP_{max} may be attributed to an expanding representation of the target muscle on the motor cortex (Ridding and Rothwell 1997). This is because more distant regions of the cortex are accessible by higher intensities of TMS. As a result, if the cortical representation of a target muscle becomes larger, it can be activated at higher TMS intensities and will increase the size of the MEP recorded from the target

muscle. Alternatively, an increased MEP_{max} but not MEPs generated at 1.2x RMT may simply suggest an increased excitability of high threshold, but not low threshold, CS pathways.

In the FES versus SS experiments in this thesis, SS did not influence CS excitability despite other literature reporting that SS increases CS excitability (Bergquist et al. 2011; Charlton et al. 2003; McKay et al. 2002; Ridding et al.2000). In a similar time-course experiment, breaks in the SS occurred every 15 minutes and CS excitability increased after 45 minutes of stimulation and reached a plateau at 60 minutes into the stimulation (McKay et al. 2002). It is possible that the more frequent breaks in the stimulation (every 5 minutes herein, as compared to every 15 minutes) were the cause for the lack of SS-induced change in CS excitability. However, these breaks did not change the effect of FES on CS excitability. It is also possible that SS did not induce any changes in the sample selected, regardless of breaks in the stimulation. Because SS is delivered with lower pulse frequency and amplitude than FES, it may be that the smaller afferent volley generated was not substantial enough to induce any changes in CS excitability.

Overall, FES increased CS excitability in MEPs generated by high TMS intensities (i.e. MEP_{max}) while SS had no influence on CS excitability. This is likely the result of FES generating a larger afferent volley than SS, though the magnitude of the afferent volley was not directly measured herein. Alternatively, it may be that MEPs generated at 1.2x RMT are not a sensitive enough outcome measure to identify consistent changes in CS excitability induced by NMES.

4.2 Different Pulse Durations do not have Differential Effects on Corticospinal Excitability

Previous work suggests that longer pulse durations preferentially recruit afferent fibers in the tibial (Lagerquist and Collins 2008; Lin et al. 2002; Panizza et al. 1992), ulnar (Bostock and Rothwell 1997; Panizza et al. 1992; Veale et al. 1973), median (Lin et al. 2002; Panizza et al. 1992; Panizza et al. 1998) and radial (Panizza et al. 1998) nerves. Accordingly, consistent with an increase in the magnitude of the afferent volley,

longer pulse durations have been shown to increase H-reflex amplitudes recorded from soleus (Lagerquist and Collins 2008; Panizza et al. 1989; Panizza et al. 1992), extensor digitorum (Panizza et al. 1989), flexor carpi radialis (Panizza et al. 1989; Panizza et al. 1992), abductor digiti quinti (Panizza et al. 1992) and abductor pollicis brevis (Panizza et al. 1992). As such, based on the premise that longer pulse durations generate a larger afferent volley, it was hypothesized that longer NMES pulse durations would increase CS excitability more than shorter pulse durations. The results did not support this hypothesis; CS excitability increased following NMES at all three pulse durations, but these increases were not different between pulse durations. Because the effect of larger pulse durations preferentially recruiting afferent fibers had not previously been demonstrated in the common peroneal (CP) nerve, to determine if there was an effect on the afferent volley, experiments were conducted in which M-wave and H-reflex recruitment curves (MH-RCs) were collected from tibialis anterior (TA). The results supported the findings from other muscles (Lagerquist and Collins 2008; Panizza et al. 1989; Panizza et al. 1992); H-reflex amplitudes generated from TA were larger with relatively longer pulse durations.

As such, it was concluded that although longer pulse durations increase the afferent volley to the spinal cord (as measured indirectly via H-reflexes), there is no differential influence of pulse duration on CS excitability (as measured via MEPs). It may be that pulse duration has an effect at the CS level, but that MEPs are not sensitive enough to measure the effect on CS excitability, or it may also be that due to the CS pathway being more complex than H-reflex circuitry, the effect of pulse duration is not large enough to influence change in CS excitability.

4.3 Tying the Results Together

The findings from each of the research chapters shed some light on the best parameter of NMES for increasing CS excitability. Chapter 2 (time-course of change in CS excitability during FES versus SS) revealed that FES increased CS excitability in high threshold CS pathways, while SS did not influence CS excitability. Further, whether FES was delivered with or without breaks did not alter the changes in the results. Chapter 3

(effect of pulse duration on CS excitability and H-reflex amplitude) revealed that although pulse duration had an effect on the afferent volley, there was no effect of pulse duration on CS excitability; long and short pulse durations were not different in their influence on CS excitability.

The effect of various NMES parameters on CS excitability is important to consider for rehabilitation; increased excitability induced by NMES has been shown to result in lasting functional improvements (Everaert et al. 2010; Fraser et al. 2002), so when NMES is used in rehabilitation it may be beneficial to apply it in a way that best increases CS excitability. Longer pulse durations, although they may not influence CS excitability, increase the contribution of central pathways to muscle contractions, which recruit motor units in a natural order, and thus may reduce NMES-related fatigue (Lagerquist and Collins 2010). Frequencies of 100Hz evoke the greatest increases in CS excitability (Mang et al. 2010), as do stimulation amplitudes above motor threshold (Chipchase et al. 2011). Chapter 2 revealed that whether there were breaks in the stimulation every 5 minutes was not important for the effects on CS excitability. Thus, when NMES is used in rehabilitation to generate fused muscle contractions, and produce functional movements for people with spinal cord injury or stroke, a stimulation protocol of 1ms pulse duration ([to potentially reduce fatigue] Lagerquist and Collins 2010), 100 Hz frequency (Mang et al. 2010) and amplitude above motor threshold (Chipchase et al. 2011) will generate the greatest increases in CS excitability and concomitant recruitment of central pathways for muscle contractions. SS is used to prime the nervous system for training (Hoffman and Field Fote 2007), though the results from Chapter 2 suggest that this may not be effective for the purpose of increasing CS excitability, as there were no changes in CS excitability detected. Additionally, where SS is shown to increase CS excitability (McKay et al. 2002), the effect takes longer than FES to accumulate (45 minutes [McKay et al. 2002] versus 24 minutes [Mang et al. 2010]), so SS is less timely than FES for the purpose of increasing CS excitability.

In both Chapter 2 and 3, FES was shown to increase CS excitability, though the measures in which these changes were observed were different. When FES was applied over the median and ulnar nerves (in Chapter 2) at the wrist, only increases in MEPs at

high TMS intensities (i.e. at MEP_{max}) were observed, while those at low TMS intensities (i.e. at 1.2x RMT) did not change. However, when FES was applied over the CP nerve at the knee (in Chapter 3), increases in MEPS were observed at low TMS intensities (i.e. at 1.2x RMT). In these experiments MEP_{max} was not reached consistently for all participants due to limitations in the TMS intensity, and thus were not analyzed, so cannot be commented on. The difference in results between the two experiments is likely due to the different muscles tested, as research indicates that different parts of the body respond to NMES differently (Mang et al. 2011). For example, NMES-induced increases in CS excitability for the lower limb are not localized to the muscle activated and there are "global" increases in CS excitability that extend to neighboring muscles, while the NMES-induced increases in CS excitability for the upper limb have been shown to increase selectively for the muscle activated without any effect of neighboring muscle groups (Mang et al. 2010). Additionally, the FES was delivered for 60 minutes in Chapter 2 (with breaks every 5 minutes as well as continuously for 60 minutes, in a separate session), whereas in Chapter 3 the FES was delivered for 30 minutes (with one break at 15 minutes). It may be that the differences in length of stimulation impacted the results, as between Chapters 2 and 3, the NMES duration and pauses were not controlled for. This could potentially impact results should the increases in CS excitability accumulate differently based on how prolonged the stimulation was, or how frequently breaks in the stimulation were dispersed.

4.4 Increasing Corticospinal Excitability for Rehabilitation

For increasing CS excitability, there are many techniques other than NMES to consider for rehabilitative purposes. As described in the General Introduction, these include repetitive TMS ([rTMS] Hoogendam et al. 2010), paired associative stimulation (Stefan et al. 2000) and transcranial direct current stimulation (Uy and Ridding 2000). In rehabilitation, lasting improvements in function are accompanied by increased CS excitability (Everaert et al. 2010). Thus, increasing CS excitability in rehabilitation has the potential to enhance beneficial neuroplasticity. Ideally, a technique to increase CS

excitability that has a large effect size, is fast acting, safe and has long-lasting effects should be used.

The largest and longest-lasting effects on CS excitability are observed with rTMS. Specifically, 30 minutes of quadripulse stimulation, a high intensity sub-type of rTMS, induced 300% increases in MEPs for 90 minutes post stimulation (Hamada et al. 2007). Whether this is practical for rehabilitation is questionable due to the specialized equipment and high TMS intensities required. Should the high-TMS intensity of quadripulse stimulation be a concern, conventional rTMS would be more appropriate as the TMS intensity is lower (below motor threshold) and the time of application is similar (~20 minutes), although the effects are not as large ([~40% increase] Jung et al. 2008). Should time of application be of importance for a rehabilitative procedure, facilitatory theta burst stimulation might be the most appropriate as the stimulation duration is around 3 minutes to generate a larger effect that rTMS evokes ([~200% increase in CS excitability] Huang et al. 2005). However, the effects are not long-lasting and do not persist for even 20 minutes after the stimulation is over (Zafar et al. 2008). The limitations of rTMS include the risk of pain and headaches (Wassermann 1998); safety guidelines are not fully established (Wu et al. 2012; Huang et al. 2005); there is risk of excessive coil heating and specialized, expensive equipment is required.

Given that NMES is used in rehabilitation to generate functional contractions, reduce muscle atrophy (Bergquist et al. 2011), maintain bone density and improve cardiovascular health (Sheffler and Chae 2007), applying NMES in a way that maximizes increases in CS excitability at the same time could enhance the benefit that patients receive from this type of treatment. Although the duration of stimulation for NMES to increase CS excitability is longer than rTMS, CS excitability is shown to increase by a similar amount ([~50%] Bergquist et al. 2011; Knash et al. 2003; McKay et al. 2002), and NMES is a practical technique for clinical use due to increased accessibility of equipment and safety. The limitations to NMES include that it may be painful for some people, treatment times are lengthy and optimal parameters of application must be determined. Additionally, due to the inter-individual variability in the effect of NMES on CS excitability, the technique may not be effective for all individuals (Chipchase et al.

2011b; Kaelin-Lang et al. 2002). Overall, if the aim is fast and long lasting changes in CS excitability, I recommend quadripulse TMS. If the aim is accessible equipment and safety, I recommend NMES delivered using the parameters outlined in Section 4.1.3.

Whether increasing CS excitability is a beneficial technique for rehabilitation requires further research, though the technique appears to be promising; FES-induced increases in CS excitability are shown to be correlated with long-lasting increased function which is attributed to a strengthened CS pathway (Everaert et al. 2010). In people who have had a stroke, this strengthening of CS pathways is a result of the undamaged adjacent areas of the cortex shifting in representation to that of the affected muscle, and thus being recruited for movement of the impaired muscle (Everaert et al. 2010). This is thought to be a result of the combination of afferent input from the FES and efferent output from the descending voluntary command, which induces a Hebbian learning type of plasticity (Everaert et al. 2010). As such, whether the increases in CS excitability induced by NMES alone will strengthen the CS pathway requires further investigation. Typically the short term induced increases in CS excitability from NMES-alone return to baseline by 1 hour after the stimulation is over (Khaslavskaia et al. 2002), however when applied repeatedly and combined with voluntary drive, these increases in CS excitability appear to accumulate over time, and result a conversion from transient to long-lasting increases in CS excitability and lasting improvements in function (Everaert et al. 2010). As such, it appears that increasing CS excitability for rehabilitation is a promising tool with beneficial results when combined with voluntary movement.

4.5 Limitations and Future Directions

Aside from rehabilitative limitations of TMS outlined above, there are several limitations of using TMS for CS excitability research. Firstly, because MEPs are measured from motoneuron discharge located far from the TMS input, there are several segmental influences, both spinal and supraspinal, that can alter the amplitude of the MEP (Burke and Pierrot-Deseilligny 2009). Should changes in facilitation or inhibition occur at any level between the motor cortex and the muscle (in either direction), the

amplitude of the MEP could be affected. One such segmental factor that affects MEP amplitude is neurotransmitter release between the CS neurons and the spinal motoneurons (Petersen et al. 2003). As a result of the potential for multi-segmental influences on MEPs, brainstem electrical stimulation could be used in conjunction with MEPs (Kaelin-Lang et al. 2002) to better determine the locus of change and identify the presence of sub-brainstem influences on the MEP. A second limitation is that TMS is not very focal (Burke and Pierrot-Deseilligny 2009). This may result in unintentional activation of cortical areas distant from the area of interest. This is important to consider, as CS excitability can be influenced even by subthreshold stimulation, as can occur under areas far from the center of the figure eight coil (Burke and Pierrot-Deseilligny 2009). Thirdly, MEPs are sensitive to the direction of the TMS current (Di Lazzaro et al. 2008; Terao and Ugawa 2002) so precautions must be taken to ensure that the orientation of the coil is consistent between participants and measurements. A final consideration is that MEPS are biased to fast-conducting CS neurons, which only represent a small fraction of the CS pathway, and many slow-conducting CS neurons are not activated at all by TMS (Di Lazzaro et al. 2008). As such, changes in CS excitability for slower-conducting neurons will not be observed by TMS.

The assumptions about the magnitude of the afferent volley that reach the cortex are likely the largest limitation of the studies herein. It was assumed in Chapter 2 that the afferent volley generated by FES was larger than that generated by SS because of the greater overall number of pulses delivered and amplitude of stimulation. It was also assumed in Chapter 3 that the afferent volley was greater with longer pulse durations because the H-reflex was larger with longer pulse durations. In both chapters, we are confident that the assumptions of the size of the afferent volley are valid as previous research indicates that higher pulse amplitude (Chipchase et al. 2011), frequency (Mang et al. 2010) and duration (Lin et al. 2002) increase CS excitability to a greater extent than lower pulse amplitudes, frequencies and durations; for CS excitability to increase to a greater extent a larger afferent volley must be transmitted to the cortex. Therefore, based on these previous findings, we are confident in these assumptions that the afferent volley was larger with FES as compared to SS, as well as

with longer pulse durations as compared to shorter pulse durations. Regardless, a future direction would be to directly measure the amplitude of the afferent volley in these types of experiments. This can be accomplished using microneurography, for example. Additionally, future studies including people who have had spinal cord injury or stroke are a possibility to determine whether changing the parameters of the NMES will transfer to those populations.

4.6 Closing Remarks

The human sensorimotor cortex exhibits remarkable neuroplasticity due to changes in activity (Ridding et al. 2000). As a result of the application of NMES, this neuroplasticity can manifest as an increase in CS excitability which may have beneficial applications for neurorehabilitation (Chae et al. 2008; Liberson et al. 1961; Mang et al. 2010; Merletti et al. 1978) and has been shown to generate lasting functional improvements (Everaert et al. 2010; Fraser et al. 2002). Although there are various studies that investigate the effects of NMES on CS excitability, the NMES parameters used are varied, resulting in gaps in the literature for direct comparisons between different NMES parameters (Bergquist et al. 2011; Chipchase et al. 2011). The experiments in this thesis have demonstrated that to best increase CS excitability with NMES, a FES stimulation protocol (see Chapter 2) of pulses of a variety of durations (see Chapter 3) at 100 Hz frequency (Mang et al. 2010) delivered at amplitudes above motor threshold (Chipchase et al. 2011) will generate the greatest increases in CS excitability.

4.7 References

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