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Neuromuscular Electrical Stimulation and the Central Nervous System

by

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Abstract

Neuromuscular electrical stimulation (NMES) is a common therapeutic tool for persons with movement disorders. The manner in which NMES generates muscular contractions has traditionally been attributed to the depolarization of motor axons underneath the stimulating electrodes, a purely peripheral mechanism, which does not involve the central nervous system (CNS). During NMES however, sensory axons are also recruited, initiating an afferent volley which can affect both spinal and cortical centers. This thesis is focused on identifying how this afferent volley influences NMES-evoked contractions and CNS excitability. Four projects are described in which NMES was delivered to generate plantar-flexion contractions. The first goal was to establish the influence of stimulus pulse width on the central recruitment of motoneurons. Contrary to previous findings, changing the pulse width did not significantly alter maximal soleus H-reflex amplitudes; however, wider pulses resulted in a leftward shift of the H-reflex recruitment curve and increased H-reflex amplitudes on the ascending limb of the recruitment curve. The second goal was to examine the effect of stimulus pulse-width on electromyographic responses and torque during NMES. During 20 Hz NMES, wide pulse widths depressed motor-waves (M-waves) and enhanced H-reflexes, generating larger contractions with a relatively greater central contribution, than when narrow pulses were used. The third project compared the torque produced during NMES-evoked contractions before and during a complete anesthetic block of the tibial and common peroneal nerves. Results from this project showed that

contractions arising from a combination of central and peripheral mechanisms fatigue less than contractions that develop from the recruitment of motor axons alone. The final project investigated how spinal and corticospinal excitability associated with the soleus muscles are affected following NMES, voluntary contractions, or a combination of both. It was found that a combination of voluntary contractions and electrical stimulation induced plastic changes in the spinal circuitry of the stimulated muscle without affecting cortical circuitry or inducing any contralateral effects. Collectively, these experiments highlight that wider pulse widths induce a greater reflexive recruitment of motoneurons which contributes to the evoked torque during NMES, and that the evoked afferent volley reduces fatigue and influences spinal circuitry plasticity in the plantar-flexors. Methods to enhance the afferent volley during NMES are only beginning to be tested in clinical populations and future experiments will determine the potential efficacy for persons with movement disorders.

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List of Abbreviations

ACT% - Activation percentage
ANOVA -Analysis of variance
CNS - Central nervous system
CON - Control
EMG - Electromyography
FES - Functional Electrical stimulation
H-reflex - Hoffmann reflex
H_{max} - Hoffmann reflex maximum
H_{max}/M_{max} - Hoffmann reflex maximum and motor wave maximum ratio
$H_{5\%Mmax}$ - Size of the Hoffmann reflex when the motor wave is 5% of maximal
ITT - Interpolated twitch
K+ - Potassium
LSOL - Left soleus
MEP - Motor evoked potential
MVC - Maximal voluntary contraction
M-wave - Motor wave
M_{max} - Motor wave maximum
M_{Hmax} - Size of the motor wave when the Hoffmann reflex is maximal
MVF - Maximum voluntary force
Na+ - Sodium
NMES - Neuromuscular electrical stimulation
NMJ - Neuromuscular junction
PIC - Persistent inward current
RSOL - Right soleus
TA - Tibialis anterior
TES - Transcranial magnetic stimulation
TMF - True maximum force
TMS - Transcranial magnetic stimulation
TNMES - Intermittent Neuromuscular electrical stimulation over the right tibial nerve
V+TNMES - Intermittent, voluntary, isometric contractions of the right plantar-flexors in conjunction with intermittent neuromuscular electrical stimulation over the right tibial nerve
VOL - Intermittent, voluntary, isometric contractions of the right plantar-flexors
WPS - Wide pulse stimulation

1.0 General introduction

Electrical stimulation is commonly used for the treatment of many conditions that affect muscles and the nervous system, in particular for persons with damage to the central nervous system (CNS). Damage to the CNS, such as that which occurs following spinal cord injury (SCI) or stroke, often results in motor impairment which disrupts the ability to voluntarily generate muscular contractions. Even if voluntary movements are not possible, nerve and muscle are still electrically excitable tissues making muscular contractions possible when external electrical stimulation is applied. This form of stimulation is referred to as neuromuscular electrical stimulation (NMES).

The therapeutic applications of NMES are numerous, including prevention of muscle atrophy, increased circulation, increased bone density, and treatment of spasticity (71). In addition, NMES can be used to activate paralyzed muscle in a specific combination and sequence to accomplish functional tasks, termed functional electrical stimulation (FES) (57). The first application of FES was for the treatment of foot drop (51); however, since that time a number of systems have been developed to assist with tasks of daily living such as standing, walking (48), grasping (66), bladder function (35) and breathing (70) (for reviews of NMES and FES see 71, 74, 63). NMES systems deliver current to nerves through surface or implanted electrodes. Implanted electrodes offer advantages over surface electrodes by eliminating pain caused by activating cutaneous receptors, eliminating skin resistance and allowing for greater muscle selectivity and lower stimulation currents. Despite these advantages and the

success of many implanted systems, surface stimulation is still widely used due to the higher cost of implanted systems and their invasive nature. While the benefits of NMES are numerous, limitations remain.

Typically, NMES is delivered using 200 μs pulse widths at constant frequencies of 10-50 Hz (71). The ensuing contractions are traditionally believed to arise from the activation of distal motor axons beneath the stimulating electrodes (34, 58, 71) and the central recruitment of motoneurons is seldom considered. During contractions generated via the direct depolarization of motor axons underneath the stimulating electrodes (peripheral mechanism) motor units are recruited in a non-physiological manner, causing increased fatigue and limiting the usefulness of NMES in rehabilitation (26). In recent years however, it has been noted that when NMES is delivered using wider pulse widths and higher frequencies than traditionally used (1ms;100 Hz; WPS-wide pulse stimulation) contractions arise from *both* peripheral and central mechanisms (for review see 16). The central recruitment of motoneurons is believed to be caused by the activation of sensory axons, particularly Ia afferents (14, 15, 16), and should thus activate motor units in the normal physiological recruitment order (3, 29) allowing for the most fatigue resistant motor units to be recruited first. This is quite different from the non-physiological recruitment of motor units occurring during peripheral activation of motor axons. Aside from decreasing fatigue, increasing the afferent volley during NMES may be advantageous for rehabilitation because of the beneficial plastic changes it can induce within the CNS. It has for instance, been reported that NMES leads to plastic changes

within cortical structures (41, 67) which have been linked to improved motor re-learning (65).

It is the main objective of this thesis to create a better understanding of how the afferent volley generated during NMES influences the CNS. All experiments were performed on healthy, neurologically intact adults. The experiments described in Chapters 2 and 3 were designed to evaluate how stimulus pulse width used during NMES influences the recruitment of motoneurons. Chapter 4 includes experiments describing how the afferent volley influences fatigue during NMES. Chapter 5 investigates the influence of the afferent volley on plasticity at cortical and spinal sites. Collectively these experiments will improve the current understanding of how the human nervous system responds to the afferent volley evoked by NMES and may lead to improved rehabilitation techniques.

This introductory chapter provides an overview of: 1) the electromyographic measures used throughout this thesis; 2) the current knowledge regarding peripheral and central mechanisms whereby NMES generates muscular contractions and; 3) plasticity within the CNS induced by NMES

1.1 Electromyographic Responses to Peripheral Nerve Stimulation

Two synchronous electromyographic responses can be observed when stimulating a nerve containing both sensory and motor axons; 1) a motor wave (M-wave) and; 2) the reflexively generated Hoffmann reflex (H-reflex) (31). The M-wave is generated by the direct depolarization of motor axons leading to

neurotransmitter release at the neuromuscular junction (NMJ). This causes muscle membrane depolarization and muscle contraction that can be recorded when using surface electromyography (EMG). This response does not involve central recruitment of motoneurons. It is traditionally believed that only the M-wave contributes to muscular contractions during tetanic NMES (see Section 1.2.1).

The H-reflex, considered the electrical equivalent of the stretch reflex is a result of the depolarization of large diameter afferents, leading to neurotransmitter release at the motoneuron synapse. Depolarization of α-motoneurons above threshold will then initiate an efferent volley causing release of neurotransmitter at the NMJ. This, in turn, will result in depolarization and contraction of the muscle fibers that can be recorded as an H-reflex when using EMG. A simplified schematic representation of the monosynaptic H-reflex pathway is shown in Figure 1.1a. A sample EMG trace showing the soleus M-wave and H-reflex is shown in Figure 1.1b. Increasing the stimulus intensity will recruit additional Ia afferents and motor axons causing greater H-reflex and M-wave responses (54) until the maximal H-reflex is obtained (H_{max}, representing maximal reflex activation). With even higher stimulus intensities, the maximal M-wave is obtained (M_{max}, representing maximal direct motor axon activation). Increasing stimulus intensities also increases antidromic propagation along motor axons. At stimulus intensities above that which evokes H_{max} the H-reflex amplitude will decline, typically being abolished at M_{max}. By gradually increasing stimulus intensity from levels that evoke a small H-reflex up to H_{max}

and finally $M_{max,}$ an H-reflex recruitment curve can be obtained. The amplitude of each H-reflex and M-wave from this recruitment curve can then be normalized to the amplitude of the maximal M-wave and represented as an M vs H curve as shown in Figure 1.2.

Originally, the H-reflex was believed to be purely monosynaptic due to the brief latency from stimulation to onset of the waveform (53). Since that time, it has been shown that oligosynaptic pathways contribute to the later portion of the H-reflex waveform (10). Thus, only the first component of the H-reflex waveform is due to the monosynaptic Ia effects and later portions may include oligosynaptic pathways from not only Ia spindle afferents but also group II muscle spindle afferents, Ib afferents from Golgi tendon organs and cutaneous afferents.

1.1.1 Implications of axonal properties for NMES

Pulse widths of 500-1000 μs are recommended when evoking the H-reflex (32, 61, 62) since they preferentially activate sensory axons over motor axons (20, 76). Such preferential recruitment is credited to sensory axons having a longer strength-duration time constant and a lower rheobase than motor axons (62). Axons having a greater strength-duration time constant will be more easily stimulated when using wider pulse widths and lower intensities than axons with a shorter strength-duration time constant. This is why it is often possible to recruit an H-reflex with stimuli below motor threshold (M-wave threshold). This difference between motor and sensory axons has been suggested to be due to a greater persistent Na^+ conductance on sensory axons (52). Thus, wider stimulus

pulse widths should increase the recruitment of sensory axons and maximize the afferent volley that reaches spinal motoneurons during NMES. This has not, however, been adequately tested in humans. To date, only one study has investigated whether differences in ion channel conductance between sensory and motor axons translate into enhanced H-reflex recruitment over a full range of stimulus intensities when using wider stimulus pulse widths (61). These previous results were based on recruitment curves that did not reach H_{max} due to limitations in stimulator output and, therefore, may be invalid. *It is the 1st objective of this thesis to compare the recruitment of motoneurons via the H-reflex pathway when using a range of stimulus pulse widths.*

1.1.2 Methods of evaluating the H-reflex

The recruitment of the H-reflex is often depicted as normalized to M_{max} via the construction of an M vs H recruitment curve. Figure 1.2 shows a normalized soleus M vs H recruitment curve collected from a single subject and illustrates three methods commonly used to evaluate the excitability of the H-reflex pathway, namely the H_{max}:M_{max} ratio, the H-reflex with accompanying M-waves of 5%M_{max} (H5%M_{max}), and the slope of the ascending limb from the M vs H recruitment curve. It is common to evoke an H-reflex accompanied by a small, stable M-wave such as with H5%M_{max}. This allows for evaluation of facilitation or inhibition of the H-reflex, while using the M-wave as a measure of stimulus consistency (77). To assess the gain of the H-reflex, a common method is to fit the ascending limb of the recruitment curve using the least sum of squares method (37, 50) (see Figure 1.2).

1.2 Motor Unit Recruitment During NMES

1.2.1 Generating Contractions from Peripheral Mechanisms

If the electric field is strong enough to adequately depolarize the membrane of motor axons when NMES is applied, voltage-gated ion channels will open and allow extracellular sodium to enter the cell. This opening of voltage gated ion channels will cause the beginnings of the cascade of ion exchange that defines the action potential (30). In motor axons this action potential will travel both proximally towards the motoneuron cell body (antidromic) and distally towards the neuromuscular junction (orthodromic). Antidromic propagation usually results in annihilation of the action potential at the cell body, whereas orthodromic propagation will result in the release of the neurotransmitter acetylcholine at the neuromuscular junction, thereby causing depolarization and contraction of muscle fibers. The greater the numbers of depolarized motor axons, the greater the neurotransmitter release at the NMJ and the larger the number of activated muscle fibers. A single pulse of NMES will not produce the maximal electrically-evoked force from a muscle, this can only be achieved by delivering tetanic stimulation at frequencies typically greater than 10 Hz. The resultant contractions from NMES delivered at tetanic frequencies are traditionally believed to arise exclusively via the activation of peripheral motor axons beneath the stimulating electrodes (34, 58, 71). Provided that lower motoneurons in the spinal cord are intact, the delivery of NMES will activate nerve before depolarizing muscle directly because the threshold charge for

generating action potentials in muscle is much greater (>100x) than that of nerves (34, 58).

Voluntary motor unit recruitment follows Henneman's size principle with small, fatigue-resistant units recruited first and the largest, most powerful and fatiguable units recruited last (3, 29). NMES on the other hand, has traditionally been believed to recruit motor units in a reversed order compared to voluntary activation, with the most fatigable units recruited first. In reduced animal preparations large motor axons were found to have a lower rheobase, and depolarized at lower stimulus intensities compared to smaller axons (7). Since muscular contractions evoked by NMES in humans fatigue more quickly than voluntary contractions, and because fatigable motor units have larger motor axons than fatigue resistant motor units, this has been interpreted as support for a reversal of recruitment order (for review see 26). The notion of a true reversal of motor unit recruitment in humans has since been challenged with findings of both random (21, 36) and normal (73) recruitment order during electrical stimulation. Thus, the assumptions that underlie the classical hypothesis of NMES preferentially activating fast-fatiguable fibers before slower, more fatigue-resistant fibers may not be valid.

Larger, more fatiguable motor units have greater motor axon diameter and are more easily depolarized by external currents *in situ* (7). This relationship does not necessarily transfer well to the stimulation of nerves via transcutaneous NMES in vivo, however (45). Several factors such as skin conductance, fat deposition and motor axon orientation will affect the order of recruitment when

using NMES in humans; thus, assumptions based on in vitro and in situ animal experiments may not apply directly to results from in vivo human experiments. Since the first suggestion of random recruitment by transcutaneous NMES (45), several studies examining motor unit recruitment of the tibialis anterior (TA) (21) and the quadriceps (36) have supported the hypothesis of random motor unit recruitment during NMES. Interestingly, one study found Henneman-like motor unit recruitment in paralyzed muscle during median nerve stimulation (73). This finding is currently the only example of a normal recruitment order induced by NMES. Evidence is mounting to suggest that transcutaenous NMES recruits motor units randomly regardless of axon diameter and fiber type (26). Almost all previous experiments have assumed that motor units are recruited *exclusively* via the direct depolarization of distal motor axons underneath the stimulating electrodes and little consideration is given to the central recruitment of motoneurons.

1.2.2 Generating Contractions from Central Mechanisms

Since 2001 numerous experiments have found evidence showing that it is also possible to generate contractions during tetanic NMES from the central recruitment of motoneurons by employing novel stimulation parameters (14, 15, 16, 1, 8, 18, 19, 47, 59). Figure 1.3 illustrates plantar-flexion generated using two different stimulation protocols in a single subject (adapted from Collins et al., 2007 (14)). Both stimulation protocols generated the same amount of tetanic force within the first four seconds of stimulation (illustrated within the grey ellipse); however, forces continued to climb when using wide-pulse stimulation

(WPS; 100 Hz, 1000 μs pulse width) while forces decreased with conventional stimulation (25 Hz, 50 μs pulse width; see "extra" torque due to central mechanism in Figure 1.3) It was hypothesized that the generation of central torque arose due to activation of a central mechanism with a slower time course than the direct depolarization of motor axons (14).

Recruiting motor units via synaptic input from large diameter afferents, such as Ia fibers, results in a natural recruitment order that follows the Henneman size principle (3, 29). Contractions generated from a large central recruitment of motoneurons should involve lower threshold motor units and exhibit less fatigue than contractions generated via the direct depolarization of motor axons beneath the stimulating electrodes. Since wide pulses are more effective than narrow pulses for depolarizing sensory axons (2,76), Collins and colleagues (14, 15) have proposed that this central contribution to torque should be greatest when using wide pulses; however, it has not been determined how pulse width affects the central recruitment of motoneurons during tetanic NMES. *It is the second objective of this thesis to extend our previous work on stimulus pulse width and H-reflex recruitment developed in Chapter 2 by investigating the recruitment of motor axons (M-waves), the reflexive recruitment of motoneurons (H-reflex), and the development of torque during tetanic NMES* delivered using a range of pulse widths.

At high intensities, the antidromic volley in motor axons makes it likely that little or no contribution from the CNS is possible (25, 64); thus at maximal intensities, NMES evoked contractions will be driven primarily by the direct

depolarization of motor axons beneath the stimulation site. When using lower stimulus intensities however, the synaptic recruitment of motoneurons can generate up to 40% of the torque generated during a maximal voluntary contraction (MVC) (14, 16); therefore, it is possible to evoke contractions from direct activation of motor axons *and* the recruitment of spinal motoneurons during NMES, provided that the intensity of stimulation does not produce large amounts of antidromic block (14, 15, 16, 1, 8, 18, 19, 47, 59). Evidence that this phenomenon is due to central mechanisms was originally identified in two subjects by applying NMES over the triceps surae before and during a complete anesthetic block of the tibial nerve in the popliteal fossa (14). When the nerve is blocked between the stimulation site and the spinal cord, it temporarily separates the muscle from the CNS. Thus, before the nerve block, all NMES evoked torque will be the result of peripheral *and* central mechanisms; however, during the nerve block, only peripheral mechanisms are able to contribute. These experiments showed that more plantar-flexion and dorsi-flexion torque developed during NMES in the intact condition (when the CNS could contribute) compared to the blocked condition (see Figure 1.4).

By combining wide pulse widths to maximize the recruitment of sensory axons and higher frequencies to increase the rate of afferent volleys, WPS should enhance the afferent volley to the spinal cord leading to a greater synaptic recruitment of motoneurons. It is this potential for synaptic recruitment of motoneurons during WPS that may provide advantages for rehabilitation compared to conventional methods of NMES.

1.2.2.1 Synchronous reflex activation

Recruitment of motoneurons by Ia afferent inputs follows the Henneman size principle (3, 29), unlike the random motor unit recruitment that is believed to occur when directly depolarizing motor axons (26). It would, therefore, seem advantageous to maximize the H-reflex contribution during NMES in those muscles where an H-reflex is easily evoked; however, reflexes are not traditionally believed to contribute to the evoked muscular contraction during NMES. This belief is based on evidence that post-activation depression of neurotransmitter release from afferent terminals, as well as antidromic transmission along motor axons (particularly at high stimulus intensities), reduce the likelihood that transmission along reflex pathways (such as the H-reflex) can make a significant contribution to contractions during NMES. Conversely, it has recently been shown that H-reflexes are evident in the EMG during NMES at 20 Hz (47). Klakowicz et al., (47) showed that a 2 s burst of 100 Hz stimulation caused additional force to develop, which persisted when stimulation was returned to 20 Hz, consistent with the original findings of Collins et al. (14). In addition, Klakowicz et al., noted that H-reflexes were initially depressed during stimulation at 20 Hz consistent with post-activation depression of neurotransmitter release at the Ia-motoneuron synapse (33); however, torque *and* H-reflex amplitude both increased significantly after 2s of 100 Hz stimulation. Figure 1.5 illustrates this phenomenon. The partial recovery of the H-reflex that follows the 100 Hz stimulation in Figure 1.5 illustrates that transmission along reflex pathways can contribute to contractions during NMES. This recovery may

be due to post-tetanic potentiation, an increase in neurotransmitter release at the Ia-motoneuron synapse, which has been associated with higher frequencies of stimulation (75). The involvement of persistent inward currents (PICs) in motoneurons and/or increased cortical excitability may also play a role.

1.2.2.2 Persistent inward currents

It was previously believed that motoneurons were passive conduits for synaptic input with no intrinsic ability to contribute to the current required for their recruitment and discharge. It is now known that the motoneurons have voltage-dependent ion channels capable of generating strong inward currents that persist as long as the membrane potential remains above the activation threshold (69, for review see 28). The currents generated from these non-inactivating ion channels have been termed PICs. PICs can lead to sustained depolarizations called plateau potentials that play an important role in regulating motoneuron firing (28). PICs can be triggered by large diameter afferent input and experience a 'warm up' phenomenon after repeated stimulation that can lead to a sustained motoneuron discharge in the absence of synaptic input and is especially prevalent in low threshold, fatigue resistant motor units (5,6). WPS should recruit large diameter sensory axons more effectively than conventional narrow-pulse stimulation at lower frequencies, and thus, enhance the afferent volley to motoneurons. It has therefore, been hypothesized that WPS recruits motoneurons by activating large diameter sensory axons triggering the development of PICs in spinal interneurons and/or motoneurons (14, 15, 16). In support of this hypothesis, WPS delivered at intensities below motor threshold (thereby

activating only sensory axons) can generate contractions as large as 40% of a person's MVC (16,19). Findings of sustained contractions and motor unit firing patterns that were dissociated from the stimulus pulses during NMES (49) and during tendon vibration (9) have been described previously. This behaviour is consistent with the activation of PICs in motoneurons or interneurons. Similar asynchronous motor unit firing due to NMES were later described by Collins *et al.* (14). Acting through a similar mechanism, afferent-driven activation of PICs has also been reported to underlie the sustained contractions that develop during and after periods of tendon vibration (24, 44). At this time however, further experiments are required to conclusively determine if WPS activates PICs.

1.2.3 Clinical implications

Damage to the CNS such as SCI or stroke causes a number of complications, stemming predominantly from inactivity. Following SCI affected muscle fibers will atrophy and transition to behave more like fast-fatigable fibers (11, 34, 55, 72). Additional complications include reduced circulatory capacity, Type II diabetes, cardiovascular disease and osteoporosis (34). The most common method to combat these problems is through the application of NMES; however, poor muscle mass and a greater proportion of fatigable fibers results in weak contractions that fatigue rapidly. In addition, traditional methods of NMES that recruit motor units randomly (in a non-physiological recruitment order) will further decrease the fatigue resistance of electrically-induced contractions. Previous experiments have been directed towards identifying stimulation parameters that maximize motor unit recruitment and decrease fatigue during

NMES (23, 40). Such efforts have been interested in optimizing the recruitment of motor axons directly beneath the stimulating electrodes by manipulating pulse widths and frequencies, but do not consider the possibility of recruiting motoneurons via central mechanisms. Typically such experiments will not exceed 600 μs and 60 Hz stimulus parameters (40). Increasing the central contribution to contractions evoked by NMES may be advantageous for rehabilitation since synaptic drive recruits motor units according to Henneman's size principle (3, 29); therefore, the slow, fatigue-resistant motor units will be recruited before the fast-fatigable motor units even at low stimulus intensities. In line with this principle, recruiting motor units via synaptic drive versus direct motor axon depolarization may improve fatigue resistance during NMES, decrease muscle atrophy and decrease the transformation from slow- to fast-twitch fiber types that occurs following spinal cord injury. This may not only be effective for SCI, but any condition involving disuse atrophy with intact motor units, such as following stroke. *It is the third objective of this thesis to investigate the fatigue resistance of NMES induced contractions using WPS before and during a peripheral nerve block. This will determine if the central recruitment of motor units delays the onset of fatigue during WPS.*

1.3 Cortical excitability

It is possible that increased cortical excitability plays a role in the development of force arising from a central mechanism during NMES (16). It is well documented that NMES can cause long lasting increases in cortical excitability (27, 41, 42, 67, 68). This increased cortical excitability may enhance

long loop reflex activation of α-motoneurons, thus contributing to centrally-generated forces (14). On the other hand, sustained motor unit discharge following NMES has been recorded in the absence of increased cortical excitability, but with concomitant increased H-reflexes (60), suggesting that spinal (rather than cortical) excitability is a contributing mechanism to centrally-generated forces. In addition, evidence of central force during NMES has been demonstrated in a population of complete SCI subjects, also suggesting that descending cortical pathways are not required for this behaviour (59). Based on this evidence, we currently hypothesize that centrally-generated forces during NMES are predominantly due to a spinal mechanism.

1.4 CNS plasticity caused by NMES

During voluntary movement the CNS receives sensory input from proprioceptors in muscle, skin and connective tissue. When learning a voluntary movement the combination of descending cortical commands and sensory input drives neuronal plasticity within the CNS. Increased excitability of cortical projections has been shown following simple (13) and complex (38) voluntary movements. Sensory information can also come from electrically-stimulated movements and electrical activation of sensory fibers during NMES. The application of NMES can cause plasticity at both cortical and spinal levels (for review see 22) and results in improved motor learning (56) as well as improved function following damage to the CNS (17). Early experiments using transcranial magnetic stimulation (TMS), transcranial electric stimulation (TES) and F-waves concluded that NMES of hand muscles induced plasticity in cortical, but not spinal

circuitry (67). Conversely, soleus H-reflexes have been shown to remain elevated for several minutes after the application of NMES to the lower leg (46). In addition, evidence of increased responses to TES has been reported following NMES over the TA muscles (41). This suggests that NMES is capable of inducing plasticity within spinal circuitry, perhaps more so when applied to the lower body.

NMES has been shown to increase the excitability of the specific region of the motor cortex associated with the muscle being stimulated. This has been demonstrated using a number of different protocols in various muscles by examining motor evoked potentials (MEPs) elicited by TMS. MEPs increased following NMES protocols which cause finger flexion/extension (2), finger flexion (12), swallowing (27) and dorsi-flexion of the foot (41, 42, 43). Clinically, it has been demonstrated that NMES improves hand function in persons with stroke (17) and SCI (4). The existence of plasticity in spinal circuitry following electrical stimulation has been debated due to inconsistent findings. Some studies have shown no effect of NMES on spinal circuitry (67, 12) while others demonstrate evidence of plasticity in spinal circuitry following NMES (41, 46).

Some experiments have combined voluntary movement with electrical stimulation to evaluate whether such a combination produces greater amounts of plasticity within the CNS. This has been explored using NMES over the common peroneal nerve (42) as well as finger flexors and extensors (2). Thus far it appears that a combination of NMES and voluntary movement produces a greater amount of plasticity within the CNS compared to NMES or voluntary activation in isolation. To date the influence of NMES and voluntary drive has

not been evaluated by stimulating the tibial nerve innervating the plantar-flexors. Therefore, *it is the fourth objective of this thesis to investigate how voluntary drive and tibial nerve stimulation, together, or in isolation, influence corticospinal and spinal plasticity in the left and right soleus muscles.*

1.5 Summary

WPS generates contractions from the peripheral activation of motor axons underneath the stimulating electrodes *and* the activation of motoneurons in the spinal cord. The central contribution to contractions during WPS may arise from a presynaptic potentiation of neurotransmitter release from large diameter afferents onto motoneurons or the activation of persistent inward current in motoneurons. Cortical mechanisms are not hypothesized to be involved in this process; however, their influence cannot be excluded. While the exact mechanism remains to be elucidated, a central recruitment of motoneurons due to WPS will likely recruit motor units in a natural order, unlike random recruitment during peripheral activation of motor axons. Using WPS may therefore, be beneficial for reducing muscle fatigue and reducing muscle atrophy for persons with movement disorders. In addition, maximizing the afferent volley during NMES by using WPS may increase the excitability within spinal and cortical centers, which may also be beneficial for rehabilitation.

1.6 Thesis Objectives:

This thesis has three main objectives: 1) to evaluate how the afferent volley is influenced by the stimulus pulse width during NMES; 2) to describe how the afferent volley affects fatigue resistance during NMES contractions and;

3) to investigate the influence of the afferent volley on spinal and cortical plasticity.

Chapter 2. This study compared the recruitment of spinal motoneurons through the H-reflex pathway using a range of stimulus pulse widths. The objective of these experiments was to determine how narrow and wide pulse widths differentially recruit motoneurons via reflex pathways when delivering single pulses, as measured via the H- reflex.

Chapter 3. These experiments evaluated if stimulus pulse width affected the amount of force generated during NMES and if a greater amount of force is associated with increased transmission via the H-reflex pathway. The objectives were to describe how pulse width affects the recruitment of motor axons (M-waves), the reflexive recruitment of motoneurons (H-reflex) and the development of torque during tetanic NMES.

Chapter 4. In this study we investigated the fatigue resistance of NMES contractions before and during a peripheral nerve block. The objective was to identify if the central recruitment of motor units delays the onset of fatigue during NMES.

Chapter 5. These experiments investigated how voluntary drive and unilateral tibial nerve stimulation influenced corticospinal and spinal plasticity associated with both the left and right soleus muscles. The objective was to determine if NMES can affect corticospinal and spinal circuitry following a single session of NMES delivered to the plantar-flexors.

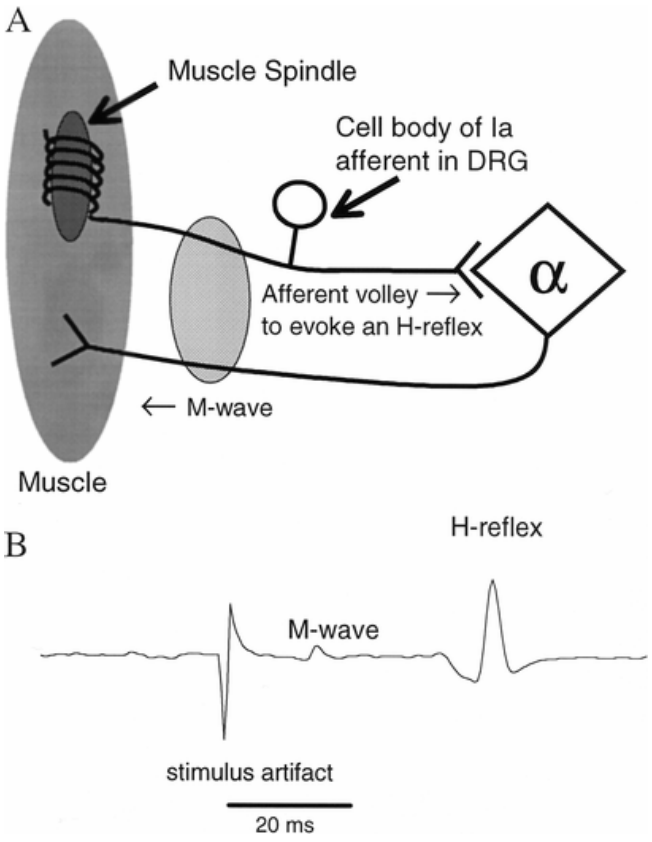

Figure. 1.1. A) Simplistic schematic of the spinal processing of the monosynaptic component of the H-reflex. The electrical stimulus used to activate the mixed peripheral nerve is shown by the *grey ellipse*. The activation of the nerve is shown to propagate orthodromically in the motor axons to evoke the M-wave, and orthodromically in the sensory axons (shown here as group Ia afferents arising from the muscle spindle) to evoke the H-reflex via a monosynaptic connection to the alpha motoneurons (α) (*DRG* Dorsal root ganglion). B) An EMG trace of the stimulus artifact, M-wave and H-reflex evoked in the soleus muscle while stimulating the tibial nerve. Adapted from Zehr (2002).

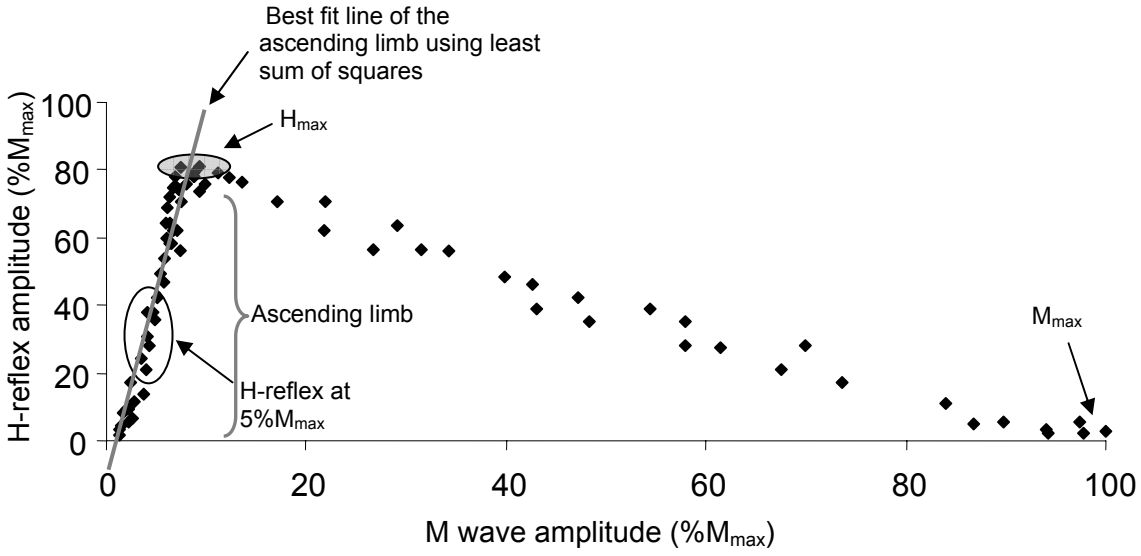

Figure 1.2. A soleus M/H recruitment curve from a single subject. The slope of the ascending recruitment curve is indicated by the grey line. H-reflex values with a concomitant M wave that is 5% M_{max} is shown in the middle of the ascending limb by the dashed ellipse. Maximal H-reflex values are shown at the apex of the ascending limb by the grey ellipse.

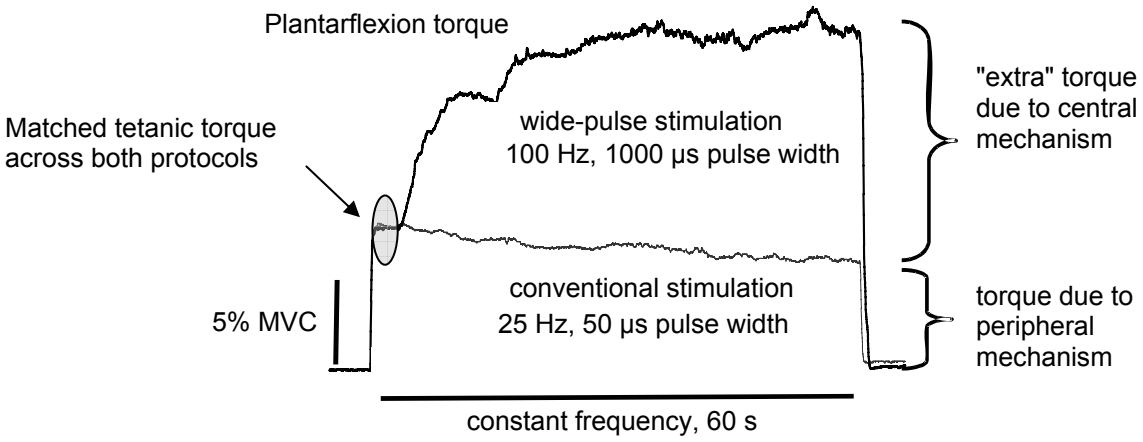

Figure 1.3. Torque recorded from the plantar-flexors during NMES delivered using conventional NMES (25 Hz, 50 μs) and wide-pulse stimulation (100 Hz, 1000 μs). The grey ellipse shows that both protocols evoked the same tetanic force in the first 3 sec of stimulation. Adapted from Collins (2007).

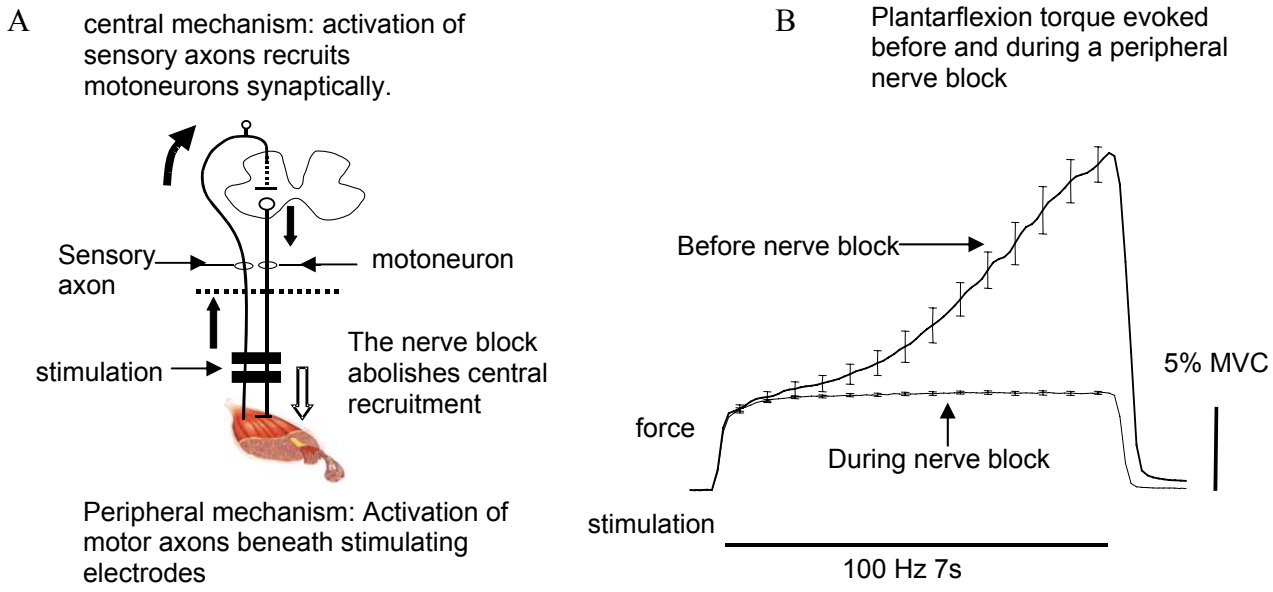

Figure 1.4. A) Central and peripheral mechanisms contribute to contractions during WPS. Motor units are recruited by the electrically evoked sensory volley (central mechanism) and by the direct depolarization of motor axons beneath the stimulating electrodes (peripheral mechanism). B) Plantar-flexion force evoked by a single subject during stimulation over the triceps surae before and during a complete peripheral nerve block. Adapted from Collins (2007).

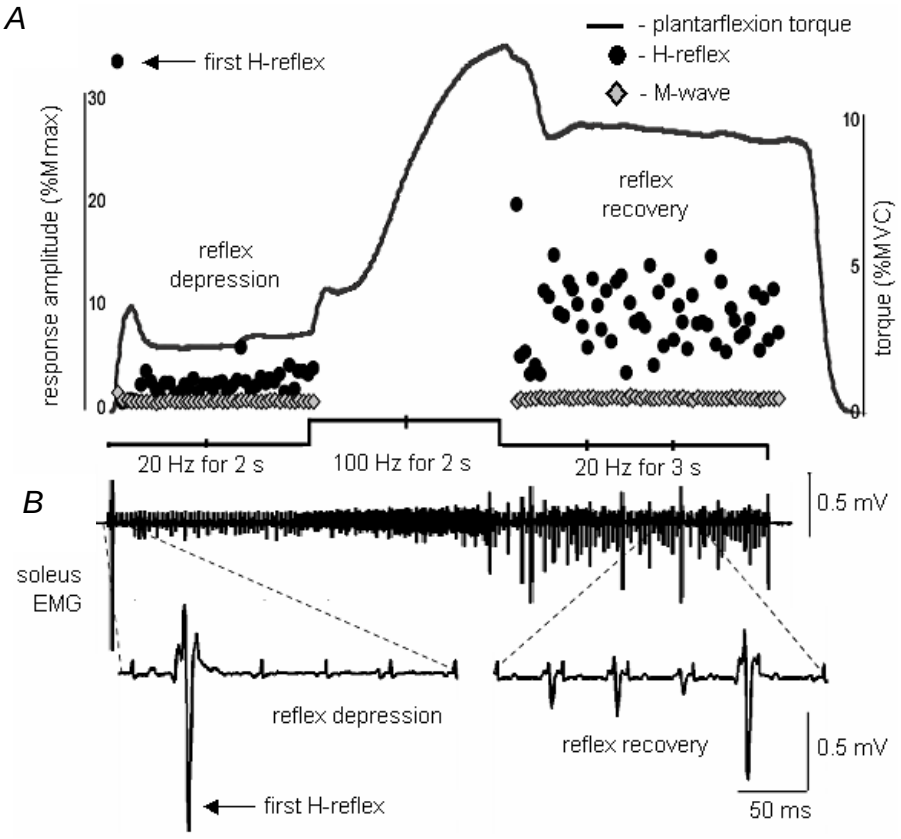

Figure 1.5. Enhanced H-reflexes contribute to the development of central torque. A) Torque, M-wave and H-reflex amplitude averaged over 5 successive stimulus trains in a single subject. M-waves and H-reflexes are not shown during the 100 Hz due to stimulus artefact interference. B) Soleus EMG recorded during a single stimulus train. Adapted from Klakowicz et al., (2006).

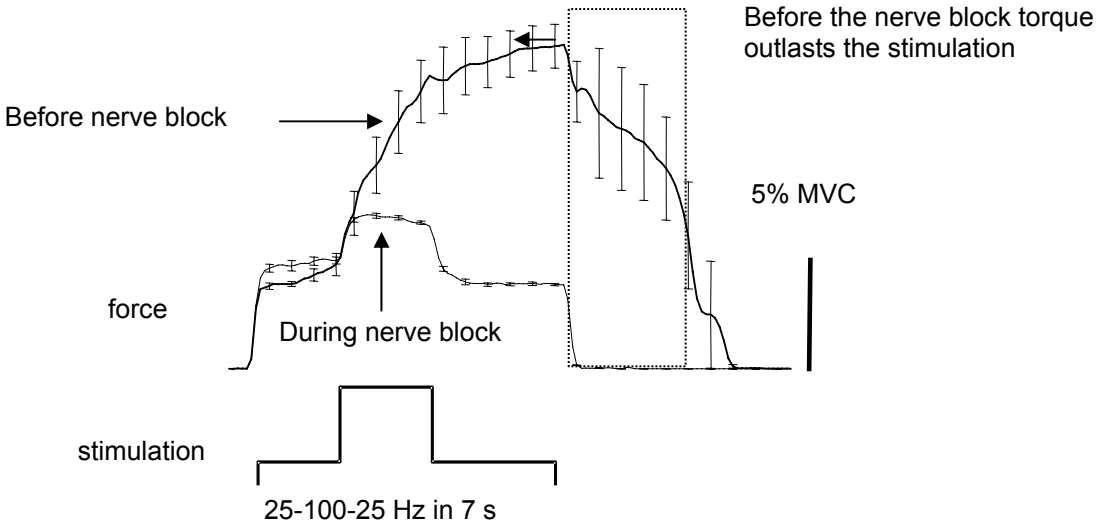

Figure 1.6. Sustained motor unit firing that outlasts the stimulation. The persistence of force that outlasts the stimulation occurs only before the nerve block when the CNS can contribute to the evoked contraction. Each trace shows the mean plantar-flexion torque evoked by five stimulus trains in a single subject. Adapted from Collins (2007).

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2.0 Stimulus pulse width influences H-reflex recruitment but not H_{max}/M_{max} ratio*

2.1 Introduction

It has been reported that pulse-widths of 500-1000 μs should be used for studies utilizing the H-reflex since they preferentially activate sensory rather than motor axons (18, 26, 33). Such preferential recruitment has been attributed to sensory axons having longer strength-duration time constants and a lower rheobase than motor axons, possibly due to a greater persistent Na^+ conductance in sensory axons (4, 24). A longer strength-duration time constant means an axon will be more easily stimulated when using wider pulse-widths and lower intensities than axons with shorter strength-duration time constants. To our knowledge, however, only one study has investigated whether the different biophysical properties of sensory and motor axons translate into enhanced H-reflex recruitment over a full range of stimulus intensities when using wider stimulus pulse-widths (26). Panizza et al., (26) reported that pulse-widths of 300-1000 μs evoked significantly larger maximal soleus H-reflexes (H_{max}) than narrower pulses; however, these results were based on recruitment curves that did not reach H_{max} for the narrowest pulse widths due to limitations in stimulator output, bringing into question the validity of these findings.

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*This chapter has been published. Lagerquist, Collins. Stimulus pulse width influences H-reflex recruitment but not H_{max}/M_{max} ratio. *Muscle Nerve* 37: 483-489, 2008.

Understanding how stimulus pulse-width affects H-reflex recruitment has implications for clinicians and researchers utilizing this technique. When investigating changes in transmission along the H-reflex pathway, it is common to use an H-reflex on the ascending limb of the recruitment curve accompanied by a small, stable M-wave. This allows facilitation or inhibition of the H-reflex to be evaluated while using the M-wave as a measure of stimulus consistency (34). Another common measure used to evaluate the H-reflex is the H_{max}/M_{max} ratio. The present experiments identify how pulse-width influences these and other commonly used measures of H-reflex excitability. In addition, a better understanding of how pulse-width affects the reflexive recruitment of motor units has implications for using neuromuscular electrical stimulation (NMES) in rehabilitation. We have proposed that during tetanic NMES, wide pulses (1000 μs) generate a larger afferent volley than narrower pulses and produce contractions with a greater reflex contribution (8-11). Activating motor units synaptically in addition to the direct activation of motor axons beneath the stimulating electrodes may result in a more physiological recruitment order, thus decreasing the muscle fatigue normally experienced with NMES (8-11). The present experiments were designed to compare recruitment of motoneurons through the H-reflex pathway using a range of stimulus pulse-widths.

We hypothesized that the H vs. M recruitment curves would shift to the left when using increasingly wider pulse-widths, resulting in a larger H-reflex for a given M-wave amplitude, due to the activation of a larger proportion of sensory axons relative to motor axons. In addition, we predicted that increasing

the pulse-width from 50 to 1000 μs would result in larger H_{max}/M_{max} ratios due to decreased antidromic block along motor axons when using wide pulses.

2.2 Materials and Methods

2.2.1 Protocol

Twelve neurologically intact persons (19 to 43 years old; 10 males and 2 females) participated with informed consent. This study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Alberta institutional review board. All experimental procedures were performed on the right leg. Subjects were seated with the right hip, knee and ankle at approximately 120, 110, and 90 degrees, respectively.

2.2.2 Electromyography

Surface electromyography (EMG) was recorded from the right soleus and tibialis anterior muscles with bipolar (2.25cm^2) Ag-AgCl electrodes (Vermed Medical Inc. Vermont, U.S.A) placed 1 cm apart. A common reference electrode was placed over the tibial anterior crest of the right leg. EMG signals were pre-amplified 500-1000 times and band-pass filtered at 10-3000 Hz (NeuroLog system, Digitimer Ltd. London U.K.).

2.2.3 Maximal voluntary contractions

Prior to data collection, each subject performed between three and seven practice maximal voluntary plantar flexion contractions (MVCs) until consistent maximal contractions were achieved. Subjects then performed a single MVC lasting 3-5s. Isometric torque and EMG were averaged over a 0.5s period centered around the point of maximal torque produced during this MVC.

Subjects were provided with visual feedback of their torque production and received verbal encouragement to perform maximally during each MVC.

2.2.4 Electrical stimulation

The right tibial nerve was stimulated using bipolar surface (2.25cm^2) Ag-AgCl electrodes (Vermed Medical Inc. Vermont, U.S.A) placed over the popliteal fossa at the site that evoked a response (M-wave or H-reflex) at the lowest stimulation intensity. Rectangular pulses of 50, 200, 500 and 1000 μs were delivered from a constant-current stimulator (DS7A, Digitimer Ltd. London U.K). A previous study by Panizza and colleagues (26) found that 2000 and 3000 μs pulse durations depress the H-reflex response significantly compared to 1000 μs pulses. Therefore, we did not exceed pulse durations of 1000 μs in the present study. Stimulation current was measured with a current probe (mA-2000 Noncontact Milliammeter, Bell Technologies, Orlando FL, U.S.A) to confirm that M_{max} amplitudes plateaued with increasing levels of stimulation. To properly quantify the current at various stimulation pulse-widths, data were sampled at 5, 10, 50, and 100 KHz when using pulse-widths of 1000, 500, 200, and 50 μs respectively. H vs. M recruitment curves were constructed from responses to 60 stimuli delivered for each of the pulse widths. When generating recruitment curves obtained with different pulse-widths, the stimulus current required is markedly different. Expressing the data as H vs. M recruitment curves enabled comparisons to be made between recruitment curves independent of differences in stimulation current. The stimulation was delivered randomly every 3 to 5 seconds at intensities ranging from below M-wave and H-reflex threshold to two

to three times the minimum current required to evoke M_{max}. Subjects never reported discomfort during testing at any pulse duration. To maintain similar levels of motoneuron excitability subjects held a background contraction of 5% maximal EMG output using visual feedback of soleus EMG low-pass filtered at 3Hz.

2.2.5 Data analysis

Three characteristics of each H vs. M recruitment curve were quantified: 1) maximal M-wave and H-reflex amplitude; 2) H-reflex recruitment gain; and 3) H-reflex recruitment relative to M-wave recruitment.

2.2.6 Maximal M-wave and H-reflex amplitude

M-waves and H-reflexes were measured peak-to-peak. H_{max} was calculated by averaging the three largest H-reflexes for each pulse width. M_{max} was taken to be the single largest M-wave for each pulse width. H_{max}/M_{max} ratios were calculated.

2.2.7 H-reflex recruitment gain

To assess the gain of the H-reflex, a linear regression using the least sum of squares method was fitted to the middle portion of the ascending limb of the H vs. M recruitment curve when the H-reflexes were between 25%-75% of H_{max}. This method has been used previously to assess the gain of the H-reflex (19, 22, 23).

2.2.8 H-reflex relative to M-wave recruitment

Three measurements were made to assess recruitment of the H-reflex relative to the M-wave. Y-axis intercepts were calculated from the linear

regression equation as a measure of H-reflex threshold (14, 22, 23). The size of the H-reflex when the M-wave was ~ 5%M_{max} ($H_{5\%Mmax}$) was calculated for each H vs. M recruitment curve using those reflexes that were evoked with an M-wave of between 3% to 7% of M_{max}. Between 10 to 17 H-reflexes fell within this range for a given subject and were included in the average. The size of the M-wave during a maximal H-reflex (M_{Hmax}) was calculated as the average amplitude of the M-waves that accompanied the three largest H-reflexes for each pulse width.

2.2.9 Statistics

Separate repeated-measures analysis of variance (ANOVAs) were used to determine significant group differences for each dependant variable (H_{max}, M_{max}, H_{max}/M_{max} ratio, recruitment curve slope, y-axis intercept, $H_{5\%Mmax}$, and M_{Hmax}) between pulse-widths. When appropriate, post-hoc analyses were performed using Tukey's HSD. Data are reported as mean ± standard deviation. An α level of $p \leq 0.05$ was used to evaluate statistical significance.

2.3 Results

Across the group neither H_{max} nor H-reflex gain changed with pulse-width. The H vs. M recruitment curve, however, shifted to the left along the x-axis when wider pulse widths were used.

Figure 2.1A displays H vs. M recruitment curves collected using 50 and 1000 μs pulse-widths from a single subject. H_{max}/M_{max} ratios were similar for the two pulse widths; however, the H vs. M recruitment curve shifted to the left when 1000 μs pulse- width stimulation was used. Figure 2.1B shows data from

the middle 50% of the ascending limb of the H vs. M recruitment curves shown in Figure 2.1A with linear regressions. The slopes of these regressions are an indication of the recruitment gain of the H-reflex and were similar for the two pulse-widths displayed.

2.3.1 Maximal M-wave and H-reflex amplitude

Across the group, M_{max} was not significantly different when using different pulse widths. Similarly, neither H_{max} amplitude nor H_{max}/M_{max} ratios were significantly different between pulse-widths (Table 2.1).

2.3.2 *H-reflex recruitment gain*

The slope of the ascending limb of the H-reflex vs. M-wave recruitment curve was not significantly different between pulse widths (Table 2.1).

2.3.3 H-reflex recruitment relative to M-wave recruitment

The y-intercepts from linear regression analysis of recruitment curves obtained using 50 and 200 μs pulse widths were lower than 500 and 1000 μs stimulation ($p \leq 0.01$) (Figure 2-2A). $H_{5\%Mmax}$ was approximately twice as large ($p \leq 0.02$) when a 500-1000 μs pulse width (~30%M_{max}) was used when compared to 50 μs stimulation (~15%M_{max}) (Figure 2.2B). There was no significant difference between $H_{5\%Mmax}$ values using 200 μs and any other pulse width. The size of the M-wave when the H-reflex was maximal (M_{Hmax}) was significantly smaller during 1000 μs than 50 μs stimulation ($p \leq 0.03$, Figure 2.2C).

2.4 Discussion

Pulse-width did not affect maximal response amplitude or H-reflex gain; however, the H-reflex recorded with an accompanying M-wave of 5% was

significantly smaller when using narrow pulse widths (50 μs) than wider pulse-widths (500 and 1000 μs). In addition, the size of the M-waves when H-reflexes were maximal (M_{Hmax}) were significantly larger when using 50 compared to 1000 μs pulse-widths. Lastly, H-reflex threshold, as measured by the y-intercept from the linear regression of the ascending limb of the H versus M recruitment curve, decreased when using 50 and 200 μs pulse-width stimulation compared to 500 and 1000 μs. Collectively, these changes, in conjunction with no change in slope or maximal H-reflex amplitude, reflect a shift of the H vs. M recruitment curve to the left when wider pulse widths are utilized.

2.4.1 Relative recruitment of sensory and motor fibers

For almost 60 years it has been known that altering stimulus pulse-width affects the recruitment of sensory and motor nerve fibers differently. Using pulses of 1000 μs to stimulate spinal roots of the bullfrog, Erlanger and Blair (12) demonstrated that recruitment threshold is lower for sensory than motor fibers. When narrow pulse widths were used they noted that the motor fibers became more easily recruited than sensory fibers. Hence, their strength-duration curves for motor and sensory axons crossed. Strength-duration curves collected using narrow and wide pulse-widths for the soleus H-reflex (i.e., sensory axons) and M-wave (i.e., motor axons) in humans overlap in a similar manner (24, 25). Using the data from Lin et al., (24) we estimate that strength-duration curves of M-waves and H-reflex data obtained from tibial nerve stimulation would overlap at approximately 300 μs. This estimate agrees with the results of Veale (33) who showed in the human ulnar nerve that pulse-widths of 1000 μs selectively

stimulate sensory fibers at threshold, whereas pulses less than 200 μs selectively stimulate motor fibers. Our measure of H-reflex threshold was the y-intercept from the linear regression analysis (Figure. 2.1B) which showed that 50 and 200 μs stimulus pulses resulted in lower y-intercepts (i.e., higher thresholds relative to the M-wave) than data collected using 1000 μs. This suggests that when using 50 and 200 μs, we stimulated at a point in the strength-duration curve relationship that favored fewer sensory axons reaching threshold compared to wider pulse-widths.

Work devoted to exploring differences in strength-duration time constants of motor and sensory fibers in humans has focused on several different nerves including the median (27, 28), radial (28), ulnar (2, 27) and tibial (24, 27) nerves. Although methodologies differed between experiments, the results consistently place strength-duration time constants for sensory axons higher than motor axons. For the median nerve, Panizza et al. (28) reported these as 71 μs and 232 μs for motor and sensory axons, respectively. Lin et al., (24) reported those associated with soleus M-waves and H-reflexes as 444 and 644 μs, respectively. These differences in strength-duration time constants result in the preferential recruitment of motor axons when stimulating with narrow pulse-widths and sensory axons when stimulating with wider pulse-widths (18, 20, 24, 27, 28, 33). Our data showed constant slope and constant H_{max} values accompanied by decreased H-reflex threshold and M_{Hmax} when stimulus pulse-widths were wider. The shift in the H vs.M recruitment curve reported in the present experiments is consistent with differences in strength-duration time

constants between motor and sensory axons. Our data suggest that stimulus pulse-widths of 500-1000 μs are equally effective at recruiting the large-diameter sensory afferents (mostly Ia fibers) responsible for generating the H-reflex.

2.4.2 Pulse width and the H-reflex

Contrary to a previous study (26) we found no difference in $H_{max,}$ M_{max} or H_{max}/M_{max} ratios between pulse widths. Panizza and colleagues (26) reported that pulse durations of 100 μs produced significantly smaller soleus H_{max} amplitudes than wider pulse-widths (300-1000 μs); however they noted that "the amplitude of the H-reflex with 100 μs duration stimulus typically did not peak in the voltage range of the stimulator." Our data suggest that had they been able to deliver more current, the amplitude of the H-reflex would have continued to increase to the same as that obtained with wider pulse-widths.

2.4.3 Limitations to the size of H_{max}

The decrease in the amplitude of the H-reflex at stimulus intensities above that which evokes H_{max} has been attributed to collision of the reflex motor output with antidromic transmission in motor axons (15**,** 17, 30). Hence, this collision is believed to limit the size of H_{max} and abolish the H-reflex at M_{max}. If antidromic collision is the only limiting factor of H_{max} amplitude, H_{max} should have increased in the present experiments with the leftward shift in the H vs. M recruitment curves when using wider pulse-widths. Since the size of the M-wave, and hence the antidromic volley, was smaller at H_{max} when using 1000 μs ($14\%M_{max}$) compared to 50 μs ($20\%M_{max}$) pulses it is reasonable to believe that factors other than antidromic collision must limit the size of the H-reflex

amplitude, including H_{max}. These factors may include oligosynaptic IPSPs (3) and presynaptic inhibition of Ia terminals (6, 34). In addition, it is possible that antidromic activation of motor axons and orthodromic activation of sensory axons associated with other muscle groups produce inhibition to the soleus motoneuron pool. In support of the idea that factors other than antidromic collision limit the size of the H-reflex, it has been noted that in the soleus muscle, H_{max} is often reached before any substantial volley is generated in the motor axons (29). Measurement of the maximal monosynaptic discharge of a motoneuron pool from the decerebrate cat hindlimb is frequently ≤50% prior to post-tetanic potentiation (7). Some human subjects can achieve 100% activation of the soleus motoneuron pool via electrical stimulation of the tibial nerve; however, values are also typically ~50% (31). Taken in combination, these findings support the idea that factors other than antidromic collision limit the size of H_{max}. Our results are consistent with the interpretation that the unpotentiated soleus H-reflex is limited to ~50%M_{max} and this limitation is independent of pulse-width.

2.4.4 Implications

When conducting experiments to investigate changes in the H-reflex pathway, it is recommended that an H-reflex is used on the ascending limb of the recruitment curve with a small but stable M-wave (34). Another common measure used to evaluate changes in transmission along the H-reflex pathway is the H_{max}/M_{max} ratio. Our results show that stimulus pulse-width, although having no effect on H_{max}, has a strong effect on $H_{5\%Mmax}$. Our results suggest that to

obtain the largest possible H-reflex with a small M-wave, pulse-widths of 1000 μs should be used.

We have proposed that wide pulse stimulation (1000 μs) may be advantageous for NMES compared to conventional, more narrow stimulus pulse widths (8-11). The rationale is that a larger afferent volley should be generated with wider pulses, resulting in a contraction that develops due to a greater synaptic recruitment of spinal motor neurons. The synaptic activation may be advantageous over the direct depolarization of motor axons beneath the stimulating electrodes since synaptic activation follows a natural physiological recruitment order (5,16) and stimulation of motor axons is prone to a reversed **(**13, 32**)** or random recruitment order (1, 21). A physiological recruitment order via synaptic activation of spinal motor neurons may result in the recruitment of fatigue resistant motor units at lower intensities than when using narrow pulse-widths. Thus wide pulse-width stimulation may be more beneficial than traditional narrow pulse-width stimulation to reduce fatigue during NMES contractions and decrease disuse atrophy (8). Our results suggest that at stimulus intensities near 5%$M_{max,}$ the recruitment of sensory axons (predominantly Ia) is greater when using pulse-widths of 1000 μs than with narrower pulse-widths. Thus, using pulse-widths greater than traditional NMES protocols may be beneficial.

Table 2.1. Group means and standard deviations (SD) of H_{max}, M_{max}, H_{max}/M_{max} ratio and slope, calculated from recruitment curves collected using different pulse-widths.

Dependent Variable	*50 µs pulse width*	*200 µs pulse width*	*500 µs pulse width*	*1000 µs pulse width*
H_{max}(mV)				
mean	3.3	3.4	3.6	3.7
SD	2.7	2.2	2.3	2.7
M_{max} (mV)				
mean	6.9	7.3	7.3	7.3
SD	4.0	4.1	4.0	4.1
H_{max}/M_{max}				
mean	0.48	0.46	0.48	0.51
SD	0.24	0.24	0.21	0.21
Slope				
mean	2.8	3.3	3.6	3.6
SD	3.1	4.7	4.2	5.7

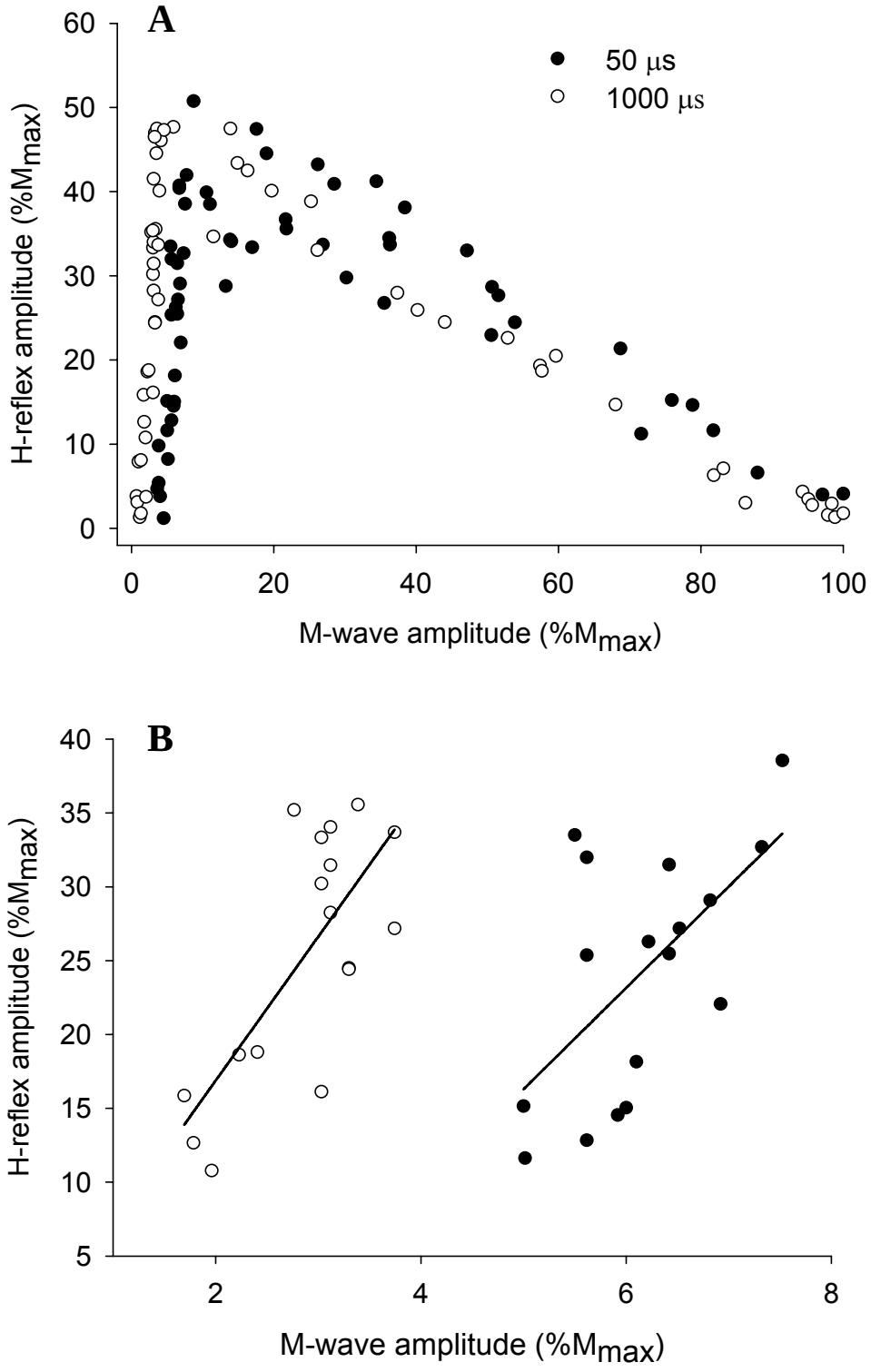

Figure 2.1. H vs. M recruitment curves collected from a single subject using 50 and 1000 μs pulse widths. A) Data collected over the full range of stimulus intensities. B) Data selected from the ascending limb of the same recruitment curves shown in A when the H-reflex was between 25%-75% H_{max}. The linear regressions are indicated by solid black lines in B.

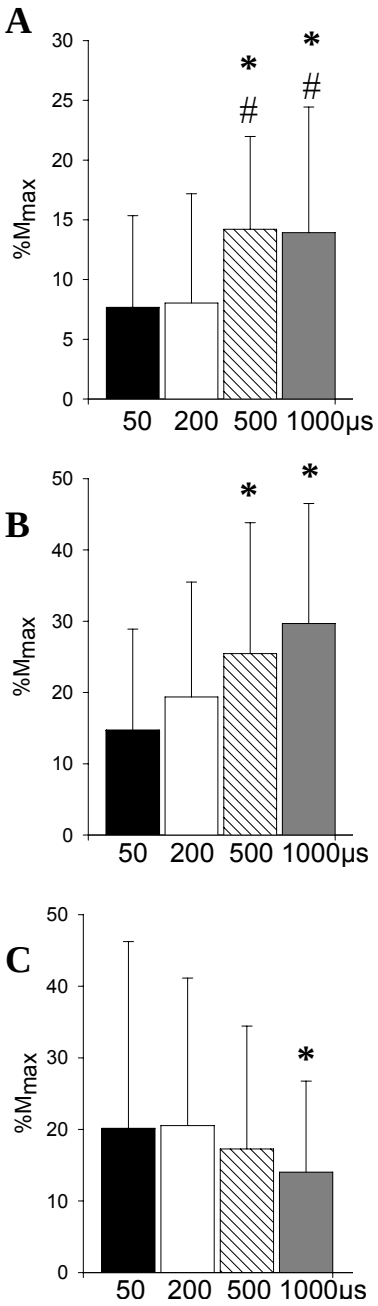

Figure 2.2. Group data showing changes in the recruitment of the H-reflex with different pulse widths. A) Y-intercepts from the linear regression of the ascending limb of the H vs. M recruitment curve. B) The size of the H-reflex when the M-wave was ~ 5%M_{max} ($H_{5\%Mmax}$). C) The size of the M-wave when the H-reflex was maximal (M_{Hmax}). Asterisks (*) indicate significant differences ($p<0.05$) compared to 50 µs and number signs (#) indicate differences compared to 200 µs stimulation.

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3.0 Influence of stimulus pulse width on M-waves, H-reflexes and torque during tetanic neuromuscular stimulation

3.1 Introduction

Introduction

Neuromuscular electrical stimulation (NMES) is often used to enhance function and reduce muscle atrophy for people with movement disorders. NMES for rehabilitation is typically delivered using pulse widths of 200 to 400 μs at frequencies between 20 to 50 Hz (37). This method of stimulation recruits motor units by depolarizing motor axons beneath the stimulating electrodes (29, 42, 50) and thus generates contractions at least in part through the summation of twitches associated with successive motor waves (M-waves). However, NMES also depolarizes sensory axons, generating an afferent volley that can recruit motor units reflexively and contribute to the evoked contraction (1, 5, 10-12, 16, 33, 44). This central contribution to electrically-evoked contractions has been confirmed by experiments involving an anesthetic nerve block where the same intensity and pattern of NMES generated significantly more torque before the nerve block when the central nervous system (CNS) could contribute, than during the nerve block, when only the activation of motor axons could contribute (5, 10, 11). The present experiments were designed to identify the influence of stimulus pulse width on the recruitment of motor axons (M-waves), the reflexive recruitment of motoneurons (H-reflexes) and isometric torque during NMES.

When NMES is delivered to generate tetanic contractions suitable for rehabilitation, post-activation depression of neurotransmitter release from

afferent terminals (14, 28, 47) and antidromic transmission along motor axons (21, 27, 47) (particularly at high stimulus intensities) both reduce the likelihood that transmission along reflex pathways can make a significant contribution to the evoked contractions. However, H-reflexes *can* contribute to contractions during NMES (33, 45) and are augmented following a brief period of NMES at 100 Hz (33). Theoretically this H-reflex contribution should be greater when NMES is delivered using wider pulse widths, as wide pulses depolarize sensory axons more effectively than narrow pulses (18, 35, 54). We have shown that wide pulses generate significantly more torque than narrow pulses during NMES even when stimulus intensity was adjusted to account for differences in charge (11). The increased torque using wide pulses was attributed to a greater reflexive recruitment of motor units due to the larger afferent volley, however M-waves and H-reflexes were not measured (11). We have also shown that larger H-reflexes were evoked for a given sized M-wave when using wider (200-1000 μs) compared to narrow (50 μs) pulses when single pulses were delivered to construct H-reflex versus M-wave recruitment curves (35). Whether the same relationship exists between pulse width and H-reflex recruitment when NMES is delivered at frequencies suitable for rehabilitation has not been tested. Similarly, the relationship between pulse width and M-wave amplitude during NMES has not been explored.

The order in which motor units are recruited during NMES is still unclear. Experiments have shown a reversed (26, 52), random (19, 22, 30, 34) or near normal (51) recruitment order. Regardless, it is generally agreed that a non-

physiological recruitment order during contractions thought to be driven primarily by M-waves accounts for the rapid fatigue associated with NMES (26, 52). Our working hypothesis is that NMES delivered using wide pulse widths generates contractions with a greater central contribution than those evoked using narrow pulses. Since synaptic drive recruits motor units from smallest to largest according to Hennemans's size principle (2, 25) we have proposed that using wide pulses, relatively high frequencies and low intensities may be advantageous for rehabilitation since it should maximize the central contribution to contractions evoked by NMES (1, 5, 10-12, 16, 33, 44). The present experiments extend our previous work on stimulus pulse width and H-reflex recruitment (35) and investigate the recruitment of motor axons (M-waves), the reflexive recruitment of motoneurons (H-reflex) and the development of torque during tetanic NMES.

In the present experiments we delivered NMES for 7 seconds in a pattern (2 s at 20Hz, 3 s at 100 Hz, 3 s at 20 Hz) we have previously used to study contractions evoked by NMES. This stimulation pattern allowed us to investigate M-waves, H-reflexes and torque during 20 Hz NMES (a typical frequency for NMES) as well as investigate the effects of delivering 2 seconds of 100 Hz stimulation at different pulse widths. Two seconds of 100 Hz NMES using 1000 μs pulse widths leads to a sustained increase in torque (1, 5, 10-12, 16, 33, 44) and H-reflex amplitude (33) during subsequent 20 Hz stimulation, due to a central recruitment of motor units. Currently we tested three hypotheses: 1) M-wave amplitudes during 20 Hz NMES will be depressed compared to those

evoked by single pulses; however this depression will be unaffected by pulse width; 2) H-reflexes will be depressed by NMES at all pulse widths compared to H-reflexes evoked by single pulses; 3) wider pulse widths (200, 500 1000 μs) will increase H-reflex amplitude and torque *post-100 Hz* versus *pre-100 Hz* while the narrowest pulse width (50 μs) will not. Stimulus intensity was adjusted to evoke M-waves of similar amplitude with each pulse width, thus recruiting a comparable proportion of motor axons. In this way, differences in H-reflex amplitudes between pulse widths reflect differences in the relative recruitment of sensory axons versus motor axons. Low stimulus intensities were used so that we could record H-reflexes with minimal obstruction by block along motor axons (21, 27, 47). The results of these experiments provide further insight into the central and peripheral recruitment of motor units during NMES.

Materials and Methods

Subjects

Eighteen people with no known neurological impairment participated with informed consent. Four participants withdrew from the study due to discomfort during NMES; thus, analyses were conducted on data from fourteen participants (19-43 years old; 12 males and 2 females). Data from twelve participants were collected during the same experimental sessions as data that were part of a companion study (35). The only data common to the present study and the companion study (35) were maximal evoked M-wave (M_{max}) values that were used to normalize each subject's EMG data. This study was approved by the Human Research Ethics Board at the University of Alberta.

Protocol

All experimental procedures were performed on the right leg. Subjects were seated with the right hip, knee and ankle at 90, 110 and 90°, respectively. Both feet were supported and the isometric torque generated by the right plantar-flexors was transduced using a System 3 Dynamometer (Biodex Medical Systems, Shirley, New York).

Electromyography

Surface EMG was recorded from the right soleus and tibialis anterior muscles with bipolar (2.25 cm^2) surface electrodes (Vermed Medical, Bellows Falls, Vermont). EMG signals were pre-amplified 500-1000× and band-pass filtered at 10-3000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England).

Maximal voluntary isometric contractions (MVCs)

At the beginning of each experiment subjects performed between 3-7 maximal, voluntary, isometric contractions (MVCs) of the plantar-flexors until three consistent maximal contractions (no more than 5% variability) were achieved. The average torque produced in the 0.5 second period centered on the point of maximal torque during the largest of the three contractions was used to establish individual MVC values. This MVC value was used to normalize each subject's torque during the tetanic stimulation trials. Subjects were provided with visual feedback of their torque production and received verbal encouragement to perform maximally during each MVC.

Electrical stimulation

The right tibial nerve was stimulated using bipolar surface (2.25cm^2) electrodes (Vermed Medical, Bellows Falls, Vermont) placed over the popliteal fossa at the site that evoked a response (M-wave or H-reflex) in soleus at the lowest stimulation intensity. Rectangular pulses of 50, 200, 500 and 1000 μs were delivered from a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, England). Stimulus intensity was adjusted based on the amplitude of the M-wave evoked by single pulses. Two stimulus intensities were used for each pulse width: 1) motor threshold (an M-wave of ~1-2% M_{max}); 2) and an intensity that evoked an M-wave of 5% M_{max}. Subjects were instructed to relax and not contribute to the evoked contractions. In each trial, a single pulse width was used in which three single pulses were delivered 5 seconds apart ~10 seconds before 5 trains of NMES were applied. The NMES pattern used in the present study was 20 Hz for 2 s – 100 Hz for 2 s – 20 Hz for 3 s, i.e., 20-100-20 Hz for 7 seconds (see Figure 1). Five stimulation trains, 45 seconds apart, were delivered using each of the four pulse widths (50, 200, 500 and 1000 μs) and two intensities (motor threshold and 5% M_{max}). The order of testing was randomly selected by drawing lots.

Data analysis

The amplitudes of M-waves and H-reflexes evoked by single pulses and during periods of 20 Hz NMES were measured peak-to-peak and normalized to M_{max}. M_{max} was taken to be the single largest M-wave evoked by single pulses delivered at supramaximal intensities for each pulse width, as described in the

companion paper (see 35). Torque recorded during the 7 second NMES trains was normalized to each subject's MVC torque. The amplitudes of M-waves, H-reflexes and torque were calculated for each NMES train and averaged over the five trains in each trial. M-wave, H-reflex and torque values were calculated during the period 1.25-1.75 seconds into the initial 20 Hz stimulation (*pre-100 Hz*; see Figure 1). These M-waves and H-reflex amplitudes were compared to those obtained with single pulses to assess the influence of pulse width on EMG responses during NMES of a typical frequency. In addition, the *pre-100 Hz* values were compared to values obtained 1.25-1.75 seconds after the 100 Hz stimulation (*post-100 Hz*) to assess the influence of 2 seconds of 100 Hz NMES on torque and EMG responses. Lastly, *post-100 Hz* EMG responses were compared to *pre-100 Hz* and single pulse values to assess the recovery of M-wave and H-reflex amplitudes. Group data were obtained by pooling mean data from each subject. EMG responses were not quantified during the 100 Hz stimulation due to the interference from overlapping of M-waves, H-reflexes and stimulus artifacts. Data were sampled at a minimum of 5 kHz using a custom-written program (LabView, National Instruments, Austin, TX) and stored on a computer for analysis.

Statistics

To assess differences in torque between data collected during *pre-100* and *post-100 Hz* we performed a 2x4 repeated measures ANOVAs with "Time" having two levels (*pre-100 Hz* and *post-100 Hz*) and "Pulse" having four levels (50, 200, 500 and 1000 μs). To assess M-waves and H-reflexes obtained with

single pulses to *pre-100 Hz* and *post-100 Hz* values at the four pulse widths, two separate 3x4 repeated measures ANOVAs with an additional level of Time (single pulse) were performed. Tests for normality using the Shapiro-Wilks tests showed that H-reflex data were non normally distributed; therefore we performed a Log_{10} transform on H-reflex data prior to performing the ANOVA. We were specifically interested in Time x Pulse interactions, and thus significant main effects are only reported when no significant interaction was present. The α level was set at $p \leq 0.05$. When appropriate, post hoc analyses were performed using Tukey's honestly significant differences test. Data are reported as mean ± standard deviation.

Results

Data recorded from a single subject during NMES delivered at motor threshold and 5% M_{max} are shown in Figures 1A and 1B, respectively. For this subject the 50 μs stimulation (black traces) did not generate more torque *post-100 Hz* compared to *pre-100 Hz*. In addition, the EMG from the 50 μs trials show relatively stable M-waves with little or no H-reflex present in either *pre-100 Hz* or *post-100 Hz* windows. In contrast, the same subject showed elevated torque *post-100 Hz* compared to *pre-100 Hz* when 1000 μs pulse widths were used (grey traces). During these 1000 μs trials both M-waves and H-reflexes were smaller during 20 Hz stimulation compared to responses evoked by single pulses; however, H-reflex amplitude recovered from the initial depression during the *post-100 Hz* stimulation.

M-waves

Stimulus intensity was adjusted so that the amplitudes of M-waves evoked by single pulses were similar and statistical analysis confirmed that there were no significant differences in M-wave amplitude between pulse widths for the two stimulus intensities ($p > 0.05$) (see "single pulses", Figure 2). During 20 Hz NMES however, M-wave amplitude was influenced by pulse width.

M-waves collected when stimulating at motor threshold showed a significant Time x Pulse interaction [$F_{(6,72)} = 8.4$; $p = 0.000001$]. Post-hoc analysis showed that M-waves were not significantly different during 20 Hz NMES (*pre-100 Hz* or *post 100 Hz*) compared to single pulses when using 50 and 200 μs pulse widths ($p \geq 0.97$). However, increasing the pulse width to 500 and 1000 μs caused M-waves to depress on average 53% ($p < 0.0001$) compared to those evoked by single pulses (Figure 2A).

M-waves at the higher intensity of 5% M_{max} also revealed a significant Time x Pulse interaction [$F_{(6,48)} = 10.8$; $p = 0.000001$]. Post-hoc analysis revealed that 50 μs was the only pulse width to not show significant depression of the M-wave during 20 Hz NMES compared to single pulse values ("50" in Figure 2B). In contrast, a significant M-wave depression (63% on average) occurred during 20 Hz NMES when using 200, 500 and 1000 μs pulses ($p < 0.0001$) (see Figure 2B). At both stimulus intensities and at all pulse widths, M-wave amplitude during NMES was unaffected by the 2 seconds of 100 Hz stimulation, as there were no significant differences between M-wave amplitudes *pre-100 Hz* to *post-100 Hz*.

H-reflexes

H-reflexes at motor threshold showed a significant Pulse x Time interaction [$F_{(6,72)} = 7.8$; $p = 0.000002$]. Post-hoc analysis revealed that H-reflexes evoked by single pulses were significantly larger than both their respective *pre-* and *post-100 Hz* values when using 200, 500 and 1000 μs pulse widths ($p < 0.04$) but not 50 μs ($p > 0.90$) (see Figure 3A). In addition, H-reflexes evoked by single pulses were on average 82% smaller ($p < 0.0001$) when using 50 μs pulses than H-reflexes generated by single pulses with 200, 500 and 1000 μs pulse widths (see "50" and "single pulses" in Figure 3A). At motor threshold H-reflexes showed a significant increase from *pre-* to *post-100 Hz* (on average 194%) when using 200, 500 and 1000 μs pulse widths ($p \leq 0.02$); however H-reflex amplitude did not increase significantly when using 50 μs pulse widths ($p = 0.2$) (see Figure 3A).

H-reflexes obtained during the higher intensity of 5% M_{max} showed a significant Time x Pulse interaction [$F_{(6,66)} = 13.5$; $p = 0.000001$]. Post-hoc analysis showed that H-reflexes evoked by single pulses were larger than both their respective *pre-100 Hz* and *post-100 Hz* values at all pulse widths ($p > 0.04$) (see Figure 3B). H-reflexes evoked by single pulses were on average 45% smaller ($p < 0.0001$) when using 50 μs pulses than H-reflexes generated by single pulses with 200, 500 and 1000 μs pulse widths (see "50" and "single pulses" in Figure 3B). H-reflexes increased on average 225% from *pre-* to *post-100 Hz* when using 200, 500 and 1000 μs pulse widths ($p \leq 0.002$); however H-

reflexes did not increase significantly when using 50 μs pulse widths ($p = 0.2$) (see Figure 3B).

Torque

Torque data at motor threshold showed a significant Time x Pulse interaction [$F_{(3,39)} = 5.5$; $p = 0.003$]. Post hoc-analysis revealed that torque increased significantly *post-100* Hz (on average 55%; Figure 4A) when pulse widths of 200, 500 and 1000 μs were used ($p \leq 0.03$) but that 50 μs pulses did not significantly increase torque ($p = 0.9$). Similarly, when stimulating at the higher intensity of 5% M_{max} a significant Time x Pulse interaction was found [$F_{(3,39)} = 6.2$; $p = 0.002$]. Again, torque increased significantly *post-100 Hz* (on average 38%; Figure 4B) when pulse widths of 200, 500 and 1000 μs were used ($p \leq 0.0005$) but 50 μs pulses did not significantly increase torque ($p = 0.9$).

Discussion

The present experiments demonstrate that 20 Hz NMES resulted in a depression of M-wave amplitudes compared to M-waves evoked by single pulses when using 200-1000 μs but not 50 μs pulse widths. Also, compared to single pulses H-reflexes were initially depressed during 20 Hz NMES (*pre-100 Hz*) at all pulse widths and partially recovered following the 2 seconds of 100 Hz (*post-100 Hz*) when using 200, 500 and 1000 μs but not 50 μs pulse widths. In conjunction with increased H-reflexes, torque was significantly greater *post-100 Hz* versus *pre-100 Hz* NMES when using all pulse widths except 50 μs. Thus, increased torque *post-100 Hz* was associated with decreased M-wave and increased H-reflex amplitudes. These findings support our working hypothesis

that NMES delivered using 200-1000 μs pulse widths generates contractions that have a greater central contribution than those evoked using narrower pulses.

M-waves during NMES

M-wave amplitude is used as an indicator of changes in muscle fiber excitation during experiments investigating human muscle fatigue (20). A reduction in M-wave amplitude during NMES has been demonstrated at a number of frequencies when using supramaximal stimulation (20-70 Hz, (3); 50-80 Hz, (4); 40 Hz, (17)), however, potentiation of M-waves has also been found to occur at supramaximal intensities while using 10 and 20 Hz stimulation (13, 15) as well as during sub-maximal 20 Hz stimulation (33). Typical pulse widths for clinical use of NMES range from 200-400 μs (47). Experimentally, pulse widths vary from 50 μs (13, 4) to 600 μs (32) and 1000 μs (12).

In the present study we compared M-waves recorded during 20 Hz NMES to those evoked by single pulses. In addition we compared M-waves during 20 Hz NMES before (*pre-100 Hz*) and after 2 seconds at 100 Hz (*post-100 Hz*). We hypothesized that M-wave amplitudes during 20 Hz NMES would depress compared to those evoked by single pulses but that this depression would be unaffected by pulse width. Contrary to our hypothesis we found a pulse width *and* intensity dependent effect on the amplitude of M-waves during NMES. During NMES at motor threshold our two narrowest pulse widths (50 and 200 μs) did not depress M-wave amplitudes compared to M-waves evoked by single pulses, while wider pulse widths (500 and 1000 μs) did. When the stimulus intensity was increased to evoke an M-wave of 5% M_{max}, 50 μs was the

only pulse width that did not depress M-wave amplitudes. A previous study found that 50 μs pulse widths delivered at 20 Hz depressed M-wave amplitude (13), although the intensity of stimulation was supramaximal and thus much higher than in the present study. Therefore, had we increased our stimulus intensity to M_{max} it is possible that we too would have observed a depression of M-waves during NMES when using 50 μs.

Mechanisms affecting the size of the M-wave may be at the level of the axon, the neuromuscular junction or the muscle fibers. However, since NMES generates action potentials in motor axons beneath the stimulating electrodes in an "all or none" manner the mechanism underlying the depression of M-wave amplitudes must be related to a differential ability of pulse widths to depolarize motor axons repetitively. Increasing the stimulus pulse width used for NMES caused a greater depression of the M-wave relative to single stimuli; thus fewer motor axons must have been recruited. Since Na^+ channels are the major determinant of threshold in axons (40) the depression of M-waves during NMES is likely related to the inactivation of voltage gated Na^+ channels. The wider pulse widths we used may have increased the inactivation of voltage gated Na^+ channels in motor axons as the inactivation time constant of classic fast voltage gated Na^+ channels is 500-1000 μs (39, 43). Thus it is likely that the wider pulse widths used in our study (500-1000 μs) inactivated a greater number of Na^+ channels leading to decreased Na^+ influx, fewer motor axons reaching threshold and smaller M-wave amplitudes.

H-reflexes and torque during NMES

H-reflex amplitude is attenuated during repetitive stimulation at rates above 0.1 Hz due to post-activation depression (47). This depression is believed to be caused by reduced transmitter release from previously activated afferent fibers (14, 28). During several seconds of NMES, a recovery of the soleus H-reflex can follow this initial depression (33, 45) and it has been hypothesized that this increased reflexive recruitment of motoneurons contributes to an increase in torque (12, 33). Collins et al. (11) examined the effect of pulse width on plantar-flexion and dorsi-flexion torque during NMES by matching the initial electrically-evoked forces at each pulse width. They showed that 1000 μs pulses evoked significantly more plantar-flexion torque at the end of a seven second train of NMES than 50 μs pulses; however, in that study EMG responses were not analyzed. Presently we show that when stimulating at motor threshold and at 5% M_{max}, pulse widths of 200 to1000 μs resulted in significantly larger soleus H-reflexes *post-100 Hz* and this was associated with increased torque. Thus the present experiments support our hypothesis that wider pulse widths (200, 500 1000 μs) increase H-reflex amplitude and torque *post-100 Hz* versus *pre-100 Hz* while narrower pulse widths (50 μs) do not.

Several mechanisms could account for the H-reflex recovery during the *post-100 Hz* period of NMES. The most likely include: voluntary activation, reduced pre-synaptic inhibition, post-tetanic potentiation and the activation of plateau potentials in spinal neurons. Voluntary activation of the plantar-flexors (7) as well as general muscle activation during tensing of the body, such as in a

Jendrassik maneuver (55), will increase the amplitude of the soleus H-reflex. In the present study however, all subjects included in the analysis reported the stimulation to be comfortable and that they remained relaxed throughout. In addition, previous experiments have documented an increase in torque using the same stimulation protocol in sleeping (10) and complete spinal cord injured subjects (44). We therefore do not believe that the increased H-reflexes and torque during NMES in the present experiments is due to voluntary activation of the plantar flexors or any other muscle group.

Reduced pre-synaptic inhibition at Ia terminals could increase H-reflex amplitude (9, 41, 49, 55, 56) thereby generating more torque from the reflexive recruitment of spinal motoneurons. In addition, post-tetanic potentiation may also increase the amplitude of H-reflexes by increasing neurotransmitter release from Ia afferent terminals (31, 36, 53). Another possibility is that NMES may activate persistent inward currents in spinal motoneurons (for review see 24) causing a sustained discharge and making them more responsive to sensory input (1, 5, 10-12, 16, 33, 45). It has been suggested that while persistent inward currents are active, motoneuron discharge may frequently become “time-locked” to each stimulus pulse as H-reflexes (33, 45). Once persistent inward currents are activated in motoneurons, discharge may also continue in a self-sustained manner, contributing to the generation of torque via activity that is asynchronous from the stimulus pulses (10). All of the aforementioned mechanisms could modify the amplitude of the H-reflex and further experiments are required to differentiate the relative contribution made by each one. The increased torque

and H-reflex amplitude *post-100 Hz* with wider pulse widths however, suggests that very narrow pulses are not as effective for synaptically recruiting motoneurons.

Relevance for NMES

Currently there is controversy regarding motor unit recruitment order during NMES as reports range from reversed (26, 52), random (19, 22, 30, 34) or normal (51) compared to synaptic activation. These discrepancies in the literature may reflect differences in the relative contribution made by motor axon recruitment (peripheral mechanism) and the synaptic recruitment of motoneurons (central mechanism) to the contractions between different studies; however, the extent to which the CNS contributes to contractions evoked by NMES is rarely considered. We have proposed that increasing the central contribution to contractions evoked by NMES by using wide pulses delivered at relatively high frequencies and low intensities may be advantageous for rehabilitation (1, 5, 10-12, 16, 33, 44) since synaptic drive from Ia afferents recruits motoneurons beginning with the smallest, according to Henneman's size principle (2, 25). These small, low-threshold motoneurons innervate muscle fibers that are the most fatigue-resistant (8). Therefore, during synaptic recruitment, slow, fatigue-resistant motor units will be recruited before the fast-fatigable motor units which may improve fatigue resistance of electrically-evoked contractions. In addition, recruiting fatigue-resistant motor units reflexively that are less accessible via direct motor axon depolarization may help protect them from atrophy and the transformation to fast twitch fiber types that occurs after periods of disuse such

as occurs following spinal cord injury (6, 38). Our results suggest that pulse widths of 200, 500 and 1000 μs are equally capable of generating contractions with a central contribution during low intensity NMES. Thus traditional NMES protocols utilizing 200 to 400 μs pulse widths at frequencies between 20 to 50 Hz (37) may also generate contractions with a contribution from the CNS provided that stimulus intensities are sub-maximal. However, it remains to be evaluated how greater stimulus intensities will influence the central contribution during NMES. Relatively low intensity NMES that is capable of activating motoneurons centrally via reflex pathways may be especially useful for patients who cannot tolerate high intensity stimulation due to heightened cutaneous sensitivity. Furthermore, electrical stimulation of sensory fibers has been shown to enhance both spinal (46) and cortical circuits (23); thus the ability to maximize the afferent volley by using wide pulse width and high frequency NMES may prove to have beneficial effects within the CNS for the rehabilitation of persons with movement disorders. In contrast, if stable M-waves and a minimal central contribution is preferred narrow pulse widths such as 50 μs should be used.

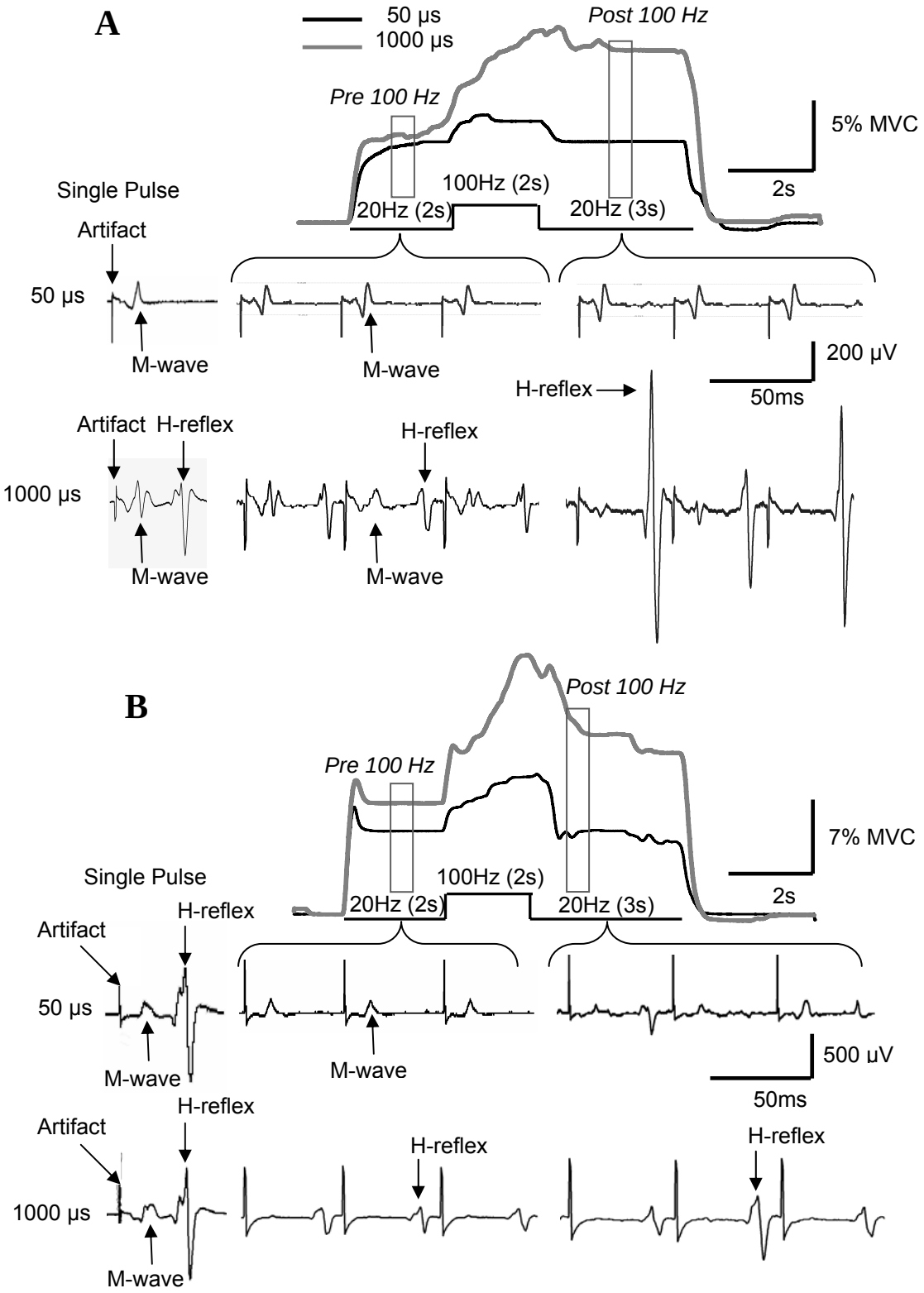

Figure 1. Single subject data showing plantar-flexion torque and soleus EMG responses recorded during the 20/100/20 Hz stimulus pattern using 50-µs (black) and 1000-µs (grey) pulse widths. Panels A and B show data collected while stimulating at motor threshold and 5% M_{max}, respectively. Vertical rectangles indicate the intervals over which data were quantified before (*pre-100 Hz*) and after (*post-100 Hz*) the 100 Hz stimulation. A sample of soleus EMG from the *pre*-and *post-100 Hz* intervals for each pulse width is displayed beneath the parentheses.

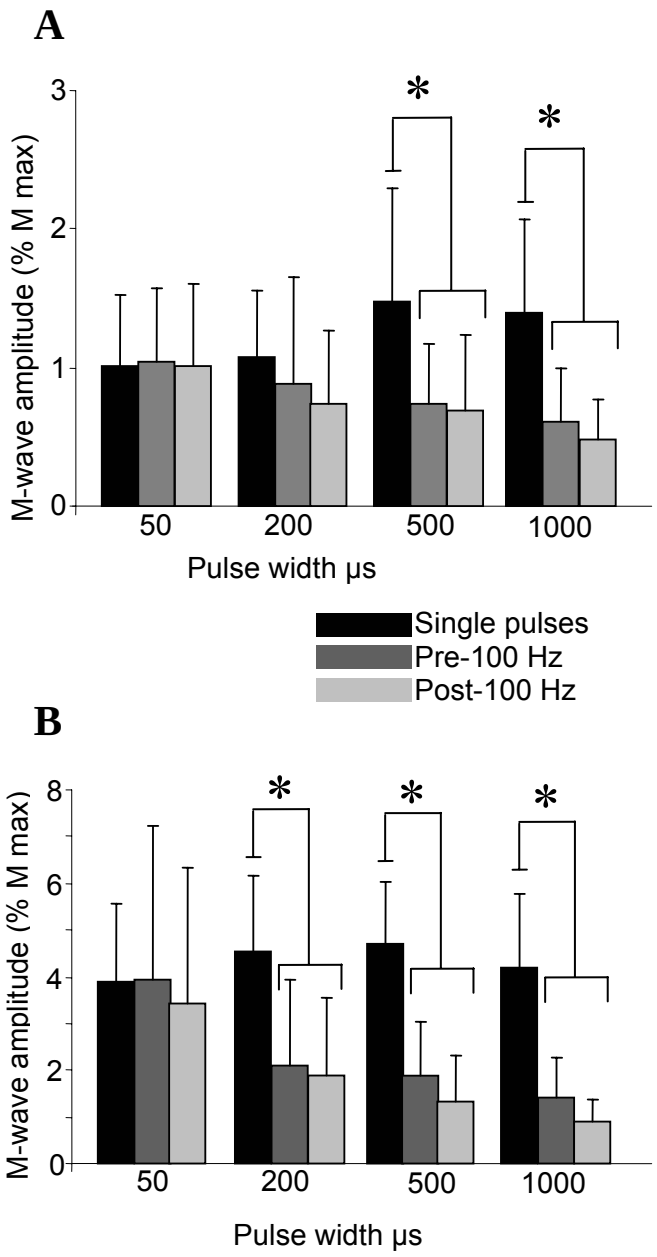

Figure 3.2. Mean group M-waves at motor threshold (A) and 5% M_{max} intensity (B) using different pulse widths. Asterisks (*) represent significant differences between single pulse data and *pre/post-100 Hz* data at each respective pulse width.

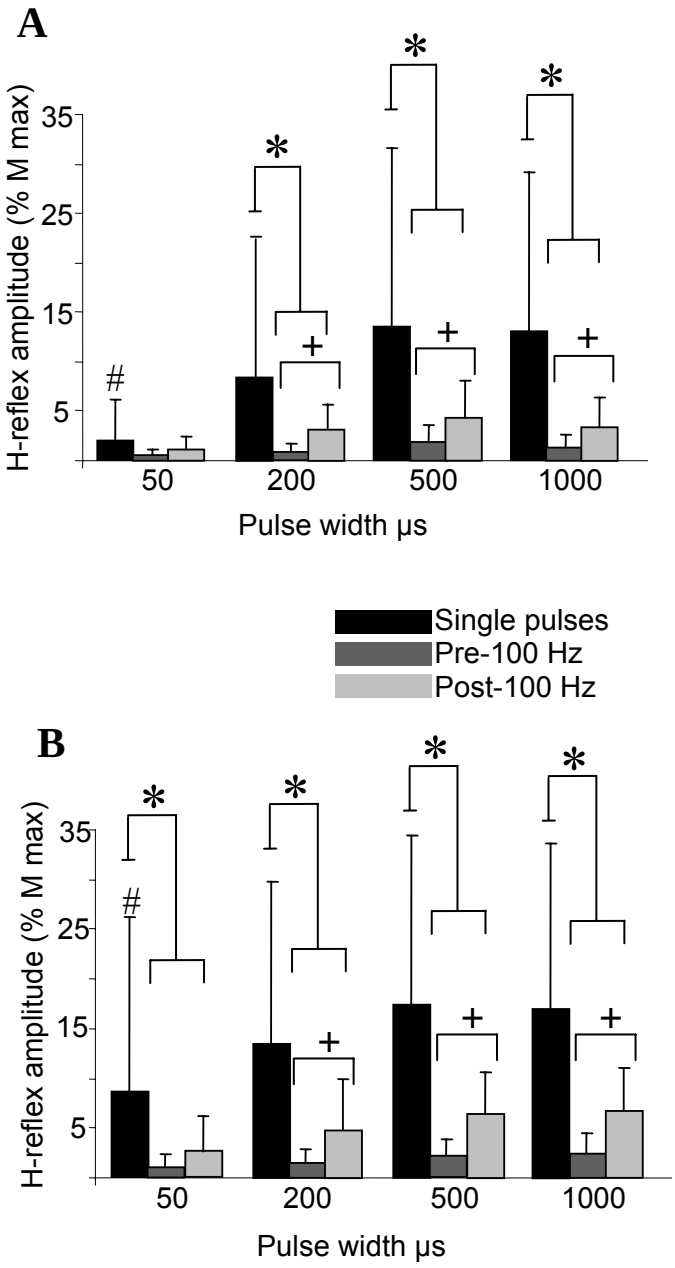

Figure 3.3. Mean group H-reflex amplitudes at motor threshold (A) and 5% M_{max} intensity (B) using different pulse widths. Asterisks (*) represent significant differences between single pulses and *pre/post-100 Hz* data at each respective pulse width. Plus signs (+) indicate significant differences between *pre-100 Hz* and *post-100 Hz* values. The number sign (#) indicates significant differences between H-reflexes obtained with single pulses relative to single pulses collected with 200, 500 and 1000 µs.

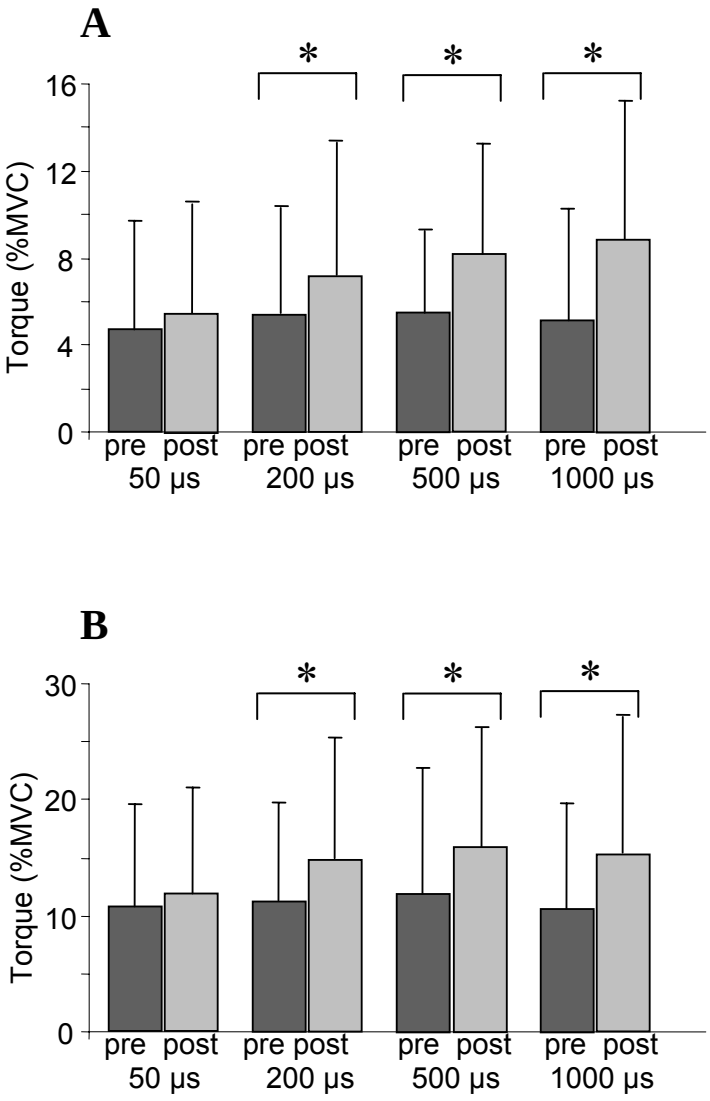

Figure 3.4. Mean group torque values from *pre-100 Hz* (dark grey) and *post 100 Hz* (light grey) intervals when using 50, 200, 500 and 1000 µs pulse width stimulation at motor threshold (A) and 5% M_{max} intensity (B). Asterisks (*) indicate a significant increase in torque from *pre-100 Hz* to *post-100 Hz* values within each respective pulse width.

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4.0 Effect of a peripheral nerve block on torque produced by repetitive electrical stimulation *

4.1 Introduction

Neuromuscular electrical stimulation (NMES) is a common rehabilitation tool for generating contractions in paralyzed muscles (23, 34, 40). While it is well known that contractions develop due to the stimulation of motor axons beneath the stimulating electrodes (23, 34, 40), the contribution made by the electrically-evoked afferent volley through the recruitment of spinal motoneurons (see 9 for review) is not as well understood. The present experiments were designed to compare torque evoked by NMES over the triceps surae before a nerve block, when the connections between the central nervous system (CNS) and periphery were intact ('Intact'), to torque generated when only the activation of distal motor axons could contribute due to a complete anesthetic block of the tibial and common peroneal nerves ('Blocked'). The goal was to provide insight into how NMES generates contractions and to test the hypothesis that torque generated during the Intact condition will show less fatigue when compared to the Blocked condition. Fatigue in humans has been defined as any exercise-induced decrease in maximal *voluntary* force produced by a muscle (17). For the present experiments, since we were not able to evaluate voluntary force during our Blocked condition and because our stimulus intensity was purposely not maximal, we will refer to fatigue as a decrease in sub-maximal electrically-evoked tetanic force.

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When NMES is delivered at high stimulus intensities the large antidromic volley in motor axons ensures that the evoked contraction will be driven largely by the direct depolarization of motor axons beneath the stimulation site with little or no contribution from the CNS; however, when using lower stimulus intensities, long stimulus trains and high frequencies the electrically-evoked afferent volley recruits motoneurons synaptically, generating up to 40% of a maximal voluntary contraction (MVC) (9, 10, 11). Thus, NMES can evoke contractions from both the direct activation of motor axons (peripheral mechanism) and recruitment of spinal motoneurons (central mechanism) (1, 3, 9, 10, 11, 12, 27, 35). This central contribution to the evoked contraction has been confirmed by applying NMES before and during a complete anesthetic block of the nerve proximal to the stimulation site (3, 10, 11). This showed that more torque developed during NMES before the nerve block, when the CNS could contribute, than during the nerve block when only the activation of motor axons could contribute and highlights the importance of considering the central recruitment of motoneurons during NMES (39).

We have shown that the central contribution involves motor unit recruitment that is time-locked to each stimulus pulse, reflecting transmission along the Hoffman-reflex (H-reflex) pathway (27) as well as motor unit discharge that is asynchronous with the stimulus pulses (10). The electrically-

evoked afferent volley elicited by low-intensity NMES has been hypothesized to recruit motor units with low voluntary recruitment thresholds (1, 3, 9, 10, 11, 12, 27, 35) as predicted for synaptic recruitment based on Henneman's size principle (2, 5, 21). These low-threshold motoneurons innervate muscle fibers that are the most fatigue-resistant (6). As described by Henneman's "size principle", voluntary contractions initially recruit small, fatigue-resistant motor units and proceeds through the larger, more fatiguable units as the intensity of the contraction increases (21). NMES on the other hand has been reported to recruit motor units in a reversed (22, 43), random (15, 19, 24, 28) or near normal order (41) compared to voluntary contractions. Whatever the exact recruitment order, it is generally agreed that the fatigue (i.e. decline in electrically-evoked torque) observed during NMES is largely due to the direct recruitment of motor axons (peripheral mechanism) (19) which does not follow Henneman's size principle. Thus, we hypothesized that electrically-evoked contractions that develop due to a combination of peripheral and central mechanisms will fatigue less than contractions evoked solely via motor axon stimulation. In contrast to our previous experiments (1, 3, 9, 10, 11, 12, 27, 35), we increased the length of the stimulus trains and decreased the pause between successive trains to evaluate the decrease in torque over time. By using stimulation patterns that incorporated both 20 Hz (a recommended frequency for NMES of the lower extremities; 40) and 100 Hz, we compared torque generated during a typical NMES frequency as well as high-frequency stimulation which enhances the central contribution to the evoked contractions.

4.2 Materials and Methods

4.2.1 Protocol

Five male volunteers (28-53 years old; 55-80 kg; 1.65-1.87 m) free from neurological and musculoskeletal disorders participated after providing informed, written consent. Since we were interested in quantifying torque generated by the combination of peripheral and central mechanisms we only studied subjects who had previously displayed torque that increased during tetanic stimulation, as this is consistent with a central contribution to the evoked contraction (1, 3, 9, 10, 11, 12, 27, 35). Central contributions typically occur in more than 85% of participants when using high frequencies and wide pulse widths (1, 3, 9, 10, 11, 12, 27, 35). Experiments were conducted at the Prince of Wales Medical Research Institute in Sydney, Australia and were approved by the University of New South Wales Human Research Ethics Committee. All experimental procedures were performed on the right leg while subjects were seated with straps to hold the foot and knee securely in place. Each subject participated in the Intact and Blocked conditions on the same day, beginning with the Intact condition. The order of protocols was randomized for each individual subject and this order was maintained across Intact and Blocked conditions. To minimize any effect of fatigue between the Intact and Blocked condition, a minimum 2 hours separated the end of testing during the Intact condition and the beginning of data collection during the Blocked condition. In addition, supramaximal twitch data were not different between any pre vs post or Intact vs Blocked conditions (see Table 4.1) suggesting that our results were not

influenced by fatigue. The right hip, knee and ankle were positioned at approximately 110°, 90° and 90°, respectively. Torque was measured with an S-type load cell (LCCB-500, Omega, Stamford, CT) attached to a custom-made foot plate designed to measure isometric plantar-flexion and dorsi-flexion torque.

4.2.2 Electrical Stimulation

Electrical stimulation was applied over the right triceps surae using two 20 cm x 5 cm flexible electrodes (Electrosurgical patient Plate 1180: Split, 3M Health care, St.Paul, MN) with the cathode and anode positioned ~10 and 20 cm distal to the popliteal fossa, respectively. Rectangular pulses of 1 ms duration were delivered from a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, England) driven by a Power 1401 data acquisition interface (Cambridge Electronic Design Limited; Cambridge, UK) controlled by a computer. Stimulation intensity was adjusted to produce ~5-10% of maximal voluntary isometric plantar flexion torque (MVCs) during 2 sec of 20 Hz NMES for all trials. The stimulus intensities used during Intact and Blocked protocols were adjusted manually based on the torque response during a 2 sec 20 Hz train. Intensities during the Intact and Blocked conditions were on average 13 mA (standard error (SE) = 2.7) and 16 mA (SE = 2.7) respectively and were not significantly different. All subjects indicated that the stimulation was comfortable during every protocol. Three stimulation protocols were used, as shown in Figure 4.1: A) 30 sec of constant 100 Hz stimulation; B) four 2 sec "bursts" of 100 Hz alternating with periods of 20 Hz stimulation; C) 30 sec of alternating 1 sec on and 1 sec off 100Hz stimulation (i.e. 15 trains). For

protocols A and B, subjects received two of the 30 sec trains separated by a 6 sec rest. For protocol C subjects received eight sets of the 15 stimulus trains with each set separated by 6 sec of rest, for a total of 120 one-sec stimulations over 275 seconds. Each protocol was delivered in both Intact and Blocked conditions. A minimum 5 minutes rest separated every stimulation protocol. The stimulus patterns used for protocols A and B have been shown in previous studies to be effective for producing torque from central mechanisms (1, 3, 9, 10, 11, 12, 27, 35). Protocol C was used to evaluate the response to intermittent (1 sec on -1 sec-off) stimulation since NMES to assist tasks such as walking (38) and cycling (16) utilize a similar pattern. Immediately before and after these stimulation patterns three single and five doublet stimulation pulses (two pulses, 10 ms apart) were delivered three seconds apart at a supramaximal intensity (150% of current necessary to generate a maximal motor response). Torque responses to supramaximal stimulation were used to assess peripheral factors related to the force generating capacity of the triceps surae muscles (see Figure 4.1). Throughout the experiments, subjects were instructed to relax and disregard the stimulation. Data were sampled at 3 kHz using Spike 2 software (Cambridge Electronic Design Limited; Cambridge, UK) and stored on computer for analysis.

4.2.3 Maximal Voluntary Contractions

Two MVCs of the plantar-flexors and dorsi-flexors lasting ~ 3 sec were performed before the nerve block. Subjects also attempted two to three plantar-flexion and dorsi-flexion MVCs to evaluate if the nerve block was complete after

administering the local anesthesia. At the end of the experimental session subjects attempted two MVCs of the plantar-flexors and dorsi-flexors to determine whether the block remained complete.

4.2.4 Nerve Block

The common peroneal and tibial nerves were localized using subcutaneous monopolar stimulation delivered via a stimulating hypodermic needle (Stimuplex A50, Braun, Melsungen, Germany) connected to a syringe containing the anesthetic. The needle was advanced and electrical stimulation was delivered to locate the site that evoked an EMG response at the lowest stimulus intensity. Local anesthetics will inhibit action potential initiation by interfering with both Na^+ and K^+ currents although the exact mechanisms are currently not known. The common peroneal nerve was blocked at the fibular head with ~ 5 ml of 2% Marcaine with adrenaline and ~ 6 ml of 2% Lignocaine with adrenaline. The longer lasting Marcaine was incorporated to ensure that the anesthetic block of the common peroneal nerve did not recover during the subsequent tibial nerve block. The tibial nerve was blocked at the popliteal fossa by injecting ~11-18 ml of 2% Lignocaine with adrenaline. The extent of the block was assessed by monitoring EMG responses to electrical stimulation and by asking subjects to perform MVCs. The block was considered to be complete when no EMG or measurable muscle twitch was evoked by electrically stimulating the tibial or common peroneal nerves at supramaximal intensities proximal to the injection site and when subjects could not volitionally produce any EMG, plantar-flexion torque or dorsi-flexion torque. All nerve blocks were

complete before data were collected for the Blocked condition**.** All participants were re-tested at the end of the experimental session to ensure that the block had not dissipated during the experiments (~ 3 hours).

4.2.5 Analysis and Statistics

Data were collected using Spike 2 software (Cambridge Electronic Design Limited; Cambridge, UK). To quantify torque, 60 torque-time integrals were calculated at equal time intervals for stimulation protocols A and B. For protocol C, 120 torque-time integrals were calculated. All torque-time integrals were calculated over a 0.5 sec interval. Statistical analyses were performed on group data to compare differences in torque time integrals at the beginning versus the end of stimulation between Intact and Blocked conditions for each protocol. For protocol A and B, each subject's second (Time 1) and 29th (Time 2) torque time integrals (1.25-1.75 sec = Time 1 and 28.25-28.75 sec = Time 2) were averaged across the first and second stimulus trains (see Figures 4.1a and 4.1b). These values were then used to calculate the mean torque time integrals at Time 1 and Time 2 for the group. For protocol C, each subject's first (Time 1) and 15th (Time 2) torque time integrals were averaged across each stimulation train (n = 8; see Figure 4.1c). These values were then used to calculate the mean torque time integrals at Time 1 and Time 2 for the group. For each subject, the percent change in torque from the beginning to the end of stimulation for each protocol was calculated using the following formula: $\left(\frac{meanTime1}{meanTime2}-1\right)\times 100$.

Kolmogorov-Smirnov and Lillefors tests for normality showed that the group data were not normally distributed. Therefore, non-parametric Wilcoxon

matched pairs tests were performed on the percent change scores between Intact and Blocked conditions for each protocol. Wilcoxon matched pair tests were also used to assess whether torque was different at Time 1 between Intact and Blocked conditions, on peak torque recorded during the supramaximal single and doublet values delivered before versus after the stimulus trains and on stimulus intensity used during Intact and Blocked conditions. The α level was set at $p < 0.05$. Cohen's d effect sizes were calculated for changes between Time 1 versus Time 2 for all protocols during both Intact and Blocked conditions. Effect size measures the magnitude of a treatment effect but unlike significance tests are independent of sample size. Cohen's *d* is defined as the difference between the means, $M_1 - M_2$, divided by the pooled standard deviation, SD, ($d = M_1 - M_2 /$ SD). Effects sizes were defined as "small, $d \leq 0.2$", "medium, $0.2 \leq d \leq 0.8$", and "large, $d \geq 0.8$" as described by Cohen (8).

4.3 Results

During the Intact condition, torque increased on average 144% from Time 1 to Time 2 across all stimulation protocols. In contrast, in the Blocked condition torque decreased on average 43% across all stimulation protocols. Torque percent change scores from the beginning to the end of the stimulation were significantly different ($p < 0.05$) between Intact and Blocked conditions for all three protocols (Intact protocol A = +125%; B = +230%; C = + 78%; Blocked protocol A = -79%; B = -15 %; C = -35%).

4.3.1 Protocol A: Constant 100 Hz stimulation

Figure 4.2a shows data from a single subject in whom torque remained relatively constant throughout the 30 seconds of 100 Hz stimulation during the Intact condition. During Blocked condition, torque dropped from an initial value of 30% MVC to 10% MVC within ~20 seconds. The group data (Figure 4.2b) show a similar pattern, with mean torque-time integrals increasing 125% from the beginning to the end of the stimulation (Time 1 to Time 2) during the Intact condition. In contrast, there was a 79% decrease in the mean torque-time integrals from Time 1 to Time 2 during Blocked condition. The effect sizes for both the increase and decrease in torque during the stimulation were large (Intact $d = 1.0$; Blocked $d = 1.3$) and the percent change scores between torque-time integrals for the Intact and Blocked conditions were significantly different ($p < 0.05$). The mean torque-time integrals at Time 1 were not significantly different between Intact and Blocked trials, indicating torque at the beginning of the stimulation was similar between conditions.

4.3.2 Protocol B: Four 2s "bursts" of 100 Hz alternating with 20 Hz stimulation

Figure 4.3a shows data from a single subject in whom torque increased from an initial value of ~5% MVC to eventually reach ~15% MVC after four bursts of 100 Hz stimulation during the Intact condition. This increase in torque did not occur during the Blocked condition. This stimulation protocol resulted in a 230% increase in the mean torque-time integral from Time 1 to Time 2 across the group (Figure 4.3b) during the Intact condition. In contrast, the mean average torque time integral decreased 15% during the Blocked condition. The effect

sizes for the increase and decrease in torque during the stimulation were large and small respectively (Intact d = 1.1; Blocked d = 0.24). These percent changes for the Intact and Blocked conditions were significantly different ($p < 0.05$). The mean torque-time integrals at Time 1 were not significantly different in the Intact versus Blocked protocol, thus torque at the beginning of the stimulation was similar between conditions.

4.3.3 Protocol C: Alternating on-off 100 Hz stimulation

The data from one subject during stimulation with protocol C are shown in Figure 4.4a. For this subject torque increased ~10% MVC from the first to the last train of stimulation during the Intact condition, whereas it decreased by ~2% MVC during the Blocked condition. On average for the group, torque-time integrals increased 78% (medium effect, d = 0.41) and decreased 35% (medium effect, d = 0.48) in the Intact and Blocked conditions, respectively. These percent changes between the Intact and Blocked conditions were significantly different ($p < 0.05$). The mean torque-time integrals at Time 1 were significantly different ($p < 0.05$) during the Intact versus Blocked protocol. Thus, there was significantly more torque at Time 1 for the Blocked versus the Intact condition.

4.3.4 Supramaximal Single and Doublet Stimulation

Peak torque evoked during the supramaximal single and doublet stimulation was not significantly different before versus after NMES for any protocol (A, B and C; see Table 4.1).

4.3.5 Sustained Plantar-flexion Torque

During the Intact condition four of the five subjects regularly had sustained plantar-flexion torque that outlasted the electrical stimulation. Examples are shown in Figure 4.3a and 4.4a indicated by the arrow labeled “end of stimulation”. This sustained activity was never present during the Blocked condition for any subject.

4.4 Discussion

In the Intact condition torque increased during all stimulation protocols on average by 144% from Time 1 to Time 2. In contrast, during the Blocked condition torque decreased on average by 43% from Time 1 to Time 2 across all protocols. Thus, results support the hypothesis that electrically-evoked contractions which develop due to a combination of peripheral and central mechanisms fatigue less than contractions evoked solely due to motor axon stimulation. The use of high stimulation frequencies increases the rate of afferent volleys reaching motoneurons while wide stimulus pulse widths increase the likelihood of activating sensory axons (14, 29, 30, 45).

During a voluntary contraction, motor unit recruitment usually begins with small, fatigue-resistant units and proceeds through the larger, more fatiguable units as the contraction increases, as described by Henneman’s “size principle” (21). It is generally accepted that NMES recruits motor units in a different order compared to voluntary contractions; however, whether motor units are recruited in a reversed (22, 43), random (15, 19, 24, 28) or near normal order (41) compared to voluntary contractions is controversial. Despite evidence

to the contrary (1, 3, 9, 10, 11, 12, 27, 35), descriptions of how contractions are generated by NMES are generally limited to the depolarization of motor axons (23, 34, 40) and the possibility of a central contribution is often not considered (7, 13, 23, 34, 40). A recent editorial has highlighted the importance of considering a central recruitment of motoneurons during NMES and that such activation has potential therapeutic advantages (39). If NMES does not involve the synaptic recruitment of motoneurons, we would not have observed any difference between torques produced in Intact versus Blocked conditions in the present study. On the contrary, torque decreased in Blocked conditions and increased during Intact conditions. Our results suggest that in the Intact state NMES generates contractions from both the direct depolarization of motor axons *and* the central recruitment of low-threshold, fatigue-resistant motor units.

Torque generated from a central recruitment of motoneurons during NMES is prominent when using high frequencies and wide pulse widths (1, 3, 9, 10, 11, 12, 27, 35) which maximize the afferent volley (14). Low stimulus intensities are especially effective at generating torque from a central recruitment of motoneurons, presumably due to a decreased probability of antidromic block along motor axons (12). Thus, recruitment of motor units *exclusively* by the direct depolarization of motor axons is likely to occur only at high stimulus intensities, when the antidromic volley will block orthodromic propagation along motor axons. As the intensity of stimulation in the present study evoked an initial contraction of ~5-10% MVC during 2 sec of 20 Hz stimulation, we expect there was minimal antidromic collision along motor axons, thus allowing

synaptically-activated motoneurons to evoke torque in part from the recruitment of small, fatigue-resistant motor units. Both protocols A and B showed well matched torques at the beginning of stimulation between Intact and Blocked trials; but since we could not match torque at Time 1 during protocol C it is possible that for this protocol subjects fatigued more during the Blocked condition due to the higher starting torque relative to the Intact condition.

The increase in NMES generated torque during the Intact condition reflects the additional synaptic recruitment of motoneurons. Our working hypothesis is that the afferent volley generated during NMES recruits according to Henneman's size principle; thus recruiting the low threshold, fatigue-resistant motoneurons first. This central contribution to the evoked torque may be due to a synchronous reflex action and/or motoneuron discharge that is asynchronous with the stimulus pulses. In contrast, contractions evoked during the blocked condition likely involve the synchronous activation of a greater proportion of fast-fatiguable motor units (15, 19, 22, 24, 28, 43) thus resulting in the decline in torque that we observed. The electrically-evoked afferent volley during NMES can generate an H-reflex and hence the synchronous, reflexive activation of motoneurons. Reflex activation of motoneurons is not traditionally believed to contribute to force generation during NMES because the H-reflex is attenuated at stimulus rates above 0.1 Hz due to post-activation depression (37). However the H-reflex can recover following this initial depression (27, 36) and may contribute to force generation during NMES in some muscles (9, 27). Another possibility is that the afferent volley generated during NMES results in the

asynchronous discharge of motoneurons due to the activation of persistent inward currents in spinal neurons. Such currents can cause repetitive firing in the absence of synaptic input and are most prominent in low-threshold, fatigue-resistant motoneurons (20, 31, 32). Electrical stimulation (10, 11, 36) and vibration (18, 25) can cause self-sustained firing in motoneurons, resulting in contractions that outlast the stimulation and are thought to be sustained by persistent inward currents (see "end of stimulation" Figure 4.3a and 4.4a). During the present experiments, four out of the five subjects produced plantar-flexion torque that outlasted the stimulation in the Intact condition only. No one produced torque that outlasted the stimulation during the Blocked condition. These results show that sustained plantar-flexion torque depends on a central mechanism but does not differentiate between a spinal or cortical origin.

Another mechanism that could contribute to the increasing torque in the Intact condition is the progressive recruitment of more motor axons during the stimulation. There is evidence that persistent inward Na^+ currents can develop in motor axons (42), which could explain a progressive depolarization and thus recruitment of motor axons; however, an increased recruitment of motor axons is not consistent with the significant difference in torque that we observed between the Intact and Blocked conditions. If the elevated torque during the Intact condition were due to increased motor axon recruitment, we would expect the same results during the Blocked condition since the ability to activate motor axons directly is not affected by the nerve block. In addition, the repetitive activation hyperpolarizes motor axons and thus decreases the likelihood of

recruitment (26, 44). It is therefore most likely that the increased torque during the Intact protocols was due to the progressive, central recruitment of motoneurons. The central recruitment of motoneurons by large diameter afferent input is known to follow Henneman's size principle (2, 5, 21), thus first recruiting motoneurons that innervate fatigue-resistant muscle fibers (20, 31, 32). The decreased torque during tetanic stimulation in the Blocked condition is likely due to the recruitment of relatively fewer motor units that are fatigue-resistant and an increase in motor axon threshold causing a loss of motor unit activation. While these mechanisms likely also contributed to a decline in torque during the Intact condition this was offset by the central recruitment of motoneurons. Our results do not suggest the presence of a decline in force generating capacity within the muscle since supramaximal single and doublet stimulation collected 2 sec following NMES were not different from those evoked before the stimulation.

4.4.1 Conclusion

In the intact nervous system, NMES can generate contractions via the recruitment of spinal motoneurons in addition to the direct depolarization of motor axons. The present data suggest that recruiting motor units via synaptic drive versus direct motor axon depolarization improves fatigue resistance during NMES compared to contractions that develop from the recruitment of motor axons alone. Recruiting fatigue resistant motor units via central mechanisms that are less accessible via direct motor axon depolarization may slow muscle atrophy and the transformation from slow to fast twitch fiber types that occurs following

spinal cord injury (4, 33). In addition, maximizing the activation of spinal motoneurons during NMES may have benefits for rehabilitation as it improves the resistance to electrically-evoked muscle fatigue.

Table 4.1. Group data showing peak torque generated by supramaximal single and doublet stimuli delivered prior to (Pre) and after (Post) each stimulation protocol for the Intact and Blocked conditions. Data are in Newton•meters with standard errors (SE).

	Intact Pre	Intact Post	Blocked Pre	Blocked Post
Single				
Mean	17.7	18.1	16.4	16.4
SE	2.9	3.0	3.2	3.1
Doublet				
Mean	30.1	30.8	29.3	29.7
SE	5.6	5.7	5.5	5.6

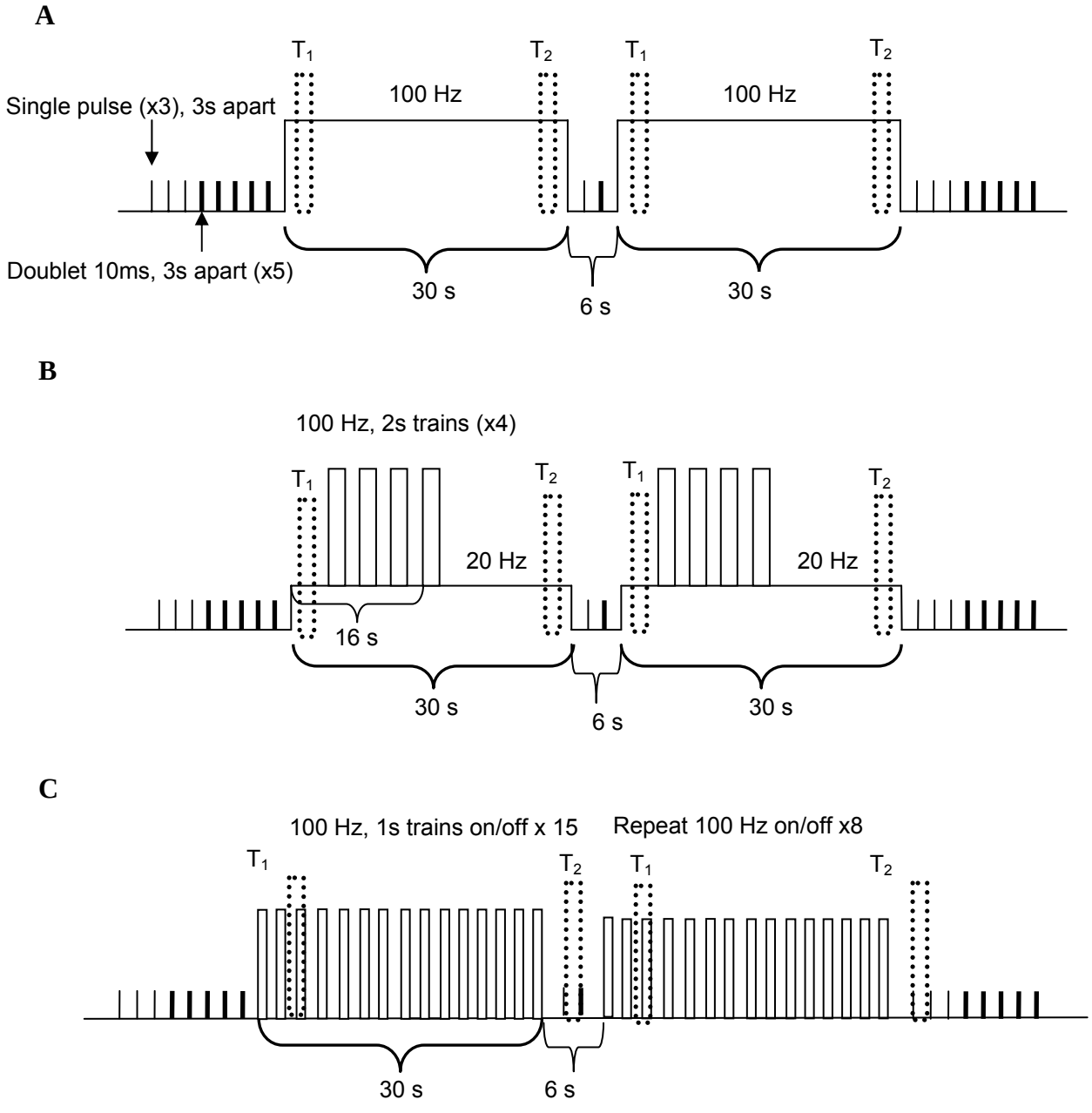

Figure 4.1. Stimulation protocols A (Constant 100Hz), B (Four 2-sec bursts of 100 Hz during 30 sec of 20 Hz, and C (alternating on-off 100Hz). Dashed boxes indicate Time 1 (T_1) and Time 2 (T_2).

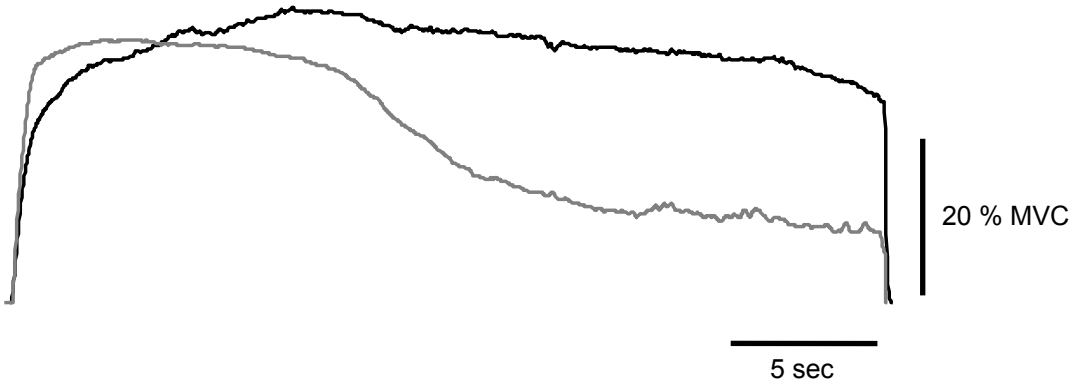

Figure 4.2a. Torque evoked during 30 s of 100 Hz stimulation (protocol A) in a single subject. Data show torque generated in the first stimulus train delivered during the Intact (black) and Blocked (grey) conditions.

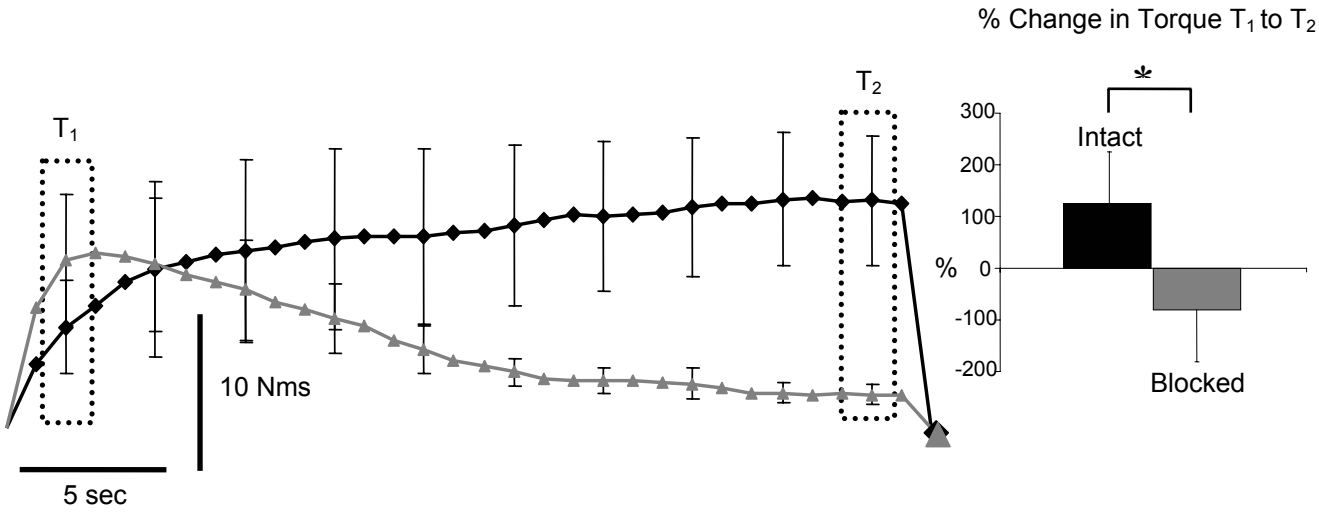

Figure 4.2b. Average torque time integrals of the group (N = 5) for protocol A during the Intact (black) and Blocked (grey) conditions. The asterisk (*) indicates a significant ($p < 0.05$) difference in the percentage change scores from time 1 to time 2 between the Intact and Blocked conditions. Error bars display standard error.

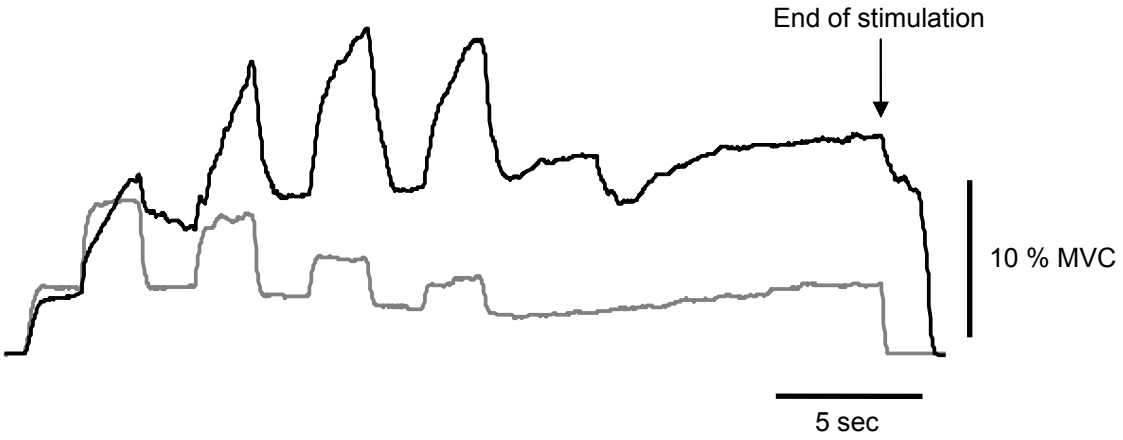

Figure 4.3a. Torque evoked during 30 s of stimulation using protocol B (four 2 sec long bursts of 100 Hz alternating with 20 Hz stimulation) in a single subject. Data show torque generated in the first stimulus train delivered during the Intact (black) and Blocked (grey) conditions.

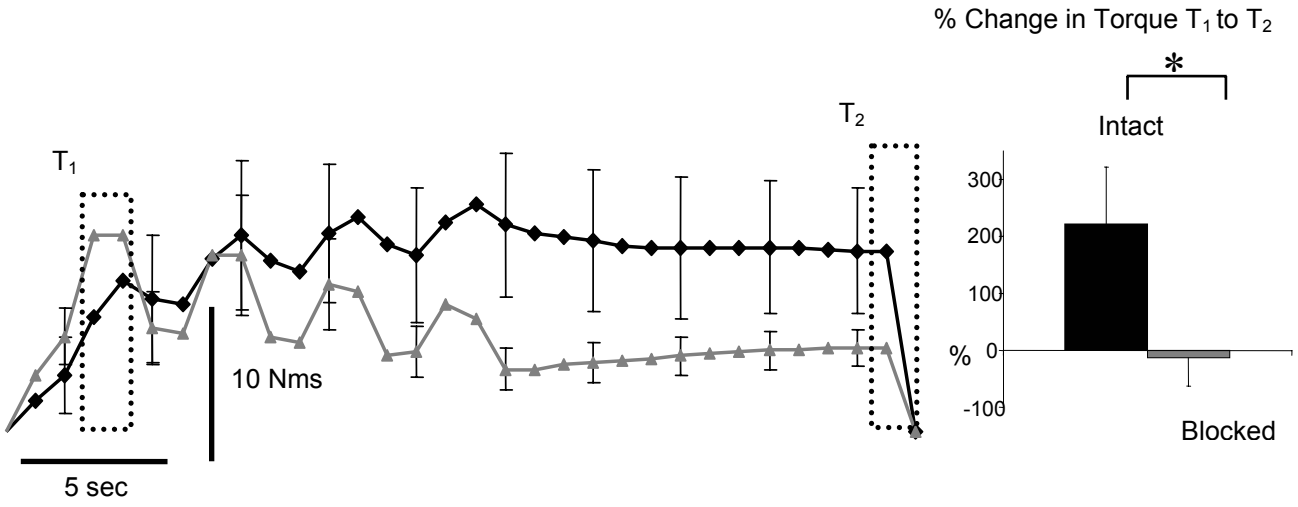

Figure 4.3b. Average torque time integrals of the group (N = 5) for protocol B during the Intact (black) and Blocked (grey) conditions. The asterisk (*) indicates a significant ($p < 0.05$) difference in the percentage change scores from time 1 to time 2 between the Intact and Blocked conditions. Error bars display standard error.

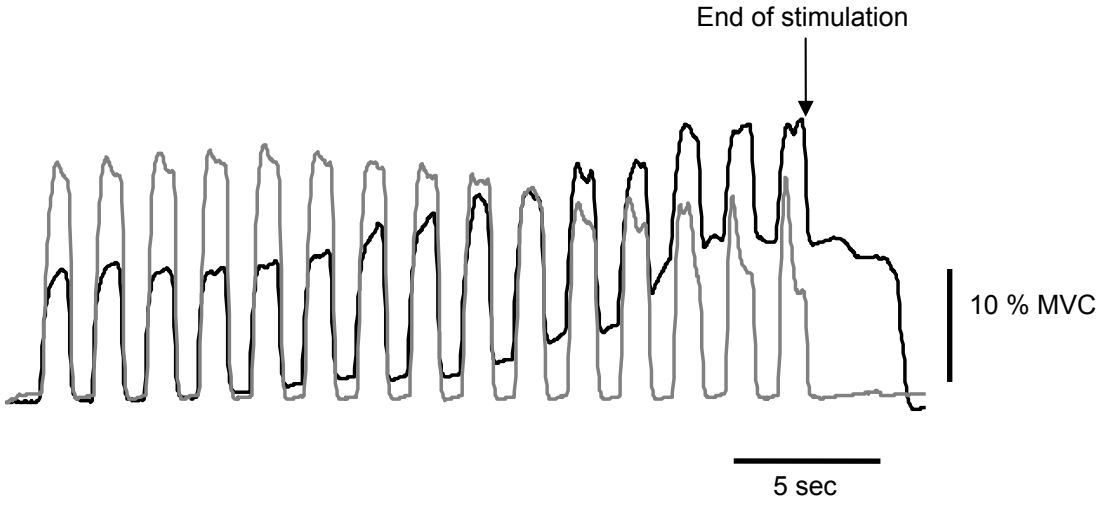

Figure 4.4a. Torque evoked during 30 s of alternating on-off 100 Hz (1sec on, 1 sec off) (protocol C) in a single subject. Data shows torque generated during the first stimulus train delivered during the Intact (black) and Blocked (grey) conditions.

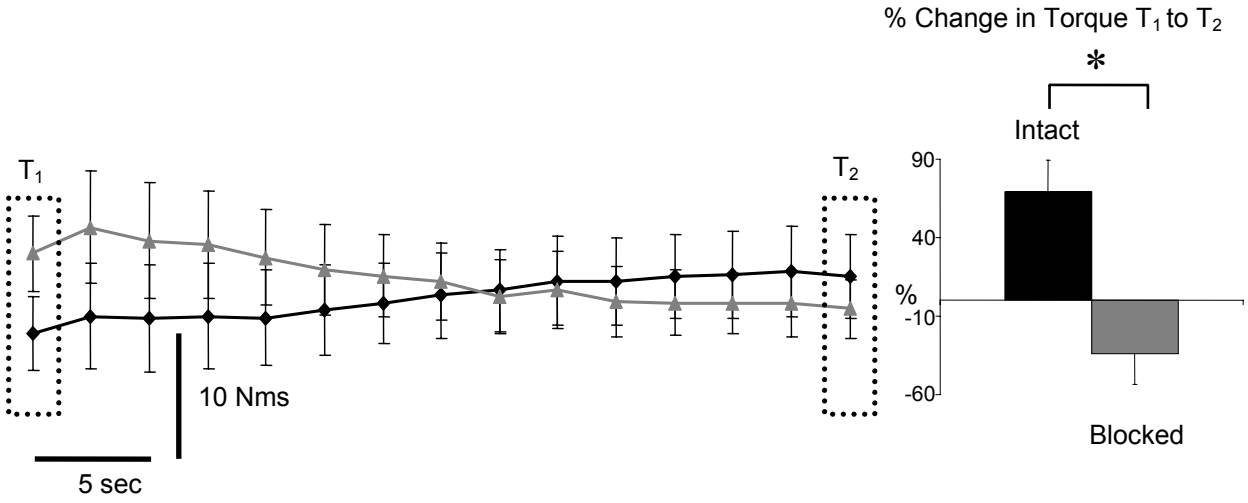

Figure 4.4b. Average torque time integrals of the group (N = 5) for protocol C during the Intact (black) and Blocked (grey) conditions. Each data point represent torque time integral values collapsed across all eight bursts. The asterisks (*) indicates a significant ($p < 0.05$) differences in the percent change scores from time 1 totime 2 between the Intact and Blocked conditions. Error bars display standard error.

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5.0 Changes in spinal but not cortical excitability following combined electrical stimulation of the tibial nerve and voluntary plantar-flexion

5.1 Introduction

Transmission across synapses and through neural circuits changes throughout one's life in an activity dependent manner. Such changes are commonly referred to as neural plasticity. The activation of the cortex in conjunction with input generated from sensory receptors drives plasticity within the central nervous system (CNS) during voluntary movements and this is believed to play an integral role in the learning of motor skills (37). It was not until 1995 that functional magnetic imaging demonstrated increased levels of activation in the adult motor cortex following the learning of skilled finger movements, indicative of plastic changes (27).

After a spinal cord injury (SCI) or stroke, plastic changes in the CNS develop which can be detrimental and contribute to the pathophysiology of the disorder (48). Neuromuscular electrical stimulation (NMES) has been found to increase the excitability of cortical projections and cause re-organization of the motor cortical map similar to the changes observed during motor learning (20, 39). NMES may, therefore, be useful for persons with movement disorders to increase CNS excitability and combat deleterious plastic changes in the CNS (16, 39, 45). The first evidence of increased motor cortex excitability due to NMES was presented in 1998 when it was found that 10 minutes of NMES delivered to the pharyngeal nerve increased excitability within the human motor cortex associated with swallowing (20). NMES has since been reported to lead to long-lasting CNS

plasticity resulting in improved function following stroke (12) and spinal cord injury (6, 7, 23).

Plasticity within the region of the motor cortex associated with the electrically- stimulated muscle has been demonstrated following finger flexion/extension (3); finger flexion (11); swallowing (20) and dorsi-flexion of the foot (28, 30, 32). Typically plastic changes are not reported to occur within spinal circuitry following NMES (11, 39). It has, however, been reported that NMES of the ankle plantar-flexors (31) and dorsi-flexors (28) can induce CNS plasticity that outlasts the conditioning stimuli and that cannot be accounted for by cortical excitability alone.

Combining voluntary movement with NMES increases cortical excitability to a greater extent than electrical stimulation or voluntary training alone. While this effect has been documented for the ankle dorsi-flexors (29, 30) and finger flexors/extensors (3), similar information does not exist for the ankle plantar-flexors; however, the particularly strong Ia connections to the soleus motoneuron pool (43) and the low strength of corticospinal connections between the motor cortex and the soleus motoneuron pool (14) may result in different responses compared to the TA and upper body muscles (14). Following CNS injury, NMES of dorsi-flexors is common to prevent foot drop during the swing phase of gait (35). NMES has also been used to activate the plantar flexors in order to increase the step clearance (1). Although the plantar-flexors are not typically stimulated for rehabilitation they are important postural muscles and contribute considerably to balance and propulsion during gait. Thus, a basic

knowledge of how NMES and voluntary movement effects CNS plasticity associated with the plantar-flexors is important and may be useful for future rehabilitation protocols.

The purpose of this study was to investigate the influence of voluntary contraction of the right plantar-flexors and NMES of the right tibial nerve on corticospinal and spinal excitability associated with the right and left soleus muscles. Functional imaging studies have shown that unilateral movements involve activation of the ipsilateral sensorimotor cortex (42); therefore, we were also interested in whether our interventions affected CNS plasticity in ipsilateral cortical or spinal circuitry. Therefore, our protocol included bilateral testing of corticospinal and spinal excitability since the influence of combining voluntary exercise and NMES on CNS plasticity in the uninvolved limb is unexplored. Each subject participated in four testing conditions conducted on different days: 1) intermittent NMES over the right tibial nerve (TNMES); 2) intermittent, voluntary, isometric contractions of the right plantar-flexors (VOL); 3) VOL in conjunction with TNMES (V+TNMES) and; 4) a control session with no voluntary contraction or NMES (CON). Corticospinal and spinal excitability were tested before and after each condition. We hypothesized that corticospinal and spinal excitability associated with the stimulated muscle would increase following TNMES, V+TNMES and VOL but not CON conditions. Furthermore, we hypothesized that corticospinal excitability for the right soleus would increase more following V+TNMES than TNMES or VOL conditions alone.

5.2 Materials and Methods

Ten persons with no known neuromuscular disorder (22 to 44 years old; 7 males) participated with informed consent. Leg dominance was determined by asking each subject which leg they preferred to kick a soccer ball with. All subjects were determined to be right foot dominant. This study was approved by the Health Research Ethics Board at the University of Alberta. Subjects were seated in the chair of a Biodex System 3 Dynamometer (Biodex Medical Systems, Shirley, New York) with the hip, knee and ankle at 90°, 120° and 90°, respectively. The ankle and foot were tightly secured to the footplate of the Biodex to measure isometric plantar-flexion torque. Subjects were asked to abstain from caffeine consumption for 12 hours prior to and for the duration of each experimental session to eliminate the possible effects of caffeine on CNS excitability (46, 47). Each session lasted approximately four to five hours.

5.2.1 Experimental procedure

Each subject participated in four experiments. The order of experiments was randomized. Data were collected before and after one of the following 40 minute conditions during each experiment: 1) intermittent NMES of the right tibial nerve (TNMES); 2) intermittent, voluntary, isometric contractions of the right plantar-flexors (VOL); 3) VOL in conjunction with TNMES (V+TNMES) and; 4) a control condition (CON) involving no contraction and no stimulation. NMES consisted of 1000 μs pulse widths delivered at 100 Hz for 5 seconds on and 5 seconds off over 40 minutes. The intensity of NMES was set to generate approximately 2-3% of each subject's maximal, voluntary, isometric plantar-

flexion torque (MVC). If necessary, stimulation intensity was adjusted over the 40 minutes to stay within this range. Every 5 minutes during V+TNMES trials subjects were asked to relax and not generate any volitional torque to ensure that TNMES elicited contractions of 2-3% MVC. Voluntary contractions during VOL and V+TNMES conditions were both ~20% MVC; thus the net torque produced during V+TNMES trails was ~22%MVC. Subjects were provided with feedback via a monitor that displayed their plantar-flexion torque. All experimental sessions were separated by a minimum of three days and were collected at the same time of day for each subject, respectively, to account for diurnal variations in CNS excitability (33, 44). The order of data collection during an experimental session was randomized on the first day of testing for every subject. This randomized order of data collection was then kept constant for each individual subject during subsequent experimental sessions. An example of one subject's randomized experimental design is illustrated in Figure 5.1. MVCs of both the left and right plantar-flexors were always performed first because those data were used to set the target EMG levels for background contractions during subsequent TMS and H-reflex testing (see below). Motor evoked potentials (MEPs) were collected bilaterally using transcranial magnetic stimulation (TMS) over the left and right motor cortex to assess corticospinal excitability for the left and right soleus respectively. Soleus H-reflexes were collected bilaterally using electrical stimulation over the left and right tibial nerves at the popliteal fossa to assess spinal excitability.

5.2.2 Electromyography

Surface EMG was recorded from the right soleus (RSOL), left soleus (LSOL), right tibialis anterior and left tibialis anterior muscles using bipolar ($2.25cm^2$) recording electrodes (Vermed Medical, Bellows Falls, Vermont). EMG signals were pre-amplified (500–2000x) and band pass filtered at 30-3000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England). All data were sampled at 2000 Hz with a 12-bit A/D converter (National Instruments, Austin, Texas).

5.2.3 Electrical stimulation

The tibial nerve was stimulated using bipolar ($2.25cm^2$) surface electrodes (Vermed Medical Inc.) placed over the popliteal fossa at the site that evoked a response (M-wave or H-reflex) at the lowest stimulation intensity. Rectangular pulses (1000 μs pulse width) were delivered from a Digitimer (DS7A) constant current stimulator. Stimulation current was measured with a current probe (mA-2000 Non-contact Milliammeter, Bell Technologies) to confirm that maximal motor wave (M_{max}) amplitudes plateaued with increasing levels of stimulation.

5.2.4 Maximum voluntary isometric contractions with interpolated twitches

Subjects performed between two to five MVCs of the right and left plantar-flexors at the beginning of each experimental session and upon completion of each 40 minute condition. Subjects performed MVCs until consistent maximal contractions were achieved (less than 5% variability on two successive trials). Each MVC lasted approximately three seconds and was

separated from the previous maximal effort by at least three minutes. Subjects were provided with visual feedback of their torque production and received verbal encouragement to perform maximally. The interpolated twitch technique (ITT) was used to estimate the degree of voluntary muscle activation during the MVCs (36) to determine whether fatigue developed during the experiments. Using the ITT, percent activation (ACT%) was calculated using the equation described by Folland and Willaims (18): ACT (%) = MVF/TMF x 100. MVF is the maximum voluntary force generated during the MVC. TMF (true maximum force - the theoretical maximum force the muscle can generate) was calculated as the MVF multiplied by the reciprocal of one minus the ratio of the "extra" force evoked by the supramaximal stimuli delivered during the MVC to the force evoked when the same stimuli was delivered after the MVC (i.e. at rest). The tibial nerve in the popliteal fossa was stimulated during (n=2), and after (n=3) each MVC. Stimulus intensity was set at 1.5 times the threshold current required to elicit a maximal M-wave (M_{max}) in soleus and the inter-stimulus interval was approximately one second.

5.2.5 Measures of corticospinal excitability

MEPs were elicited using a magnetic stimulator (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) with a figure-of-eight coil (Medtronic MC-B70, Minneapolis, Minnesota). The position and orientation of the coil was adjusted over the left and right motor cortices to find the two locations at which clear RSOL and LSOL MEPs were generated (~50-150 μV) at the lowest stimulus intensity, respectively. The coil position and orientation was guided and

recorded by a magnetic resonance imaging-guided TMS system (Brainsight; Rogue Research, Montreal, Quebec). Using this system, the same area of motor cortex for each subject was stimulated during all experimental sessions. In the present study, we placed the TMS coil to within 3mm of its optimal position for each of the four conditions. To maintain similar levels of motoneuron excitability during TMS trials, subjects held a background contraction of 5% maximal soleus EMG output using visual feedback of soleus EMG low pass filtered at 3Hz. Two measures of corticospinal excitability were evaluated: 1) active soleus MEP threshold and; 2) soleus MEP amplitude at 1.2 times the stimulator output required for eliciting active MEP threshold. Threshold intensity was determined by manually adjusting stimulator output in 1% intervals to elicit clearly discernible MEP responses (typically 50-150 μV) in at least four out of eight responses. Units for MEP thresholds are expressed as a percentage of maximal TMS stimulator output.

5.2.6 Measures of spinal excitability

M versus H soleus recruitment curves were constructed from responses to 60 stimuli delivered to the tibial nerve at the popliteal fossa. The stimulation was delivered randomly every three to five seconds at intensities ranging from below M-wave and H-reflex threshold to two to three times the minimum current required to evoke M_{max}. Subjects held a background soleus contraction of 5% low pass filtered EMG as described above when collecting M versus H recruitment curves. Three measures of spinal excitability were evaluated 1) *H-reflex recruitment gain:* A linear regression using the least sum of squares

method was fitted to the middle portion of the ascending limb of the M versus H recruitment curve when H-reflexes were between 25-75% of H_{max}. The slope of this regression was used as an indication of H-reflex recruitment gain (26, 33, 34, 47). *2) H-reflex recruitment relative to M-wave recruitment*: The size of the H-reflex on the ascending limb of the M versus H recruitment curve (H_A) was calculated using those reflexes that were evoked with an M-wave of between two to seven percent of M_{max}. Between nine to18 H-reflexes fell within this range for a given subject and were included in the average. 3) *Hmax/Mmax Ratio:* The maximal H-reflex to M_{max} ratio (H_{max}/M_{max} ratio) was calculated from the average of the three largest H-reflexes and the maximal M-wave from every subject's M versus H recruitment curve.

5.2.7 Statistics

All data were tested for normality using the Kilmogorov-Smirnov-Lilliefors test. To assess differences due to the effects of Time or Condition, separate 2x4 repeated measures analysis of variance tests were used to analyze five of our seven dependent variables (MVC, MEPs at 1.2 X threshold; H_{max}:M_{max} ratio, M versus H recruitment curve slope and H_A values). These variables were evaluated using two levels of "Time" (before and after each 40 minute condition) and four levels of "Condition" (TNMES, VOL, V+TNMES, CON). In the event of a significant main effect or interaction, post-hoc analysis was performed using the Tukey honestly significant differences test. We were specifically interested in Time x Condition interactions; thus, results from main effects of Time and Condition are not reported. There were, however, no

significant main effects of Time or Condition for any variable. All data are presented as means ± standard errors. The α-level for all tests was set at $p<0.05$. A Friedman test was used to evaluate percent activation and MEP threshold due to the non-normal distribution of the data.

5.3 Results

5.3.1 MVC's and ITTs

Neither plantar-flexor MVC torque nor percent activation values changed significantly during our experiments. There was no significant Time x Condition interaction for torque produced during MVCs of the right [$F_{(3,27)} = 0.41$; $p = 0.75$] or left [$F_{(3,27)} = 1.1$; $p = 0.4$] plantar-flexors. On average, subjects produced 275 ± 114 Nm MVC torque before and 279 ± 116 Nm after each condition. There was no change in the percent activation of the plantar-flexor muscles before versus after any of the conditions on either the right or left side (Friedman test, $p > 0.8$). The mean percent activation values were 98 ± 3% and 97 ± 2%, before versus after all conditions, respectively.

5.3.2 Soleus MEPs

None of the four conditions caused a significant change in our measures of corticospinal excitability as measured by MEP threshold or MEP amplitude at 1.2 x threshold. On average, MEP threshold values were 61 ± 12 before and 60 ± 11 % after all conditions. MEP data at 1.2 x threshold did not show any significant Time x Condition interaction for the right [$F_{(3,24)} = 1.9$; $p = 0.2$] or left soleus muscles [$F_{(3,24)} = 1.6$; $p = 0.2$]. Figure 5.2 shows group data from soleus MEPs evoked by 1.2 times threshold.

5.3.3 M versus H Recruitment Curves

Spinal excitability only changed for RSOL following the V+TNMES condition. M versus H recruitment curve data from a single subject before and after 40 minutes of V+TNMES is shown in Figure 5.3. Panel A shows the entire range of the M versus H recruitment curve (from 0-100% M-wave) and panel B shows only the ascending limb of the recruitment curve (1% to 8% M-wave). This subject's recruitment curve slope was steeper following V+TNMES (pre = 13; post = 60) and H_A values nearly tripled (from 25% to 68% M_{max}) while the H_{max}:M_{max} ratio remained constant at 80% M_{max}.

The slopes from the RSOL M versus H recruitment curve across the group showed a significant Time x Condition interaction [$F_{(3,24)} = 3.5$; $p = 0.03$]. Post-hoc analysis revealed that only the V+TNMES condition resulted in a significant slope increase for RSOL (105%; $p = 0.03$). Figure 5.4A shows group data of the M versus H slope obtained from RSOL. Recruitment curve data from LSOL were unchanged, exhibiting no significant Time x Condition interaction [$F_{(3,24)} = 0.5$; $p = 0.7$].

The amplitude of H-reflexes on the ascending limb of the M versus H recruitment curve (H_A) increased significantly for RSOL after the V+TNMES condition. H_A data from RSOL showed a significant Time x Condition interaction [$F_{(3,21)} = 6.1$; $p = 0.02$]. Post-hoc analysis revealed that only the V+TNMES condition resulted in a significant increase in H_A (52%; $p = 0.02$). Figure 5.4A illustrates the mean amplitude of the H_A group data from RSOL.

Equivalent H-reflex data from LSOL did not change (Time x Condition interaction [$F_{(3,21)} = 0.6$; $p = 0.7$]).

H_{max}:M_{max} ratios from RSOL and LSOL did not change during our experiments. There was no Time x Condition interaction for RSOL [$F_{(3,24)} = 1.3$; $p = 0.3$] or LSOL [$F_{(3,24)} = 1.2$; $p = 0.3$] H_{max}:M_{max} ratios. On average, H_{max}:M_{max} ratios collected before and after all conditions were 53 ± 20 and 51 ± 19, respectively.

Similar to the H_{max}:M_{max} data, soleus M_{max} values remained unchanged during our experiments. There was no Time x Condition interaction for either RSOL [$F_{(3,24)} = 1.4$; $p = 0.3$] or LSOL [$F_{(3,24)} = 0.4$; $p = 0.7$] M_{max} data. Average M_{max} values collected before versus after all four conditions were 8.4 ± 4.5 and 8.5 ± 3.8 mV, respectively.

5.3.4 Background EMG

Soleus background EMG during MEP was not different between conditions. There was no significant Time x Condition interaction for either RSOL [$F_{(3,27)} = 1.2$; $p = 0.3$] or LSOL [$F_{(3,27)} = 1.1$; $p = 0.4$] background EMG data. The averaged background EMG values before vs after all conditions were 5.0 ± 0.9 and 4.9 ± 0.7 % maximal EMG, respectively.

Soleus background EMG during H-reflex acquisition also did not change during our experiments. (RSOL Time x Condition: [$F_{(3,27)} = 1.6$; $p = 0.2$]; LSOL Time x Condition: [$F_{(3,27)} = 0.6$; $p = 0.6$]. Averaged background EMG values were 4.9 ± 0.8 and 5.0 ± 0.7 % maximal EMG, before versus after all four conditions, respectively.

5.4 Discussion

The present experiments were designed to determine whether a single 40 minute session of unilateral VOL, TNMES or V+TNMES of the right plantar-flexors increased the excitability of corticospinal or spinal projections to the RSOL and LSOL. Two main results are reported: 1) the excitability of the corticospinal pathways to RSOL and LSOL were not affected by any condition; 2) spinal excitability for RSOL increased following only the V+TNMES condition, with no effects on LSOL. Thus, tibial nerve stimulation induced plastic changes within spinal circuitry on the same side being stimulated only when voluntary contractions were performed at the same time. These findings did not support our hypotheses since we predicted increased cortical excitability for RSOL following TNMES, VOL and V+TNMES conditions. The lack of change in soleus MEPs with concomitant increased H-reflex amplitudes suggests that NMES affects CNS excitability differently when stimulating the plantar-flexors compared to other muscle groups.

To date, NMES has been shown to increase cortical excitability following stimulation delivered over a variety of sites (finger flexors/extensor, 3; ulnar nerve, 11 39; pharyngeal nerve, 20; common peroneal nerve, 28, 29, 32). A comparison of responses evoked by TMS and transcranial electrical stimulation (TES) in both the upper (hand muscles, 39) and lower limbs (tibialis anterior, 28) suggest that these changes are due predominantly to cortical and *not* spinal plasticity. It is believed that TMS activates pyramidal tract neurons synaptically, while TES recruits corticospinal axons directly without involving the soma of

the cortical cells (40). Thus, findings of increased responsiveness to TMS concomitant with no change in responses to TES are strong evidence for changes at a cortical but not spinal level. Previous studies have examined the influence of combining voluntary drive and NMES of the TA (29, 30) and the finger flexors/extensors (3). These studies found that cortical excitability was greater when NMES was combined with voluntary contractions compared to either NMES or voluntary contractions alone. In contrast, reports of increased cortical excitability following voluntary movements alone are mixed. Khaslavskai et al. observed a 35% increase in TA MEP values following 30 minutes of intermittent isometric dorsi-flexion exercise (29); however, Kido Thompson et al (30) reported that 30 minutes of walking had no effect on TA MEPs. These conflicting results may be due to differences in cortical drive to TA produced during isometric contractions versus walking. Capaday et al. has shown smaller soleus MEPs during the stance phase of walking than during isometric contractions; however, TA MEPs were large during the same phase of gait even when TA was inactive (10). These results were interpreted to suggest a greater cortical control of dorsi-flexors versus plantar-flexors. These differences likely reflect a greater independence of TA for discrete movements and its importance in controlling the trajectory of the foot during the swing phase of walking (38), whereas soleus is predominantly an anti-gravity, stabilizer muscle used during standing and locomotion.

Presently, 40 minutes of intermittent, isometric, unilateral plantar-flexion did not increase soleus MEPs. The particularly strong Ia connections to

the soleus motoneuron pool (43) and the low strength of corticospinal connections between the motor cortex and the soleus motoneuron pool (14) may explain why the responses in soleus are so different when compared to TA. For example, Ia connections onto the TA motoneuron pool are relatively weak and have been suggested to be under heavy influence from pre-synaptic inhibition (8) while the corticospinal projections to TA are strong (14). Consequently, H-reflexes are typically small or non-existent in the dorsi-flexor muscle groups such as the TA (8, 25). In contrast, it has been demonstrated that the percentage of soleus motoneurons recruited by the H-reflex is on average 50% M_{max} but can reach as high as 100% M_{max} in some subjects (43). While afferent spinal projections to the soleus motoneuron pool may be strong, the corticospinal projections from the motor cortex are relatively weak compared to the TA and upper body muscles (14). There may also be differences in the strength of connections to the cortex from the tibial versus common peroneal nerves; however somatosensory evoked potentials following both tibial and peroneal nerve stimulation generate robust responses of approximately the same amplitude (13). The lack of change in cortical excitability as measured by MEP responses in the present experiments may be due to differences in the descending tracts to the soleus motoneuron pool compared to other muscles that have been tested in this manner.

The H-reflex represents predominantly the efficacy of Ia connections to the motoneuron pool and this technique is commonly used for evaluating plasticity within spinal circuitry; however, few studies examining the effects of

NMES on CNS plasticity have examined muscles with strong Ia afferent-motoneuron connections. This has not permitted for an easy evaluation of H-reflexes following NMES protocols, such as in the present experiments. It has been reported that the size of the F-wave depends on motoneuron excitability (17). Consequently, a lack of change in F-wave amplitude following NMES has been taken as evidence for a lack of spinal plasticity (38); however, the H-reflex is the preferred indicator of spinal plasticity since it has been found to be more sensitive than F-waves. Specifically, the sensitivity of the H-reflex to heteronymous Ia excitation has been found to be tenfold greater than that of the F-wave (24). In addition, the motoneurons contributing to the F-wave vary greatly between responses (15) causing uncertainty about the validity of pre and post measurements. Thus, conclusions regarding motoneuron excitability from F-wave measurements should be interpreted with caution. H-reflex responses, however, are not only influenced by the excitability of the motoneuron pool, but also by pre-synaptic inhibition, especially at the Ia motoneuron synapse. Previous experiments that have investigated CNS plasticity following NMES by using TES and F-waves would not have detected changes in presynaptic inhibition in the spinal cord. Therefore, whether NMES alters presynaptic inhibition has been largely unexplored. One previous experiment has reported that NMES evoked lasting increases in soleus H-reflex amplitudes with no change in soleus MEPs (31); however, these experiments involved only three subjects during TMS trials, and were likely underpowered. Nevertheless, our findings agree with those of Kitago and colleagues (31) and extend their results

by showing an absence of cortical or spinal effects in the non-stimulated limb. In contrast to our findings, unilateral NMES of the tibial nerve has recently been reported to increase MEPs bilaterally in both the upper and lower body (21). Considerable differences in methodologies between the present experiments and those of Hayashi et al., (21) may explain these conflicting results. Hayashi and colleagues tested patients undergoing spinal surgery under anesthetic while utilizing a five second period of tetanic stimulation to the tibial nerve at the lateral malleolus. In contrast, the present experiments used healthy, awake subjects and applied 40 minutes of intermittent NMES delivered to the tibial nerve at the popliteal fossa.

5.4.1 Pre synaptic Mechanism

When evoking the soleus H-reflex the recruitment of motoneurons follow Henneman's size principle (4, 22). This is similar to the orderly recruitment of motoneurons reported during TMS (5). In the present experiments, H-reflex amplitudes were larger (~25%M_{max}) than MEP amplitudes (~5%M_{max}); however, the same soleus motoneurons recruited by TMS were likely represented within the larger H-reflex. In addition, Ia projections diffuse to every motoneuron within the soleus motor pool (2); therefore, we believe that we are examining responses in some of the same motoneurons but not all. It was, however, not possible to evaluate H-reflexes small enough to match the MEPs in all subjects since the accompanying M-wave would have been too small to ensure proper stimulus constancy.

The size of the MEP evoked by TMS is influenced by excitability of cortical neurons as well as the excitability of the motoneuron pool (40). We observed increased soleus H-reflex amplitudes following the V+TNMES protocol, with no concurrent change in soleus MEPs. This is suggestive of a presynaptic mechanism at the level of the spinal cord, since we should also have observed increased MEP amplitudes had there been a general increase in motoneuron pool excitability. The soleus H-reflex is particularly influenced by changes in presynaptic inhibition or facilitation at the Ia motoneuron synapse (for review see 41). In this manner, afferent transmission can be modulated without any influence on the postsynaptic (motoneuron) membrane; however, other presynaptic effects from type II, Ib, and cutaneous afferents cannot be excluded since the H-reflex can also be influenced by oligosynaptic pathways (9). Tetanic stimulation of group Ia afferents can produce post-tetanic potentiation (PTP) of the H-reflex (19), but the effects of PTP typically diminish within the first minute while our results lasted up to 2 hours. Previous results have demonstrated a potentiation of H-reflexes following NMES (31); however, we only showed a similar effect when NMES was paired with a voluntary contraction. We propose that the combination of increased afferent drive generated during V+TNMES in conjunction with descending supraspinal commands, functioned synergistically to potentiate Ia spinal reflex pathways at a presynaptic location during the present experiments.

5.4.2 Conclusion

To our knowledge, this is the first study to demonstrate that voluntary effort in conjunction with NMES facilitates spinal plasticity in the absence of cortical changes. Our results show that, for the soleus muscle, the combination of voluntary effort and NMES enhanced transmission through the H-reflex pathway, but electrical stimulation or voluntary training alone did not. Our findings suggest that this enhanced transmission is due to a mechanism that is presynaptic to the motoneuron membrane. Furthermore, compared to other muscles, the plantar-flexors may be unique in the way that voluntary drive and sensory feedback interacts to produce plasticity within the CNS.

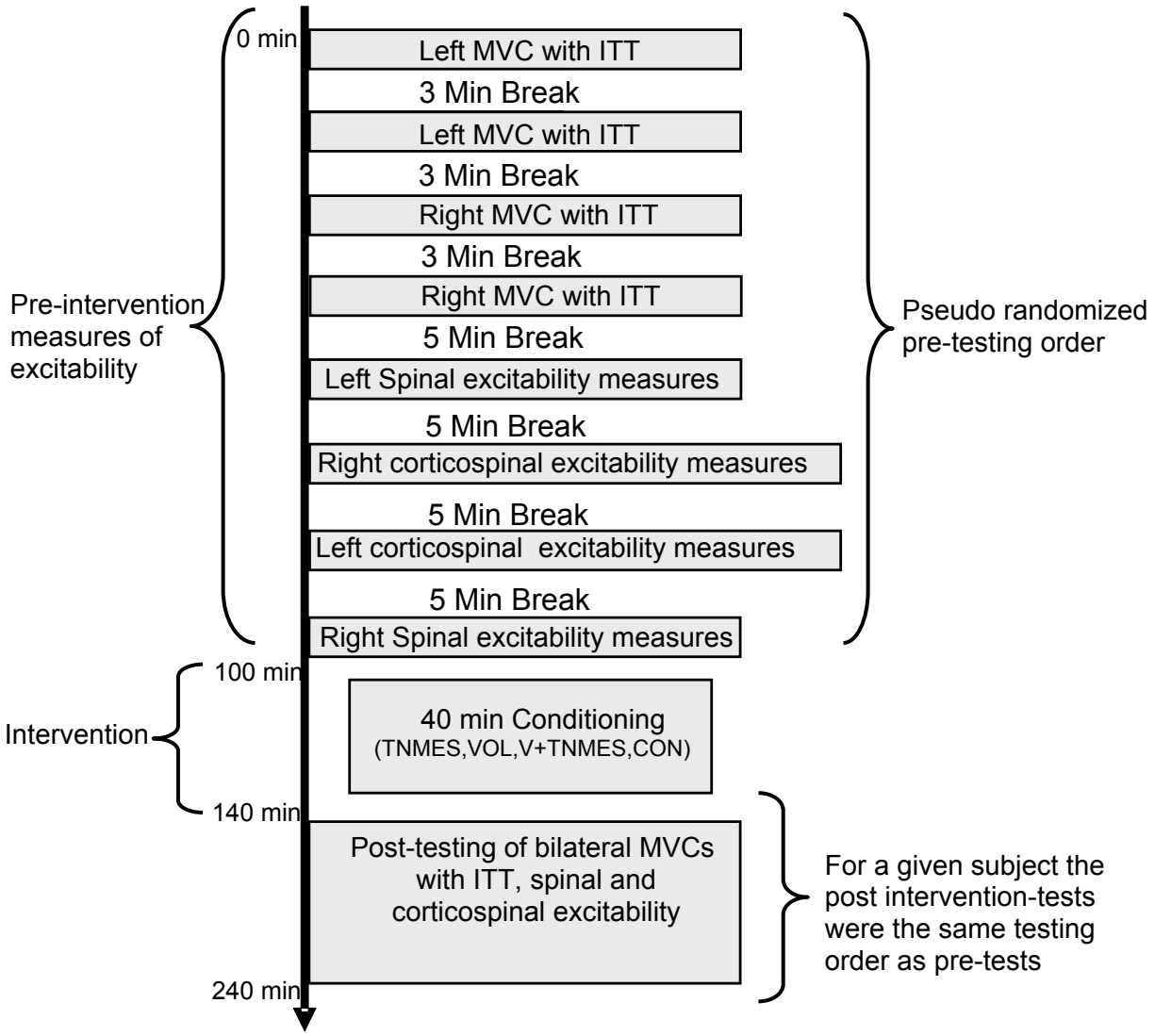

Figure 5.1. Example of randomized testing order for one subject. MVC ITT is maximum voluntary isometric contraction with interpolated twitch technique and TMS/MEP is transcranial magnetic stimulation used for collecting motor evoked potentials. MVC ITT trials were always pseudo randomized first since subjects were required to hold a 5% of maximum soleus EMG background contraction during H-reflex and TMS testing. The identical testing order was used before and after each 40 minute condition.

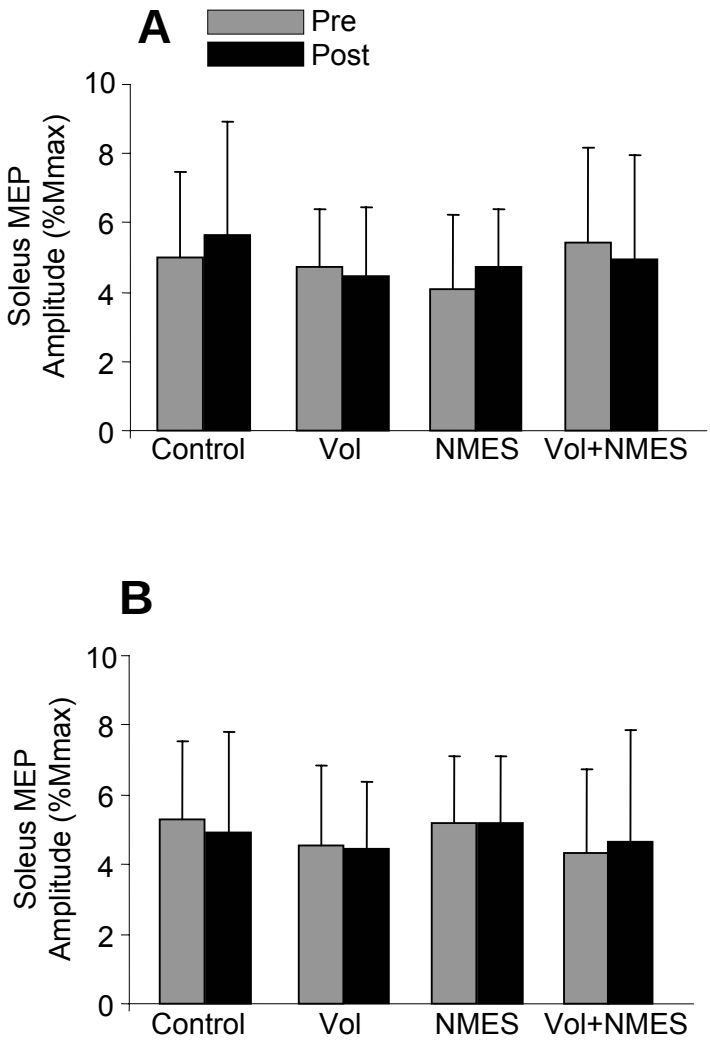

Figure 5.2. Group Soleus MEP data from the right (panel A) and left leg (panel B). Data collected before and after conditioning trials are shown in grey and black respectively Values have been normalized to each person's respective soleus $\%M_{max}$.

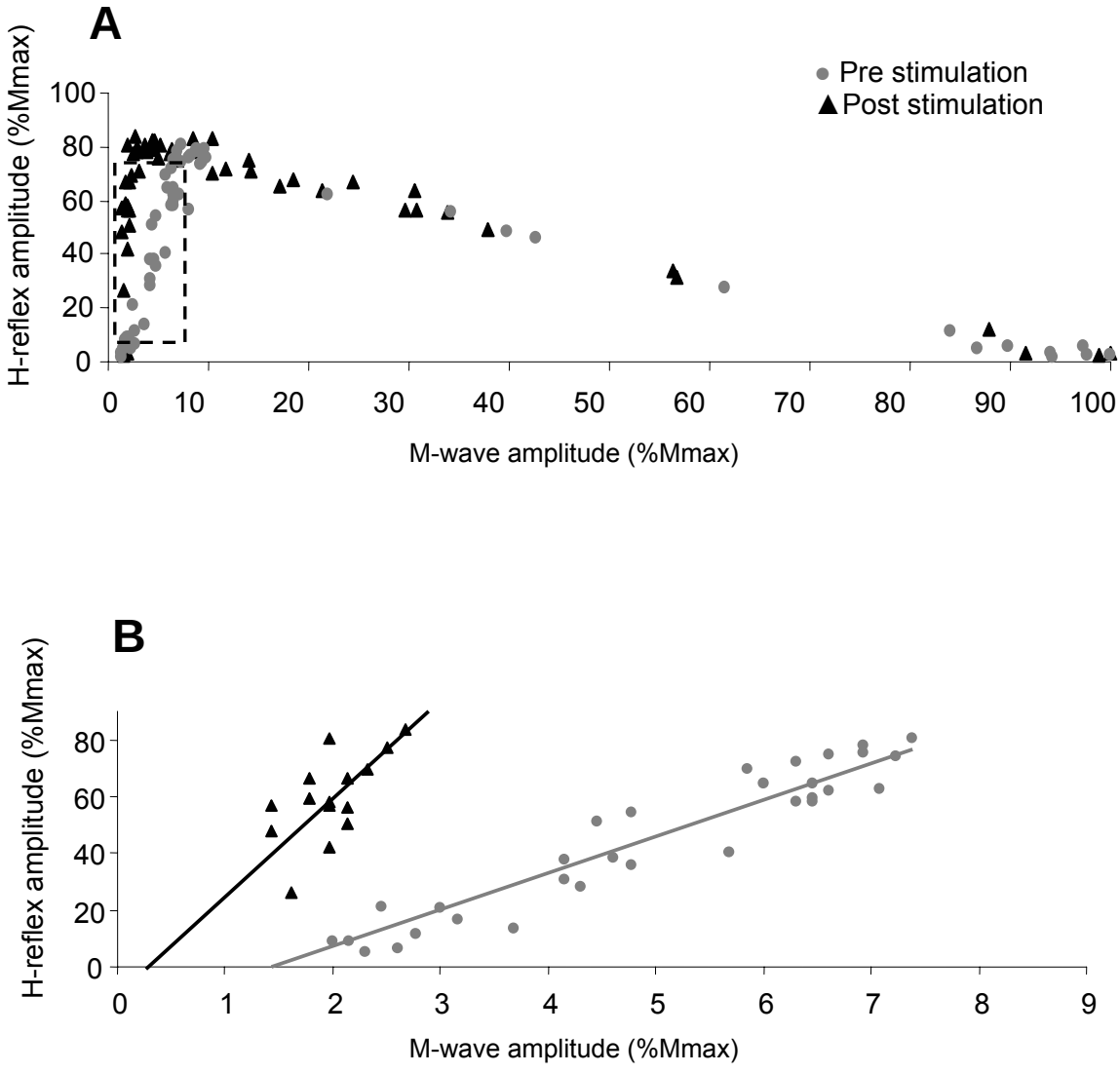

Figure 5.3. H versus M soleus recruitment curves collected from a single subject before (gray) and after (black) 40 minutes of Tibial nerve stimulation and concurrent isometric voluntary activation of the plantar-flexors. Panel A shows data collected over the full range of stimulus intensities. Panel B shows data selected from the ascending limb of the same recruitment curves (the area within the dotted box) shown in panel A when the H-reflex was between 1-8 % Mmax. The linear regressions of pre and post data are indicated by gray and black lines respectively in panel B.

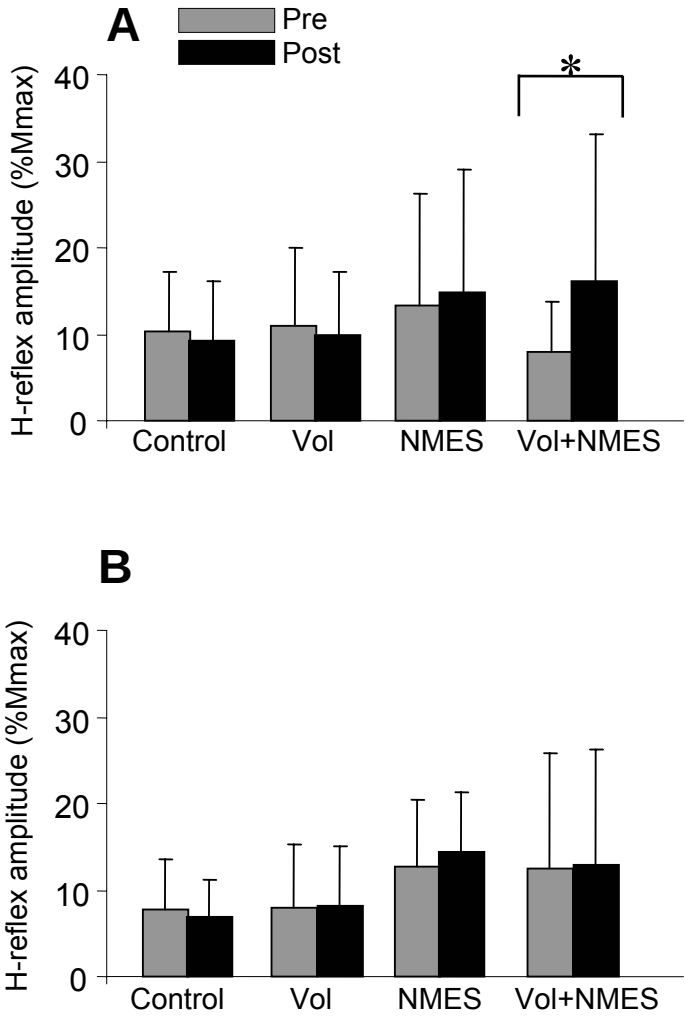

Figure 5.4. Group Soleus H-reflex slope data from the right (panel A) and left leg (panel B). Data collected before and after conditioning trials are shown in grey and black respectively Values have been normalized to each person's respective soleus $\%M_{max}$. Asterisks (*****) indicate significant differences ($p<0.05$) between pre and post values.

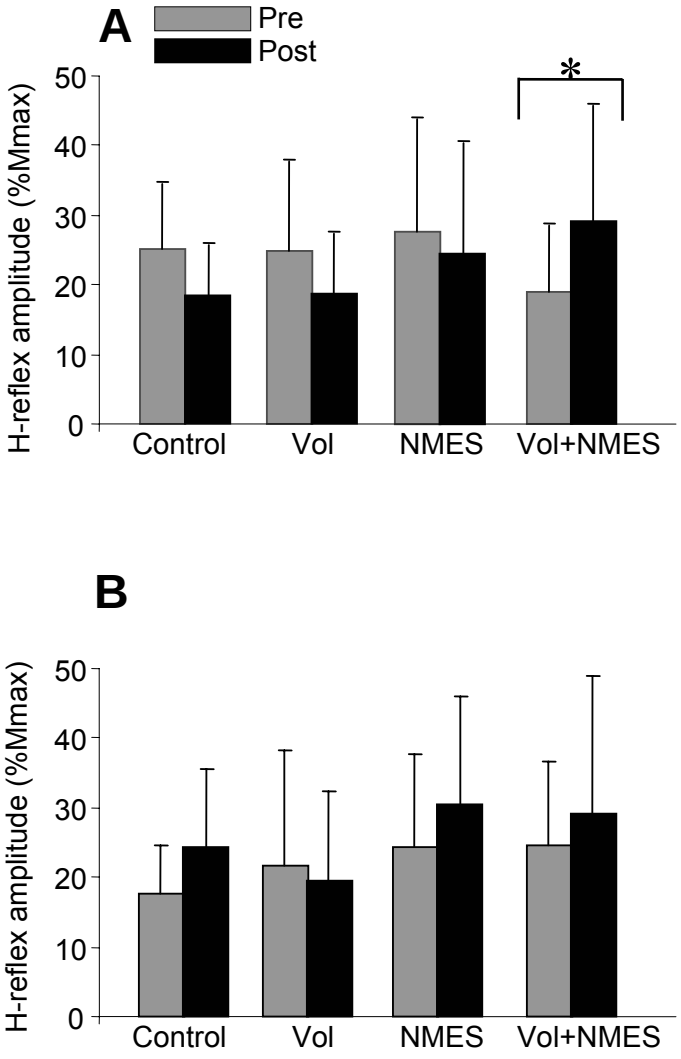

Figure 5.5. Group Soleus H5%$_{Mmax}$ data from the right (panel A) and left leg (panel B). Data collected before and after conditioning trials are shown in grey and black respectively Values have been normalized to each person's respective soleus %M_{max}. Asterisks (*****) indicate significant differences ($p<0.05$) between pre and post values.

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6.0 General Discussion

NMES is an important aspect of rehabilitation that can be used to decrease muscle atrophy and spasticity, increase circulation and bone density and generate functional movements. Although there are numerous benefits, limitations exist, particularly with regard to the unnatural recruitment order that accompanies NMES. Thus, there is a need to explore new techniques which maximize the therapeutic benefits of NMES and minimize the limitations.

Previous attempts to improve upon the limitations of NMES have centered on peripheral mechanisms without considering the influence of the afferent volley. In 1946, long duration, slowly rising currents were reported to selectively activate low-threshold motor units (14). While the possibility of a central contribution was acknowledged (14), these investigators ultimately concluded that they were dealing with peripheral effects only. Experiments in the early 1990's found that wide "quasitrapezoidal" pulses could selectively activate the axons of low-threshold motor units by selectively blocking the generation of action potentials in large motor axons (6). More recently, sub-threshold, long duration ramp prepulses were found to preferentially recruit low-threshold motoneurons (10). These studies all describe peripheral mechanisms that may account for the selective recruitment of low threshold motoneurons but the central recruitment of motoneurons is seldom considered. Contractions generated via NMES are commonly believed to arise exclusively from the activation of distal motor axons beneath the stimulating electrodes (11, 21, 27). In contrast, recent evidence suggests that enhancing the afferent volley evoked during

NMES may act on central mechanisms to decrease fatigue and enhance positive plastic neural adaptation within the CNS.

This thesis is comprised of four research projects with the common objective of evaluating how the afferent volley evoked during NMES affects muscle and the CNS. The four projects can be divided into three sections: 1) enhancing the afferent volley during NMES to increase the central recruitment of motoneurons; 2) investigating fatigue during NMES while enhancing or blocking the afferent volley, and 3) determining the influence of the afferent volley on plasticity within the CNS. The following sections summarize the main findings of this thesis and discuss limitations and future directions.

6.1 Central recruitment of motoneurons

Experiments in Chapters 2 and 3 demonstrated that low intensity NMES protocols using 200-1000 μs pulse widths recruited more motoneurons through the H-reflex pathway than using 50 μs. This increased reflexive recruitment resulted in a greater central contribution to the evoked contraction when using 200-1000 compared to 50 μs pulse widths. The H-reflex is typically facilitated following spinal cord injury and stroke (5); therefore, it may be possible to take advantage of heightened reflexive responses to generate contractions with an especially strong central contribution during NMES. It remains important to establish whether enhancing the afferent volley with wide pulse stimulation (WPS) during NMES is safe and efficacious for improving rehabilitative protocols, specifically regarding increasing the incidence and/or severity of spasticity. Spasticity is prevalent in populations with damage to the CNS and is

reported to occur in ~70% of patients with spinal cord injury (17), 35% in patients with stroke (28) and 85% in patients with multiple sclerosis (26). Spasticity is, in part, characterized by hyperexcitability of spinal reflexes (23) and has been linked to changes in motoneuron properties (7, 24). Since WPS is hypothesized to act on motoneurons via central pathways, it remains important to investigate any possible deleterious effects of such stimulation. Previous experiments utilizing WPS for short periods of time have thus far not demonstrated any increase in the incidence or severity of spasticity (3, 22). In addition, NMES protocols are typically found to reduce spasticity for persons with SCI (15), stroke (19) and multiple sclerosis (16); therefore, it is not expected that WPS will increase the occurrence or severity of spasticity. Currently, the long term effects of WPS on spasticity are unknown and merits further research. Another avenue of research which requires clarification concerns stimulus intensity. Since the present experiments utilized only low stimulus intensities, future experiments are required to explore the influence of higher stimulus intensities (such as those typically needed to generate contractions for FES assisted standing and walking). Greater antidromic block along the motor axons may decrease the torque contribution from the central recruitment of motoneurons at higher intensities; however, a large afferent volley may still be able to recruit some low threshold motoneurons via central mechanisms provided that the intensity of stimulation is not great enough to produce antidromic block along all motor axons. This might increase the fatigue resistance of the muscle during NMES.

6.2 Fatigue resistance during NMES

Experiments outlined in Chapter 4 demonstrated for the first time that NMES- evoked contractions, which develop due to a combination of peripheral and central mechanisms, fatigue less than contractions evoked solely by peripheral motor axon stimulation. These results highlight the importance of the evoked afferent volley for increasing the fatigue resistance of the stimulated muscle. Any central recruitment of motoneurons (whether it is synchronous or asynchronous) should, theoretically, result in improved fatigue resistance compared to the direct activation of motor axons underneath the stimulating electrodes. A central recruitment that causes asynchronous motor unit activation, however, should offer an advantage over synchronous activation.

Voluntary contractions make use of asynchronous firing patterns with independent firing rates for each motor unit which minimizes fatigue (20). On the other hand, contractions evoked by synchronous activation, even if originating from a central mechanism, will activate motor units without independent firing rates. Recent experiments using near-infrared spectroscopy (NIRS) demonstrated that synchronous activation of motor units during NMES of the TA muscles resulted in less tissue oxygenation than an equivalent voluntary contraction (18); thus muscle oxygenation and metabolic demands within the muscle are increased when motor units are recruited synchronously as opposed to asynchronously (18). Measuring oxygen saturation with NIRS during NMES may, therefore, provide an indirect measure of motor unit synchrony during WPS. In addition, NIRS is capable of assessing regional blood flow in the

motor cortex and may be useful for investigating changes in cortical excitability during and following NMES.

6.3 CNS plasticity

NMES using wide pulses increases excitability within corticospinal circuitry that outlasts the conditioning stimuli (finger flexors/extensor (1); ulnar nerve (2, 25); pharyngeal nerve (9); common peroneal nerve (12,13,). The mechanisms underlying plastic changes following NMES may reside at spinal or cortical levels; however, cortical mechanisms are most often suggested to dominate (12, 13, 25). Plastic changes due to NMES have been linked with functional improvement after injury to the CNS (4, 8). Results from Chapter 5 demonstrated that plastic changes occurred within spinal circuitry following NMES of the plantar-flexors. This plasticity occurred only when NMES was paired with voluntary isometric exercise and is hypothesized to be due to a mechanism that is presynaptic to the motoneuron membrane.Interestingly, previous claims that NMES causes cortical but not spinal plasticity (25) utilized techniques which were not sensitive to changes in pre-synaptic inhibition. Currently, the plantar-flexors appear to be the only muscle group that does not increase cortical excitability following NMES or voluntary exercise. Future studies should investigate other muscles where H-reflexes can be easily evoked, such as the flexor carpi radialis and the quadriceps muscles. This would contribute to understanding how NMES and voluntary contractions affect spinal and cortical circuitry in the upper and lower limbs and may elucidate if pre-synaptic mechanisms contribute to plasticity within other muscles. Presently, it is

unknown whether NMES-induced plastic changes within spinal circuitry improve functional recovery following damage to the CNS. Inducing plastic changes within spinal circuitry does not imply that positive functional outcomes must follow. In addition, it must be considered that our results may be isolated to the plantar-flexors.

All experiments described within this thesis investigated changes associated with the plantar-flexors and the soleus muscle in particular. The plantar-flexors provide a convenient model for assessing spinal reflex pathways due to the ease at which the H-reflex can be elicited in this muscle group; however, the strong Ia afferent connections to the motoneuron pool of the plantar-flexors are not typical of other muscle groups. In addition, the composition of muscle fibers within the triceps surae, especially the soleus muscle with its vast majority of fatigue-resistant muscle fibers, is not typical of other muscle groups; therefore, results described within this thesis will need to be verified in other muscle groups before generalizations can be made.

6.4 Future directions

In addition to the potential directions mentioned above, future experiments should specifically address the clinical efficacy of delivering NMES to enhance the afferent volley. For instance, two groups of participants with either complete or incomplete SCI could be randomly allotted into two groups: 1) a conventional NMES strengthening program using a stimulation protocol consisting of narrow pulse width (100 μs) and a typical tetanic stimulation frequency (20 Hz); or 2) a novel NMES strengthening program using wide pulse

width (1000 μs) and intermittent high frequency stimulation (20 Hz and 100 Hz). Measures evaluating electromyography, muscle girth, electrically-evoked strength, fatigue resistance during NMES, muscle oxygenation, bone density, spasticity and changes in function would be recorded prior to, at the mid point and upon completion of a 12 week training period. Experiments such as these are necessary in order to conclusively demonstrate whether maximizing the afferent volley during NMES is capable of exerting more positive outcomes than conventional stimulation protocols.

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