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Peripheral and central contributions to evoked contractions during neuromuscular electrical stimulation

by

Austin James Bergquist

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Dedication

To Joanna (Mrs. Bee) Broadbent,

who in her passing taught me a vital lesson in perspective.

Abstract

The present thesis examined two general questions regarding neuromuscular electrical stimulation (NMES): 1) How can the delivery of NMES be optimised to enhance synaptic motor unit recruitment via reflex pathways (*central* pathways) and 2) Can motor unit recruitment through *central* pathways improve the fatigue-resistance of NMES-evoked contractions in people with chronic motor-complete spinal cord injury (SCI)?

To address the first general question, two sets of experiments were conducted with people who were neurologically-intact (Chapters 2 and 3). Information about how motor units were recruited was provided by electromyographic responses evoked during NMES. The first two sets of experiments tested the hypothesis that NMES delivered over the nerve trunk (nNMES) would generate contractions of the *plantar flexors* (Chapter 2) and *knee extensors* (Chapter 3) with greater activity through *central* pathways compared with contractions of equivalent amplitude evoked by NMES delivered over the muscle belly (mNMES). Both hypotheses were confirmed, indicating that nNMES may hold significant advantages over mNMES for rehabilitation, and in particular for generating fatigue-resistant contractions.

To address the second general question, two sets of experiments were conducted in people with chronic motor-complete SCI (Chapters 4 and 5). The first set of experiments tested whether contractions of the paralysed plantar flexors evoked by mNMES would fatigue sooner, and to a greater extent, compared with contractions of equivalent amplitude generated by nNMES. This

hypothesis was confirmed. However, differences in fatigue-resistance between NMES sites were dependent upon the contribution of *central* pathways (H-reflexes) during the evoked contractions. When contractions were generated only through successive motor axon activation (M-waves; *peripheral* pathways), NMES site had no influence on fatigue-resistance. The second set of experiments tested the hypothesis that contractions of the paralysed plantar flexors evoked by nNMES using a short pulse duration (50 μs) would fatigue sooner, and to a greater extent, compared with contractions of equivalent amplitude evoked by nNMES using a long pulse duration (1000 μs). Data collection for this project continues, however initial data support our hypothesis. In conclusion, activity through *central* pathways is dependent upon NMES site, and holds promise for generating fatigue-resistant contractions after SCI.

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List of Abbreviations

AIS	American spinal injury association impairment scale
ANOVA	analysis of variance
CV	coefficient of variation
EMG	electromyography
FES	functional electrical stimulation
H-reflex	Hoffmann reflex
H_{max}	maximum H-reflex
H_{max}/M_{max}	H_{max}-to-M_{max} ratio
$H_{5\%Mmax}$	H-reflex amplitude when the M-wave was 5% M_{max}
H vs M	H-reflex versus M-wave
iNMES	interleaved NMES (rotating pulses between mNMES and nNMES)
M-H	M-wave-H-reflex
M-wave	motor wave
M_{Hmax}	M-wave amplitude at H_{max}
M_{max}	maximum M-wave
mNMES	NMES over a muscle belly
MVIC	maximum voluntary isometric contraction
NMES	neuromuscular electrical stimulation
$NMES_{50}$	NMES using a 50 µs pulse duration
$NMES_{1000}$	NMES using a 1000 µs pulse duration
nNMES	NMES over a nerve trunk
PTT	peak twitch torque

RMS	root mean square
RMS_{max}	maximum RMS
rmANOVA	repeated measures ANOVA
SCI	spinal cord injury
SD	standard deviation
SE	standard error
sNMES	sequential NMES (mNMES using multiple electrode pairs)
TES	therapeutic electrical stimulation

CHAPTER 1: GENERAL INTRODUCTION[1]

1.1 Preface

The focus of this thesis was two-fold; 1) to develop a further understanding of how motor units are recruited and, thus, muscle contractions are generated by neuromuscular electrical stimulation (NMES), and 2) to determine ways of improving the fatigue-resistance of contractions generated by NMES in people with chronic spinal cord injury (SCI). Chapters 2 and 3 involve experiments designed to determine whether the *site* that NMES is delivered on the surface of the skin (NMES site), either over a muscle belly (mNMES) or over a nerve trunk (nNMES), affects how contractions of the *plantar flexors* (Chapter 2) and *knee extensors* (Chapter 3) are generated. Chapters 4 and 5 involve experiments designed to determine whether the NMES *site* (Chapter 4) and NMES *pulse duration* (Chapter 5) affect the fatigue-resistance of evoked plantar flexor contractions in people with chronic SCI.

1.2 Overview of NMES

NMES can generate contractions to assist activities of daily living (82) and provide opportunities for exercise (36, 67) in people who experience paralysis due to SCI. However, the non-physiological way in which motor units are

[1] A portion of this chapter has been published.
Bergquist AJ, Clair JM, Lagerquist O, Mang CS, Okuma Y, and Collins DF. Neuromuscular electrical stimulation: implications of the electrically evoked sensory volley (invited review), *European Journal of Applied Physiology* 111:2409-2426, 2011.

recruited beneath the NMES electrodes (see Section 1.3 below) results in contractions that have limited fatigue-resistance, thereby minimising the effectiveness of NMES for restoring movement (11, 99, 118). This is particularly true for people with chronic SCI, whose muscle quality below the level of lesion may be compromised (119). Due to the inactivity imposed by the injury, muscle paralysed by SCI tends to atrophy and previously slow fatigue-resistant motor units take on characteristics of fast fatigable motor units (26, 61, 113, 120). Much research has been conducted on determining how different NMES parameters affect the evoked contractions, with a general goal of identifying how to produce the most fatigue-resistant contractions (see Section 1.3 below). This work has provided important information about generating contractions primarily through *peripheral* pathways (Figure 1-1), as the way in which NMES has been delivered in most studies tends to favor the contribution made by activating motor, as opposed to sensory, axons. Research in our laboratory has focused on identifying ways of generating contractions through *central* pathways, initiated by the activation of sensory axons (see Ref 30 for review). Generating contractions through *central* pathways recruits motor units by the synaptic activation of motor neurons (Figure 1-1), similar to motor unit recruitment that occurs during voluntary contractions, and this may be beneficial when NMES is used for rehabilitation. Specifically, increasing *central* recruitment during NMES may improve the fatigue-resistance of evoked contractions (see Section 1.3.1 below).

1.2.1 *Peripheral* and *central* contributions to evoked contractions

NMES generates contractions by the repetitive depolarisation of axons beneath the NMES electrodes. The depolarisation of motor axons produces contractions by signals traveling from the NMES site to the muscle (*peripheral* pathway), with no involvement of the central nervous system (motor volley; Figure 1-1). Motor units recruited through this pathway discharge relatively synchronously, and their discharge can be measured as an M-wave in the electromyographic (EMG) signal recorded from the muscle innervated by the stimulated nerve. In the same way that motor axons are recruited during NMES, sensory axons are also depolarised (sensory volley; Figure 1-1). The resultant sensory volley comprises signals in afferents from muscle spindles, Golgi tendon organs and cutaneous receptors (21). This sensory volley is sent to the central nervous system relatively synchronously, compared to sensory feedback generated during voluntary movements. It has been estimated that when evoked by NMES of the tibial nerve trunk in the popliteal fossa, signals in the fastest conducting Ia afferents arrive at the motor pool for soleus in ~15 ms, with the slowest arriving 6.7 - 9.4 ms later (21). The amount of temporal dispersion of the sensory volley will depend on the distance between the NMES electrodes and the spinal cord, with less dispersion for more proximal sites. During NMES, the sensory volley is sent to the central nervous system repetitively at the frequency of NMES, and can contribute to the evoked contraction by the synaptic recruitment of neurons in the spinal cord (*central* pathway; Figure 1-1). Thus, contractions produced by NMES can be generated by a combination of *peripheral*

recruitment, by the activation of motor axons beneath the NMES electrodes, and *central* recruitment, by the evoked sensory volley.

Confirmation that the *central* recruitment of motor units contributes to evoked contractions has been provided by experiments in which NMES was applied before and during a complete anaesthetic block of the peripheral nerves between the NMES site and the spinal cord (18, 31, 32, 87). In these experiments, contractions were larger before the nerve block, when the central nervous system could contribute to the evoked contraction, than during the nerve block when only transmission along *peripheral* pathways could contribute. Thus, during NMES the recruitment of motor units through *central* pathways can augment contractions generated through *peripheral* pathways, leading to the development of greater torque (extra or central torque). A *central* contribution to evoked contractions has now been shown for the triceps surae (4, 9, 31, 32, 79), tibialis anterior (31, 79), quadriceps femoris (10), wrist extensors (4) and flexor pollicis longus (18). The strength of the *central* contribution, measured as the amplitude of H-reflexes, asynchronous activity (see below) and evoked-torque, depends on the muscle being stimulated and the NMES parameters (9, 10, 31, 37, 87, 88). The first two research chapters of the present thesis investigate the possibility that the strength of the *central* contribution also depends on the NMES site (mNMES versus nNMES) for contractions of the plantar flexors (Chapter 2) and knee extensors (Chapter 3).

The sensory volley generated during NMES recruits motor units *centrally* in two distinct ways. Perhaps the most obvious form of *central* recruitment is

through the Hoffmann- or H-reflex pathway (29, 79, 88). Like the M-wave, motor units recruited through H-reflex pathways discharge relatively synchronously, although at a longer latency due to a longer pathway through the spinal cord. Thus far, evoked contractions with a robust contribution from H-reflexes have been shown for the triceps surae (9, 29, 79, 88) and quadriceps (10). On the contrary, the H-reflex contribution to evoked contractions of tibialis anterior is small (79) consistent with the weaker reflexive inputs to tibialis anterior motor neurons (73). The second form of *central* recruitment that occurs during NMES results in motor unit discharge that, unlike the M-wave and H-reflex, is not synchronised to each NMES pulse (32, 89). Such asynchronous activity, which tends to develop over time, may be initiated by pre-synaptic mechanisms, such as post-activation potentiation of neurotransmitter release and/or post-synaptic mechanisms, such as the activation of persistent inward currents in spinal neurons (32, 33). During NMES, asynchronous activity has been observed in single motor unit recordings (32, 89) and using surface EMG, where it appears as an increase in baseline activity measured between the M-wave and H-reflex (9).

1.2.2 Effect of NMES parameters on motor unit recruitment

The following sections provide an overview of how NMES pulse amplitude, pulse duration and pulse frequency influence the recruitment of motor units through *peripheral* and *central* pathways.

1.2.2.1 Pulse amplitude

Increasing the amplitude of NMES pulses (i.e. current or voltage) produces a stronger depolarising drive that travels deeper into the underlying

tissue (97, 126). Clearly, higher NMES intensities generate larger contractions by depolarising more motor axons beneath the NMES electrodes. Higher NMES intensities will also depolarise more sensory axons and send a larger sensory volley into the CNS, however, the extent to which this larger sensory volley can contribute to the evoked contraction is limited by antidromic transmission in motor axons (Figure 1-1). At high NMES intensities, antidromic transmission in motor axons blocks orthodromically transmitted signals, reducing the *central* contribution to evoked contractions (56, 108). Thus, contractions evoked at maximal intensities, that activate all the motor axons to a given muscle, will be driven exclusively by activity through *peripheral* pathways. Generating contractions with a large *central* contribution requires that the NMES be delivered at a low enough amplitude to minimise this antidromic block. In some individuals, NMES (100 Hz) delivered at or near motor threshold, when there is little or no antidromic block in motor axons, can generate up to 40% of the torque generated during a maximum voluntary isometric contraction (MVIC) almost exclusively through *central* pathways (31, 32). Overall, to evoke contractions with a large *central* contribution, the NMES intensity must be delivered low enough to minimise antidromic block, but contractions must be large and stable enough to be useful for restoring movement. Although delivering NMES at lower intensities may seem counter-intuitive when considering that benefits derived from NMES training tend to be proportional to the contraction amplitude (intensity, (117); dose, (124)), high NMES intensities can be problematic for people with hyper-sensitivity (118) or who have compromised bone density (43).

1.2.2.2 Pulse duration

Changing the duration of the pulses delivered during NMES alters the relative recruitment of motor and sensory axons. Short pulse durations (50 to 400 μs) preferentially activate motor axons (60), whereas the use of longer pulse durations (500 to 1000 μs) will recruit relatively more sensory axons (68, 77, 101, 104, 105). This differential effect of pulse duration on axonal recruitment is related to sensory axons having a longer strength-duration time constant and lower rheobase than motor axons (Figure 1-2; 101, 106, 132) and is the reason that longer pulse durations are more effective for evoking the H-reflex (86, 104). When single pulses were delivered to the tibial nerve trunk to generate soleus M-wave-H-reflex recruitment curves, the H-reflex recruitment curve was shifted to the left, relative to the M-wave recruitment curve, when using longer pulse durations (500 and 1000 μs versus 50 μs), consistent with a preferential recruitment of sensory over motor axons. Accordingly, when the M-wave was 5% of a maximal M-wave (M_{max}), H-reflexes were significantly larger when using longer pulse durations compared with shorter pulse durations (86).

A similar effect of pulse duration, consistent with changes in the relative recruitment of sensory and motor axons, occurs during repetitive NMES. During 100 Hz mNMES of triceps surae, 1000 μs pulses generated significantly larger contractions, indicative of a greater *central* contribution, compared to NMES delivered using 50 or 250 μs pulse durations, as shown for one participant in Figure 1-3a (31). In these experiments the current delivered was adjusted for each pulse duration to evoke the same torque at the beginning of each contraction. In

the same study, changing pulse duration did not alter the *central* contribution to contractions evoked by mNMES of tibialis anterior (31). A more detailed investigation that included assessment of M-waves, H-reflexes and torque during NMES over the tibial nerve trunk (88) confirmed that additional torque generated by the longer pulse duration was associated with greater *central* recruitment. When NMES was delivered at motor threshold and to evoke an M-wave that was 5% M_{max}, pulse durations of 200, 500 and 1000 μs generated larger H-reflexes and greater torque than a 50 μs pulse duration (88). This effect of pulse duration is shown for one participant in Figure 1-4 where H-reflexes and torque were larger following a period of 100 Hz NMES delivered using 1000 μs pulses compared to 50 μs pulses. Interestingly, M-wave amplitude also depended upon pulse duration. After the initial response, M-waves were depressed when NMES was delivered using 200, 500 and 1000, but not 50, μs pulse durations. Thus, the use of longer pulse durations during NMES can enhance *central* recruitment and reduce *peripheral* recruitment during NMES. However, these experiments indicate that activity through *central* pathways can contribute to evoked contractions across a range of pulse durations.

1.2.2.3 Pulse frequency

The frequency at which individual pulses are delivered within a NMES train (pulse frequency) determines the frequency at which action potentials travel along motor and sensory axons. For contractions generated through *peripheral* pathways, pulse frequency influences how torque generated through successive M-waves summates and contributes to the smoothness and strength of evoked

contractions. In general, for contractions generated through *peripheral* pathways, NMES is delivered at frequencies high enough to produce fused contractions (20-40 Hz; 3, 14), but not so high (>50 Hz) that contractions fatigue rapidly (58, 76); see Section 1.3.2.1 below). A decline in torque at higher pulse frequencies is consistent with the observation that torque tends to decline when NMES is applied at 100 Hz during a peripheral nerve block, when only *peripheral* pathways can contribute (87). In the same study, however, significantly more torque was recorded when the same NMES was delivered before the nerve block, when *central* pathways could contribute.

The influence of pulse frequency on the recruitment of motor units through *central* pathways can be complicated, as transmission across central synapses is strongly dependent upon the frequency of the sensory volley. For example, as pulse frequency increases, H-reflexes are progressively depressed due to post-activation depression of synaptic transmission (34, 115, 129). Such post-activation depression is clearly demonstrated during NMES at 20 Hz for a group of participants in Figure 1-5 (filled triangles), showing that soleus H-reflexes remained markedly depressed throughout the NMES train after the first H-reflex. In contrast, H-reflex amplitude did not change when NMES was delivered at 5 Hz, but during 10 Hz NMES H-reflexes were initially depressed and then their amplitude recovered completely by the end of the NMES train (Figure 1-5; Ref 28). Interestingly, the amount of reflex depression depended strongly on the voluntary contraction level, since depression was greatest when participants were relaxed, and was absent during contractions of 20% MVIC. In an apparent

contradiction to this frequency-dependant depression of H-reflex transmission, when NMES is delivered over the triceps surae muscle belly at high, but not low, frequencies for several seconds, large contractions can develop (31, 32). This is shown for a single participant in Figure 1-3b where torque increased the most when NMES was delivered at 100 Hz compared to NMES at 25 or 200 Hz. Across a group of 6 participants in this study, torque increased similarly during NMES at 100 and 200 Hz, but did not increase during NMES at 25 Hz. Although measuring H-reflexes or asynchronous activity at such high frequencies is difficult due to contamination of the EMG by successive NMES artefacts, the large contractions that can develop through *central* pathways (up to 40% MVIC) during high frequency NMES (31) may be due to the emergence of asynchronous activity, as occurs during tonic vibration reflexes (22, 62, 93).

In addition to the *central* recruitment that can develop during constant high-frequency NMES, *central* recruitment can be augmented when brief periods of high-frequency NMES (bursts) are delivered during longer trains at lower pulse frequencies (4, 31, 32, 37, 79, 88). Figure 1-4 shows that both H-reflexes and torque can be augmented after brief bursts of 100 Hz NMES. These *central* contractions depend on the NMES frequency, and are largest at frequencies greater than or equal to 80 Hz (37). Taken together these experiments suggest that activity through *central* pathways can contribute to evoked contractions across a range of frequencies, but that the *central* contribution may be predominantly due to H-reflexes at lower frequencies and asynchronous activity when NMES is

delivered at higher frequencies; although H-reflexes can be augmented by brief bursts of high-frequency (100 Hz) NMES.

1.3 Aspects of motor unit recruitment that affect fatigue-resistance

In each of the following sections, we describe how the orderly, temporal and spatial aspects of motor unit recruitment through *peripheral* pathways contribute to the limited fatigue-resistance of NMES-evoked contractions (92). We then describe a number of approaches that aim to improve each aspect.

1.3.1 Motor unit recruitment during NMES is not orderly

When motor units are recruited by voluntary descending drive (39, 98) or reflexive inputs (6, 20, 64, 65, 128), small fatigue-resistant motor units are recruited first, followed by larger fast-fatigable motor units, in accordance with the *size principle* (63). This recruitment order is attributed to smaller motor neurons having higher input resistances, which results in larger excitatory post-synaptic potentials for a given input, resulting in their recruitment at lower synaptic currents than larger motor neurons (125). In contrast to this well-established orderly recruitment during synaptic activation, the data available on motor unit recruitment order during NMES are less consistent (see Ref 57 for review). Initially, recruitment order was thought to be reversed compared to voluntary contractions, based on experiments involving NMES of motor axons using implanted electrodes in anesthetised cats (55, 123) and the idea that axons of larger motor units have lower axonal resistance, making them more easily

depolarised by currents applied directly to the nerve trunk (17, 123). While this view has a solid theoretical foundation, how NMES generates contractions for NMES at the surface of the skin may be quite different. In recent years, it has been suggested that motor unit recruitment during NMES follows the *size principle* (80, 97, 127) or is random with respect to motor unit type (71, 97). The general consensus seems to be that when NMES is delivered through the skin in humans, axonal activation depends both on the distance of the axons from the NMES electrodes as well as the axon diameter (2, 49, 78, 97). As a consequence, for contractions produced through *peripheral* pathways, the consensus is that there is no clear relationship between motor unit recruitment order and motor unit type (57). Thus, NMES-evoked contractions recruit relatively fewer fatigue-resistant motor units than voluntary contractions of equal amplitude, thereby contributing to the limited fatigue-resistance of NMES-evoked contractions.

1.3.1.1 Approaches to improve the orderly aspects of motor unit recruitment during NMES

Surprisingly few methods have been developed to improve motor unit recruitment order (i.e. recruit slow fatigue-resistant motor units first) during NMES. Methods that have demonstrated improved recruitment order *empirically* involve implanted electrodes that block action potentials in large motor units using either direct current (114), high-frequency (600 Hz; 5) or anodal (1, 48) NMES (*simulations* of exponentially rising waveforms have demonstrated improved recruitment order; Ref 66). In each case, action potentials are initiated at a proximal electrode and blocked at a distal electrode positioned along a nerve

trunk. To selectively activate small motor units, these methods take advantage of the reversed motor unit recruitment order of motor axons which occurs when NMES is delivered directly to a nerve trunk (55, 123). Since large motor units are more easily depolarised by externally applied currents (17, 123), large motor units are also more easily blocked at lower amplitudes of direct current, high-frequency or anodal NMES. Despite this work, the validity of achieving improved recruitment order with blocking techniques for improving-fatigue resistance of evoked contractions has not been tested thoroughly (123) and has largely been abandoned. Further, such blocking techniques are not feasible using NMES from the surface and, thus, it is unlikely that these methods will be widely incorporated into NMES rehabilitation programs.

Aside from these blocking techniques, generating contractions through *central* pathways (H-reflexes) can also recruit motor units according to the *size principle* (20, 128). Thus, generating contractions through *central* pathways may be one way to improve the fatigue-resistance of NMES-evoked contractions. Evidence that *central* contributions can improve fatigue-resistance of evoked contractions has been provided by nerve block experiments in people who are neurologically-intact (87). When NMES was delivered for up to 30 s at high frequencies (100 Hz), plantar flexion torque decreased when only motor axons could contribute (blocked condition) and increased when sensory axons could also contribute (before the nerve block). Chapters 2 and 3 of the present thesis aim to identify non-invasive ways of augmenting activity through *central* pathways, thereby recruiting motor units according to the *size principle*, during NMES.

Chapters 4 and 5 go on to test whether generating contractions through *central* pathways does indeed improve the fatigue-resistance of NMES-evoked contractions in people with chronic SCI.

1.3.2 Temporal aspects of motor unit recruitment

During voluntary contractions, motor units are recruited asynchronously with respect to each other, allowing for fused contractions to be achieved at relatively low firing rates (<20 Hz for soleus; Refs 8, 35). Such low firing rates reduce the metabolic demand placed on individual motor units (2). In contrast, during NMES, motor units discharge relatively synchronously to each other as M-waves, time-locked to each NMES pulse. Thus, to develop fused (tetanic) contractions capable of reaching near maximum torque levels, higher motor unit discharge rates are required during NMES than voluntary contractions (3), increasing the metabolic demand with respect to torque production (2, 131). Thus, although higher pulse frequencies are capable of generating greater torques, they also increase the rate of fatigue (53, 54, 70, 76). Thus, when recruiting motor units through *peripheral* pathways, it is recommended that NMES be delivered at the lowest frequency, capable of generating the desired torque, in order to minimise fatigue (3).

1.3.2.1 Approaches to improve the temporal aspects of motor unit recruitment during NMES

The majority of the research directed at improving the fatigue-resistance of NMES-evoked contractions has focused on the potential of modulating pulse frequency. Typically, NMES is delivered at constant frequencies, which have

evenly spaced intervals between pulses, to generate contractions. However, variable frequency trains, which begin with two pulses (doublet) separated by a brief interval (5 to 10 ms) followed by evenly spaced pulses at longer intervals, can augment torque development of fatigued muscle (14, 15, 27, 112). Variable frequency trains that utilise a high-frequency doublet at the onset of NMES take advantage of the intrinsic *catch-like property* of skeletal muscle (24). The catch-like property of skeletal muscle is the increased torque observed when high frequency pulses are added to the beginning of a non-tetanic train of NMES pulses (24), and may be due to either increased sarcoplasmic calcium concentration and/or increased stiffness of the series elastic elements of the muscle (25, 42, 107). Interestingly, high-frequency doublets like those at the onset of variable frequency trains also occur during voluntary contractions (7, 85) and are more prevalent during fatigue (59). Thus, the use of high-frequency doublets may be one way the central nervous system optimises torque output to overcome fatigue. Although variable frequency trains can augment torque development of fatigued muscle, there is controversy as to whether repetitive activation with variable frequency trains can generate contractions that are more fatigue-resistant than constant frequencies alone. During continuous isometric contractions of the thenar muscle with nNMES, variable frequency trains resulted in slower rates of torque attenuation, suggesting a slower time to fatigue, compared with constant frequency trains (14). However, during intermittent isometric contractions of the quadriceps with mNMES, variable frequency trains generated a greater decline in torque compared with constant frequency trains (16). Thus, it may be that optimal

NMES patterns involve the introduction of high-frequency doublets only once fatigue begins to develop (116).

Another strategy that aims to improve the fatigue-resistance of NMES-evoked contractions involves progressively increasing the pulse frequency during an NMES session (41, 74, 122). For mNMES of the knee extensors in people with SCI (74), switching from a lower (20-33 Hz) to a higher (66 Hz) pulse frequency allowed for a greater number of intermittent knee extension contractions to be completed compared with constant pulse frequencies (20, 33 or 66 Hz). This method of modulating pulse frequency is thought to overcome *low frequency fatigue*, which is the relative greater torque loss at low compared to high pulse frequencies once a muscle becomes fatigued (Ref 44; see Section 1.4.2 below). Interestingly, this effect of increasing pulse frequency seems to be dependent upon NMES intensity. During sustained isometric contractions of the thenar muscle by nNMES of the median nerve, progressively increasing the pulse frequency from 20 to 40 Hz improved fatigue-resistance compared with a constant 20 Hz frequency, but only when NMES was delivered at maximal amplitudes (to evoke M_{max}; Ref 41).

1.3.3 Spatial aspects of motor unit recruitment

Motor unit recruitment during mNMES is mainly, but not entirely (2), superficial due to the large distance from the mNMES electrodes to the deepest motor units (19, 97, 103, 109, 130). This results in an inability to recruit all of the motor units in a muscle, even at high mNMES intensities (2, 103, 109). In fact, one estimate suggests that only ~54% of the muscle cross sectional area can be

activated during NMES applied over the quadriceps muscles (2). To maximise the spatial recruitment of motor units during mNMES, Maffiuletti (2010) has suggested increasing the NMES pulse amplitude, to depolarise additional muscle fibres located at a greater distance from the electrodes (126), and moving the electrodes or varying joint angle after several contractions, both of which will change the population of superficial fibres that are recruited. Recent work indicates that spatial recruitment of motor units can also be improved upon by delivering nNMES, instead of mNMES. Motor units recruited during nNMES are distributed evenly throughout a muscle, regardless of NMES intensity (103). Figure 1-6 shows M-wave recruitment curves constructed from tibialis anterior data collected from a single participant during mNMES (panel A) and nNMES (panel B), respectively, while recording from a superficial and deep site within the tibialis anterior muscle using fine wires (103). During mNMES, the gain of the recruitment curves for the superficial and deep recording sites were markedly different. In this participant, deep regions of the muscle could only be activated at relatively high amplitudes during mNMES (in 3 of 9 participants, M_{max} could not be reached on the deep recording site during mNMES). In contrast, during nNMES, the recruitment curves were similar between the recording sites and both superficial and deep regions of the muscle could be activated at relatively low NMES intensities (M_{max} was reached on the deep recording site in every participant during nNMES). These findings regarding the spatial recruitment of motor units through *peripheral* pathways during NMES are summarised schematically in Figure 1-7. At the level of the muscle belly, motor units located

superficially within the muscle, those closest to the mNMES electrodes, are recruited preferentially during mNMES (97, 130) as depicted in panel A. At the level of the nerve trunk, recruitment of motor axons is random in relation to axon diameter (40, 94) and is likely superficial within the nerve trunk (panel B). Thus, motor unit recruitment should be randomly distributed throughout a muscle, regardless of the spatial organisation of motor unit types (filled circles; Figure 1-7B). Thus, even for contractions produced solely through *peripheral* pathways (*central* pathway contributions are omitted from Figure 1-7 for clarity), nNMES recruits motor units with a wider spatial distribution throughout the muscle. Thus, different types of motor units will be recruited by NMES at each site since the spatial distribution of different fibre types varies both within and between muscles (23, 81, 91).

1.3.3.1 Approaches to improve the spatial aspects of motor unit recruitment during NMES

In order to increase the spatial distribution of recruited motor units during mNMES, researchers have rotated NMES pulses between multiple electrodes over the muscle belly (known as *Sequential* NMES; sNMES; Ref 111). sNMES is thought to selectively activate sub-populations of motor units, rather than activate only one motor unit population repetitively beneath a single sNMES site, thereby mimicking, albeit crudely, the asynchronous motor unit discharge that occurs during voluntary contractions. Thus, sNMES improves the spatial recruitment of motor units, by increasing the populations of motor units contributing to evoked contractions through the use of multiple pairs of electrodes, and reduces the pulse

frequency required to generate contractions, since rotating pulses between pairs allows for a reduction of pulse frequency at each site, while maintaining the pulse frequency delivered to the muscle as a whole. During NMES of the quadriceps, with electrodes placed over rectus femoris, vastus lateralis and vastus medialis, knee moments declined to 50% of initial eight times faster when NMES was delivered simultaneously to each muscle (mNMES) compared to when NMES was cycled between all three muscles separately (sNMES; Ref 111). To date, sNMES has been shown to improve the fatigue-resistance for evoked contractions of the human triceps surae (102) and quadriceps (38, 95, 110, 111).

1.4 Sites of fatigue during NMES

During NMES, a decline in torque production can be due to failure in: 1) neuromuscular transmission, 2) excitation-contraction coupling and/or 3) metabolic processes (not discussed presently).

1.4.1 Neuromuscular propagation failure

Neuromuscular propagation failure involves compromised transmission at axonal branch points, neuromuscular junctions and/or at the muscle fibre membrane (46). In anesthetised animal preparations, impaired transmission at each of these sites has been associated with parallel declines in muscle force and M-wave amplitude (45, 51, 72, 83, 84, 90). As such, an approach to studying whether neuromuscular propagation failure plays a role during fatigue in humans has been to measure changes in M-wave characteristics (i.e. amplitude, duration, etc), and then to relate those changes to the decline in torque (12, 100, 120, 121).

In general, neuromuscular propagation failure during NMES is thought to contribute primarily to *high-frequency fatigue*, whereby torque declines rapidly at high frequencies (>50 Hz), due at least in part to an accumulation of extracellular potassium (69), but recovers rapidly within seconds when the frequency is reduced (13, 70). Interestingly, fast motor units are more susceptible to neuromuscular propagation failure (47, 52). Accordingly, neuromuscular propagation failure plays a more prominent role during NMES-evoked contractions in people with chronic SCI, in whom chronic disuse has led to transformation of motor units from slow to fast, compared to people with acute SCI, in whom motor unit transformations have not progressed (121). However, the contribution of neuromuscular propagation failure to NMES of muscle chronically paralysed by SCI may be minimal. During recovery from fatigue induced by 20 Hz nNMES of the plantar flexors, soleus M-waves had recovered completely, whereas plantar flexion torque recovered only minimally and remained depressed by 40%. The minimal recovery of torque was attributed to improved neuromuscular propagation, whereas the sustained 40% loss of torque was attributed to *low-frequency fatigue* (excitation-contraction coupling).

1.4.2 Excitation-contraction coupling failure

Generally, when there are minimal to no changes in M-wave characteristics, fatigue is attributed to failure of mechanisms *downstream* from the muscle fibre membrane, such as excitation-contraction coupling or metabolic processes (not discussed; Ref 46). Excitation-contraction coupling failure can occur at any one of the seven steps between 1) activation of the muscle fibre

membrane and 7) myosin binding to actin and force generation (50) and is thought to underlie a long lasting (hours to days) type of fatigue, termed *low-frequency fatigue*, characterised by a relatively greater loss of torque in response to low- compared to high- pulse frequencies (44, 69, 75). Specifically, intracellular measurements have shown that a reduction in calcium release from the sarcoplasmic reticulum may be particularly important in the development of *low-frequency fatigue* (133). The ratio between torque generated by 20 Hz and 50 or 80 Hz has been used as a measure of *low frequency fatigue* (44, 69, 96), with a decrease in the ratio indicating its presence (75).

1.5 Thesis Objectives

The present thesis examined two general questions regarding NMES: 1) How can the delivery of NMES be optimised to enhance synaptic motor unit recruitment via *central* pathways and 2) Can motor unit recruitment through *central* pathways improve the fatigue-resistance of NMES-evoked contractions?

To address the first general question, two sets of experiments (Chapter 2 and 3) were conducted with people who were neurologically-intact. The objectives of Chapter 2 and 3 were to compare activity through *peripheral* (M-waves) and *central* (H-reflexes and asynchronous activity) pathways during plantar flexor (Chapter 2) and knee extensor (Chapter 3) contractions evoked by NMES delivered over the muscle belly (mNMES) versus over the nerve trunk (nNMES). In both sets of experiments, we hypothesised that nNMES would

generate contractions with greater activity through *central* pathways, compared with mNMES.

To address the second general question, two sets of experiments (Chapters 4 and 5) were conducted in people with chronic motor-complete SCI. The objective of Chapter 4 was to compare the fatigue-resistance of paralysed plantar flexor contractions evoked by mNMES versus nNMES. We hypothesised that nNMES would generate contractions that were more fatigue-resistant than mNMES. The objective of Chapter 5 was to compare the fatigue-resistance of paralysed plantar flexors contractions evoked by nNMES using a short (50 μs) versus a long (1000 μs) pulse duration. We hypothesised that nNMES using a long pulse duration would generate contractions that were more fatigue-resistant than nNMES using a short pulse duration.

Together, the experiments in this thesis provide evidence that activity through *central* pathways: 1) is dependent upon NMES site (mNMES versus nNMES) and 2) holds promise for generating fatigue-resistant contractions in people with chronic SCI.

1.6 Figures

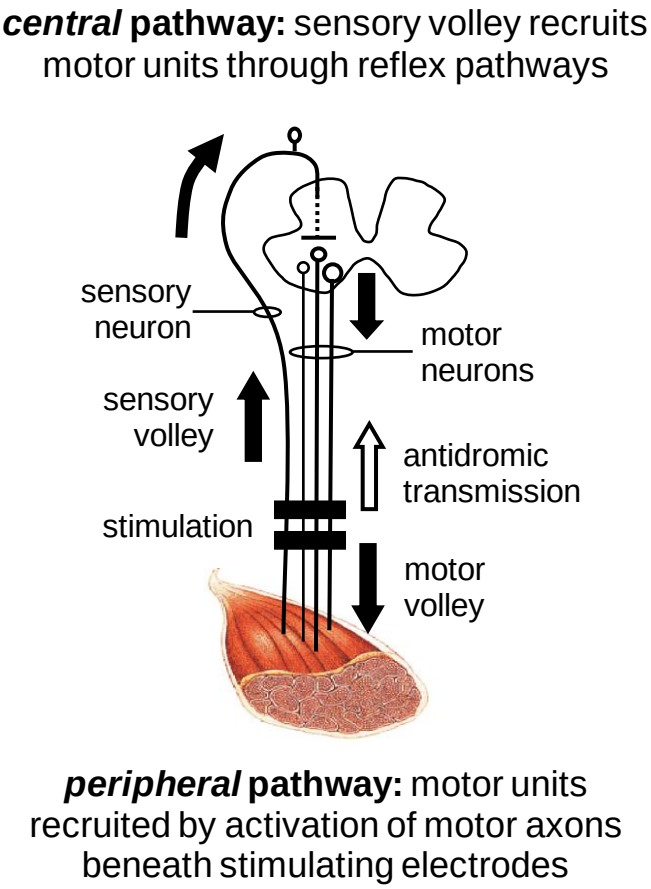

Figure 1-1 Schematic of *peripheral* and *central* pathways. Motor units are recruited by the NMES-evoked motor and sensory volleys initiated by depolarisation of axons beneath the NMES electrodes. The contribution from the evoked sensory volley is limited by antidromic transmission in motor axons at high NMES intensities. (Adapted from Ref 30)

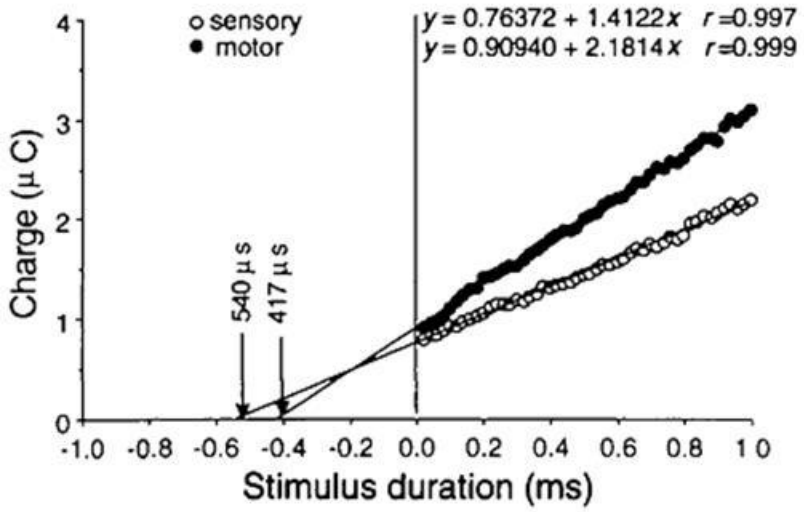

Figure 1-2 Relationship between stimulus charge and stimulus pulse duration for sensory and motor axons in a single human participant. The differences between the strength duration time-constants are represented by the x-axis intercept, as highlighted by the arrows. Rheobase is represented by the slope of the regression line shown in the top right of the panel. (Adapted from Ref 101)

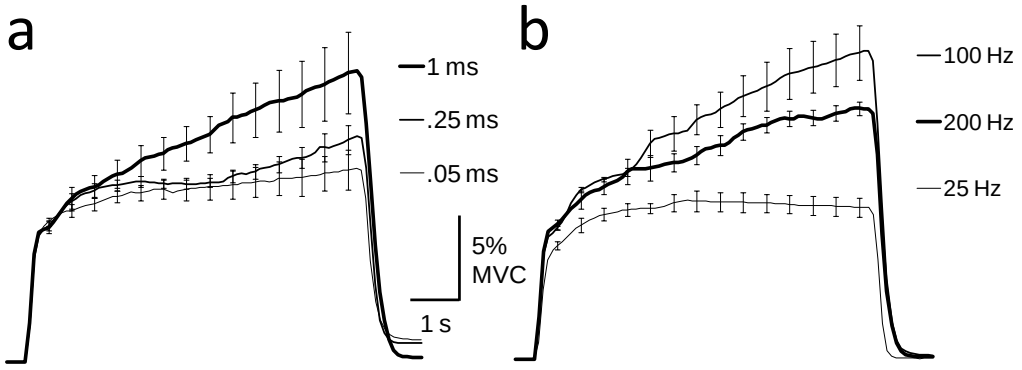

Figure 1-3 The effect of pulse duration and pulse frequency on the *central* contribution to mNMES-evoked contractions of the plantar flexors. a Mean (n = 5) torque responses evoked by mNMES (100 Hz) using pulse durations of 50, 250 and 1000 µs in a single participant. b Mean (n = 5) torque responses evoked by mNMES (1000 µs) using pulse frequencies of 25, 100 and 200 Hz. Error bars represent one standard error. (Adapted from Ref 31)

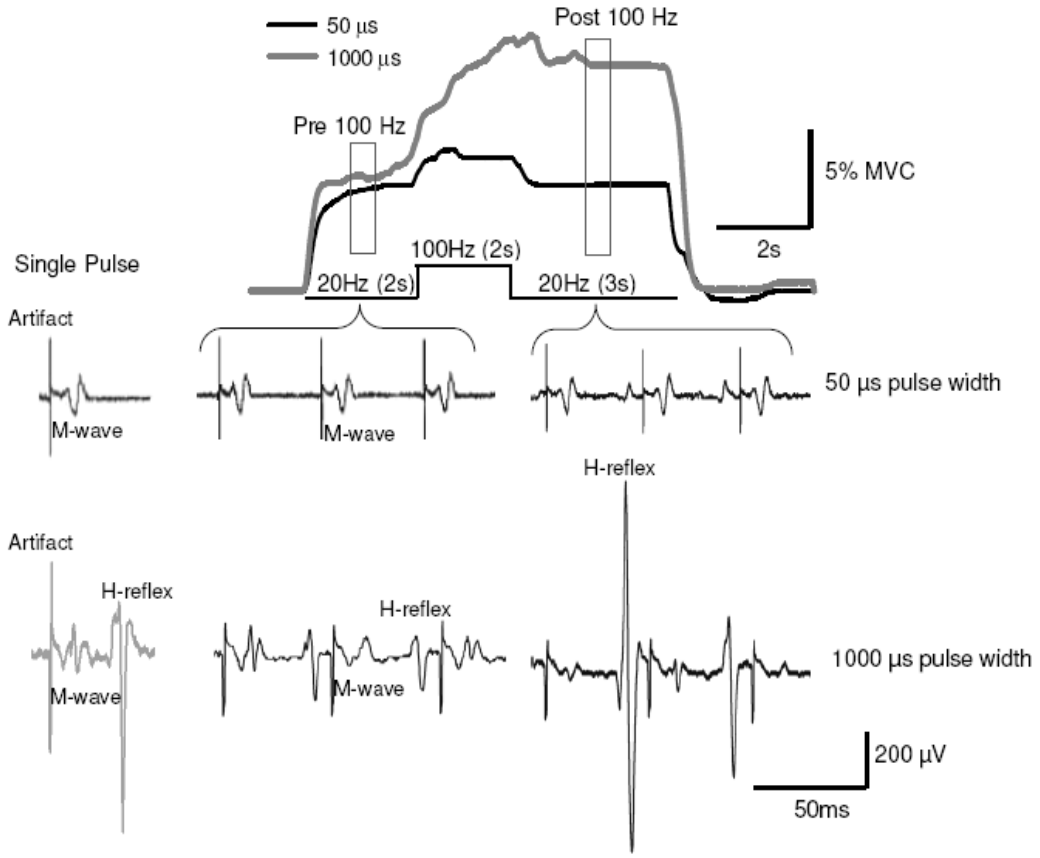

Figure 1-4 Plantar flexion torque and soleus EMG evoked by nNMES delivered at motor threshold in a pattern (20-100-20 Hz for 2-2-3 s, respectively). Vertical rectangles indicate the region from which torque and H-reflex data were sampled. A sample of soleus EMG for each pulse duration is displayed beneath the parentheses. Following NMES at 100 Hz, torque and H-reflexes were enhanced when using a 1000, but not a 50, µs pulse duration. (Adapted from Ref 88)

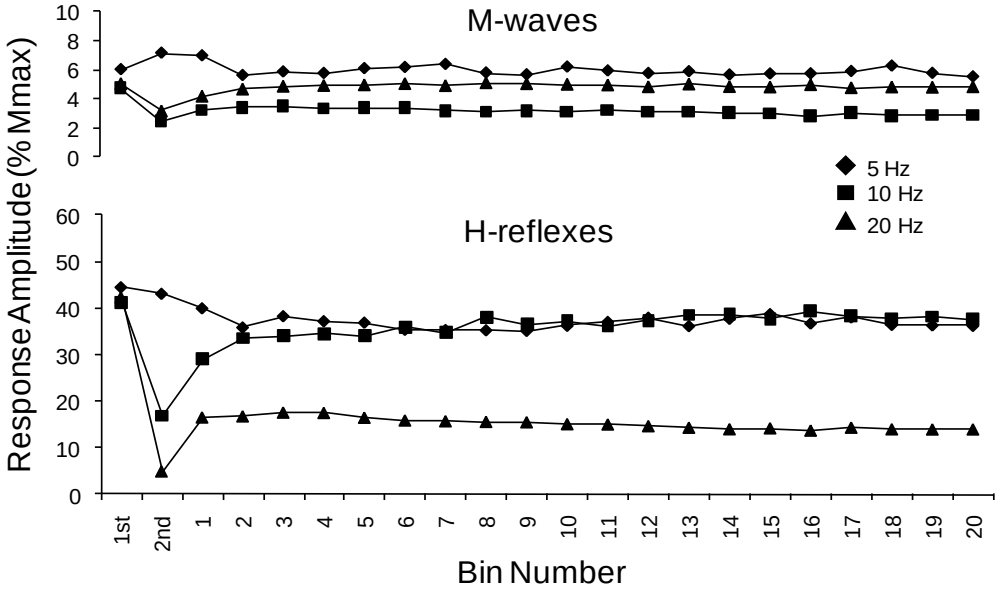

Figure 1-5 The effect of pulse frequency on the *central* contribution to nNMES-evoked contractions of the plantar flexors. Group data (n = 11) depicting recovery of H-reflexes during nNMES to evoke an M-wave of ~5% M_{max}) delivered over the tibial nerve trunk at 5, 10 and 20 Hz while seated participants held plantar flexion contractions of 12 ± 4% MVIC. The first two pulses are an average of three responses from each participant. The subsequent bins represent data averaged over 0.5 s intervals. (Adapted from Ref 28)

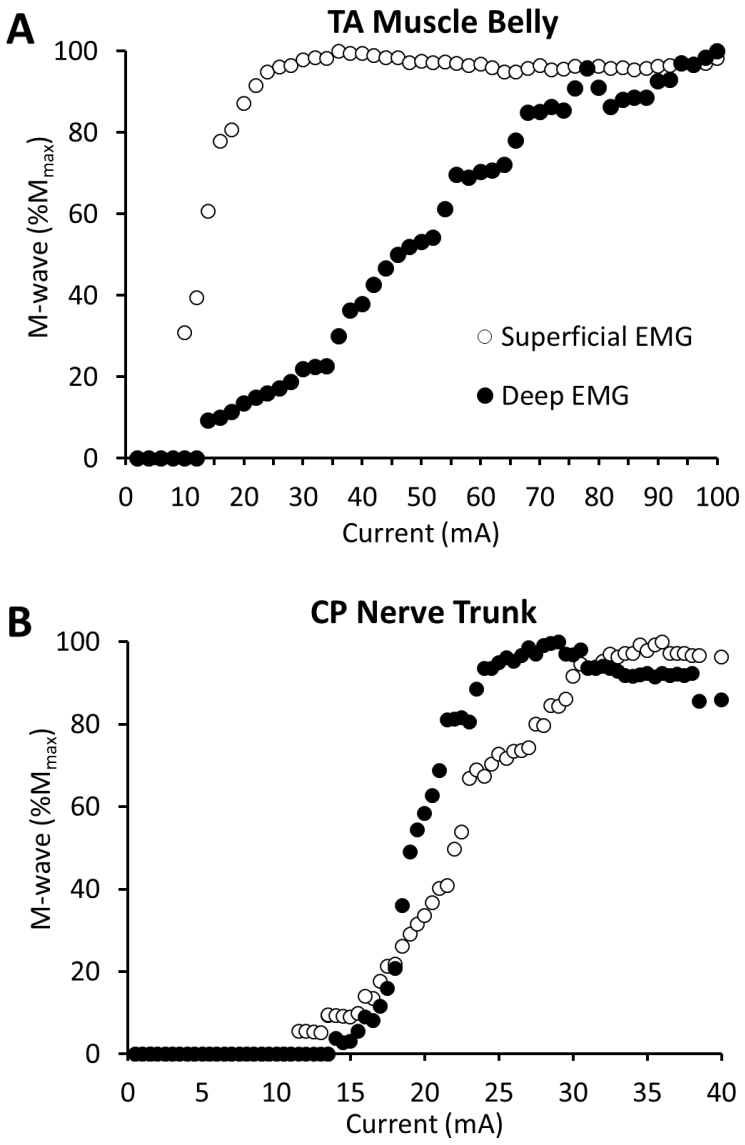

Figure 1-6 M-wave recruitment curves recorded from a superficial and deep region within tibialis anterior using fine wires when mNMES (panel A) or nNMES (panel B) was applied. (Adapted from Ref 103)

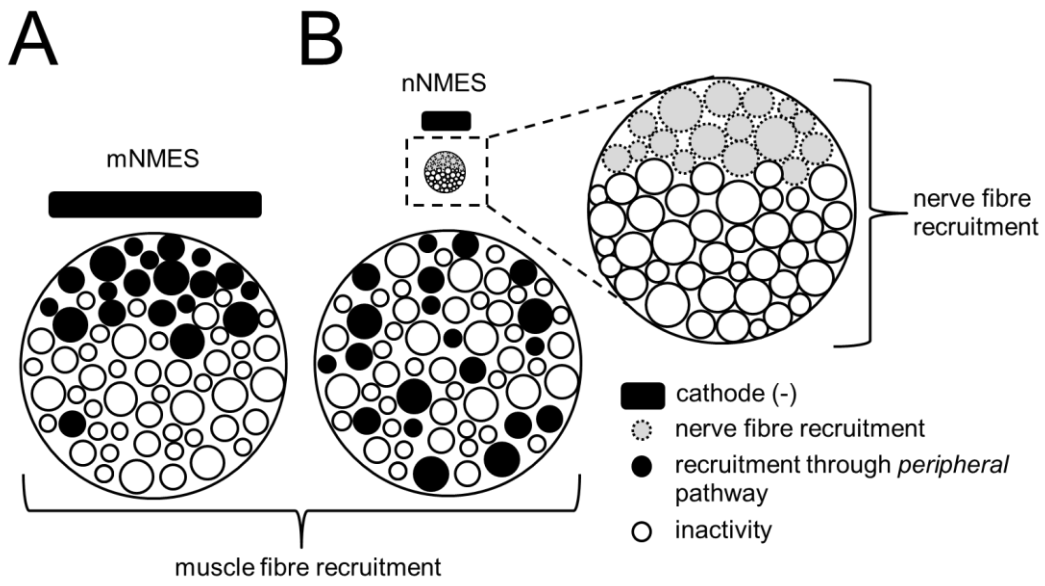

Figure 1-7 Proposed spatial motor unit recruitment through *peripheral* pathways during mNMES (A) and nNMES (B) of a hypothetical hetergeneous muscle. Contributions through *central* pathways have been omitted for clarity.

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CHAPTER 2: MOTOR UNIT RECRUITMENT WHEN NEUROMUSCULAR ELECTRICAL STIMULATION IS APPLIED OVER A NERVE TRUNK COMPARED WITH A MUSCLE BELLY: TRICEPS SURAE[2]

2.1 Introduction

Neuromuscular electrical stimulation (NMES) is commonly used to alleviate muscle atrophy and restore movement following spinal cord injury (SCI) (46). NMES is often applied through electrodes placed on the skin over a *peripheral* nerve trunk or over a muscle belly. For example, NMES over the common peroneal nerve has been used for years to restore dorsiflexion during the swing phase of gait (48) and NMES over the quadriceps muscles is used to produce walking, rowing and cycling movements (6, 29, 38, 49). In the present experiments, we utilised surface electromyographic (EMG) recordings to establish whether different neural pathways contribute to contractions generated when NMES is applied over a *peripheral* nerve trunk (nNMES) compared with NMES applied over a muscle belly (mNMES).

NMES initiates contractions by the excitation of axons under the NMES electrodes (4) and can recruit motor units in 3 distinct ways (16). The most direct form of motor unit recruitment utilises a *peripheral* pathway via the activation of motor axons and does not involve the central nervous system. Depolarising motor axons generates an M-wave in the EMG and recruits motor units synchronously at

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a predictable, *time-locked*, latency following each NMES pulse. Generating contractions through this *peripheral* pathway tends to recruit motor units randomly in relation to motor unit type (13, 24, 50) which may limit the efficacy of NMES for maintaining muscle quality, as fatigue-resistant motor units will be activated less compared with when recruitment is orderly. This relative inactivity leaves fatigue-resistant motor units vulnerable to disuse atrophy. Additionally, the non-physiological recruitment order and synchronous discharge of motor units contributes to the rapid fatigue that is problematic when NMES is used to restore movement (46).

In addition to activating motor axons, NMES also activates sensory axons and this can contribute to the evoked contraction by recruiting motor units in two distinct ways (16). One form of this *central* recruitment is through the H-reflex pathway. Similar to recruitment during the M-wave, motor unit recruitment during the H-reflex is time-locked to each NMES pulse, but occurs at a longer latency due to the longer pathway through the spinal cord (43). The other form of *central* motor unit recruitment results in *asynchronous* motor unit discharge that is not time-locked to each NMES pulse (17, 41). It has been suggested that this asynchronous activity is brought about by the activation of persistent inward currents in spinal neurons (17). Both forms of *central* recruitment produce contractions synaptically and therefore likely follow the *size principle* (28), whereby the lowest threshold and most fatigue-resistant motor units are activated first. Increasing the recruitment of low threshold motor units may help reduce the atrophy and fibre type transitions associated with SCI and subsequent inactivity

(8, 25). Additionally, increasing *central* motor unit recruitment during NMES may improve the fatigue resistance of NMES-evoked contractions (40).

The relative contributions made by *central* and *peripheral* pathways to NMES-evoked contractions may differ for nNMES compared with mNMES (5). During nNMES of the plantar flexors, H-reflexes were prominent in the soleus EMG when contractions were 3-10% of maximum voluntary isometric contraction (MVIC) torque. Conversely, during contractions of similar amplitude evoked by mNMES of the plantar flexors, M-waves dominated the EMG and there was little H-reflex activity. From these data it would seem that nNMES generates contractions with a greater *central* contribution than mNMES. However, these data were recorded in only 4 participants, and no statistical analyses were performed. Despite the apparent lack of an H-reflex during mNMES, a contribution from the central nervous system to contractions evoked by mNMES has been established (12, 17, 18, 40). Torque was significantly reduced when mNMES was applied during an anaesthetic nerve block proximal to the mNMES site, when only activation of motor axons could contribute to the evoked contractions. To reconcile the lack of an H-reflex during mNMES with the clearly demonstrated *central* contribution, we have suggested that asynchronous motor unit activity may provide the majority of the *central* contribution during mNMES (5). To date, a contribution from asynchronous motor unit activity to contractions evoked by NMES has not been quantified.

The present experiments were designed to compare the contributions made by *central* and *peripheral* pathways to motor unit recruitment for plantar flexor

contractions of similar amplitude generated by nNMES compared with mNMES. We studied the triceps surae muscle group because we have data suggesting that motor units are recruited differently between nNMES and mNMES of this muscle group (5). Additionally, there is growing interest in stimulating these muscles for rehabilitation of gait for people who have had a stroke or incomplete SCI (3, 34, 44). Accordingly, we were also interested in characterising motor unit recruitment during larger, more functionally relevant, contractions than have been studied previously (5, 37). Contractions of ~10-40% MVIC torque were examined as this encompasses the range of plantar flexion torque (20-30% MVIC) estimated for walking (2). We anticipated that NMES at both sites would generate contractions through *peripheral* and *central* pathways, but that the relative contributions would differ. Specifically, we hypothesised that contractions evoked by nNMES would have smaller M-waves and larger H-reflexes compared with mNMES. We also hypothesised that mNMES would produce more asynchronous activity than nNMES, given that we have shown mNMES can produce contractions through *central* pathways (11, 17, 18, 40) without the presence of H-reflexes (5). The results of the present experiments contribute to our understanding of how NMES generates contractions and confirms that nNMES and mNMES of the plantar flexors generate contractions with markedly different contributions through *central* and *peripheral* pathways.

2.2 Methods

2.2.1 Participants

Fourteen human participants with no known neurological or musculoskeletal impairments (20 to 48 yrs of age; 10 males and 4 females) volunteered after providing informed, written consent. Four of these participants (2 males and 2 females) did not complete the experiments and their data were not included in the analysis. One of these participants withdrew because they found the NMES uncomfortable before an adequate contraction could be evoked. Two participants were excluded because we could not activate the triceps surae without strong co-activation of the tibialis anterior muscle during nNMES in the popliteal fossa. Another participant was excluded because the latency of their H-reflex was such that accurate peak-to-peak measurements were not possible due to contamination by the subsequent NMES artefacts during 20 Hz NMES. Two of the 10 participants whose data were grouped for the statistical analyses were completely naïve to NMES. These experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta.

2.2.2 Protocol

All participants took part in one experimental session which lasted between 1.5 and 2.5 hours. All procedures were performed on the right leg. Participants were seated in the chair of a Biodex Dynamometer (System 3, Biodex Medical Systems, Shirley, New York) with the hip at 110°, the knee at 120° and the ankle at 90° with the lateral malleolus aligned with the axis of the

dynamometer. The foot was secured to the Biodex footplate to measure isometric plantar flexion torque.

2.2.2.1 Electromyography

Surface EMG was recorded from the right soleus and tibialis anterior muscles using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT) in a bipolar configuration. The electrodes were placed parallel to the predicted path of the muscle fibres with approximately 1 cm inter-electrode distance (Figure 2-1). A common reference electrode (not shown) was placed over the tibia or patella of the right leg. EMG signals were amplified 1000 times and band-pass filtered at 30 to 3,000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

2.2.2.2 Maximum voluntary isometric contractions (MVICs)

Prior to the trials involving NMES, participants performed MVICs of the triceps surae by plantar-flexing the ankle against the footplate to increase torque to a maximum, and held this contraction for 3 to 5 s. Participants were provided with visual feedback of their torque production on a computer monitor and received verbal encouragement to promote maximal performance during each MVIC. Each participant completed 2 to 3 MVICs until peak plantar flexion torque differed by less than 10% between trials. Each MVIC was separated by at least 3 min of rest to minimize fatigue. After collecting MVICs, participants were no longer provided any feedback of their torque production for the remainder of the experiment.

2.2.2.3 Neuromuscular electrical stimulation (NMES)

Plantar flexion contractions were generated by either nNMES or mNMES (Figure 2-1) using a constant-current stimulator and 1 ms rectangular pulses (DS7A Digitimer, Welwyn Garden City, UK). A 1 ms pulse duration was used as long pulse durations generate contractions with a larger *central* contribution than short pulse durations (17, 18, 39). NMES current was measured using a current probe (mA 2000 Non-contact Milliammeter; Bell Technologies, Orlando, Florida). nNMES was delivered through two adhesive gel electrodes (2 x 3 cm; Vermed Medical, Bellows Falls, VT) placed on the skin of the popliteal fossa with an inter-electrode distance of 1 cm. Electrodes were placed on the site at which a single pulse evoked a soleus EMG response (M-wave or H-reflex) at the lowest intensity. mNMES was delivered through 2 flexible adhesive electrodes (4 x 16 cm; Electrosurgical Patient Plate 1180: Split, 3M Health Care, St. Paul, Minnesota) trimmed to fit over the triceps surae muscles of each participant. The anode was placed over the lateral and medial gastrocnemii at the point of approximately the largest circumference. The cathode was placed over the soleus, just distal to the gastrocnemii. If contractions of the peroneus muscles were observed through visual inspection and palpation during NMES at either site, the electrodes were re-positioned medially and/or were cut smaller to more selectively activate the triceps surae muscles.

2.2.2.4 M-wave-H-reflex (M-H) recruitment curve

Separate M-H recruitment curves were constructed for nNMES and mNMES from soleus EMG responses to 50 NMES pulses. Stimuli were delivered

randomly every 3 to 5 s at current levels ranging from below M-wave and H-reflex threshold to 1.5 times the minimum current required to evoke the largest M-wave (M_{max}). To maintain similar levels of motor neuron excitability during collection of the recruitment curve data (14), participants held a background contraction of ~10% of the maximal rectified soleus EMG using visual feedback displayed on a computer monitor. However, the 3 to 5 s inter-stimulus interval may be too short to completely avoid the effects of post-activation depression on H-reflex amplitude, even while holding a background contraction (47) and thus, H_{max}-to-M_{max} ratios in the present study may be slightly underestimated.

2.2.2.5 NMES patterns

NMES was delivered in two patterns as illustrated by the dotted lines in Figure 2-2: 1) a constant frequency pattern of 20 Hz for 8 s and 2) a step frequency pattern of 20-100-20 Hz for 3-2-3 s for each phase, respectively (adapted from Ref 17). The 20 Hz frequency was chosen because it was the highest frequency that allowed for H-reflex analysis between NMES artefacts (50 ms inter-stimulus interval). This frequency is also within a recommended frequency range (18 to 25 Hz) for NMES of the lower limb (46). The step frequency pattern was chosen because it allowed us to examine contractions evoked by NMES at 20 Hz before and after a period of 100 Hz NMES, which has been shown to enhance the *central* contribution to the evoked contractions (18, 37). The constant frequency pattern then also acted as a control, allowing us to determine the effects of the 100 Hz step on torque and motor unit recruitment.

2.2.2.6 NMES intensity

NMES was delivered at two intensities. Low intensity NMES was delivered to evoke a peak torque of ~10% MVIC during the interval 2 to 3 s into the NMES in 10 participants ($Time_1$; see Figure 2-2). The mean current for this low NMES intensity was 7.8 ± 0.9 mA for nNMES and 28.3 ± 1.9 mA for mNMES. Higher intensity NMES was delivered to generate between 20% and 40% MVIC torque at $Time_1$. The mean current for this higher intensity NMES was 8.4 ± 0.8 mA for nNMES and 34.2 ± 2.7 mA for mNMES. For all trials, if the NMES was uncomfortable, the experimental session was concluded. Four participants found the NMES uncomfortable before a contraction of 20% MVIC torque could be achieved. Data from 1 participant who received NMES to evoke a contraction of ~40% MVIC were excluded from the group statistical analyses as there was strong co-activation of the tibialis anterior muscle during nNMES (Figure 2-5). Therefore, data that were grouped for analyses were obtained from 5 participants with higher intensity NMES. Of these 5 participants, 4 received NMES to evoke ~20% MVIC torque and 1 received NMES to evoke ~30% MVIC torque.

2.2.3 Data acquisition & analysis

A single trial of NMES consisted of 5 repetitions of a NMES pattern with 45 s between each repetition. For each NMES site, trials were collected using both patterns at both intensities. The order of trials was randomised for each participant. Throughout the NMES trials, participants were asked to remain relaxed and refrain from contributing voluntarily to the evoked contractions.

Data were sampled at 5 kHz using custom written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analyses that were conducted using custom written Matlab software (The Mathworks, Natick, MA). MVIC torque was calculated by averaging data over a 500 ms window centred on the peak plantar flexion torque recorded during the largest MVIC. Recruitment curves were generated by plotting peak-to-peak M-wave and H-reflex amplitudes as a function of NMES intensity. The single largest H-reflex (H_{max}) and M-wave (M_{max}) from each recruitment curve were used to calculate the H_{max}-to-M_{max} ratio. To determine whether the gastrocnemii were equally well activated during nNMES and mNMES, peak twitch torques from the recruitment curve data evoked by similar sized M-waves were compared. Data were compared between sites for NMES intensities from 60-100% M_{max} when no H-reflex was present during nNMES. Torque during M-H recruitment curves and NMES was normalized to that recorded during each participant's MVIC. The amplitude of each M-wave and H-reflex during 20 Hz NMES was measured peak-to-peak and normalized to each participant's M_{max}. EMG during 100 Hz NMES was not quantified due to contamination by NMES artefacts.

To quantify asynchronous motor unit activity, we calculated the root mean square (RMS) of the EMG immediately before each H-reflex during 20 Hz NMES (see Figure 2-2A, lower left trace). From this value, we subtracted the baseline RMS of the EMG with each participant at rest prior to each NMES trial. The intervals over which asynchronous activity was quantified were determined on an individual basis by the onset latency of the largest H-reflex (H_{max}) recorded during

the recruitment curve for nNMES. An interval duration of 10 to 12 ms was chosen because it was the only period of time when asynchronous activity was not contaminated by the NMES artefact, M-wave or H-reflex. In some instances during mNMES, large M-wave amplitudes prevented the EMG from returning to baseline by the H-reflex onset. To address this and prevent over-estimation of the RMS calculation, all data in the intervals over which asynchronous activity was quantified were fit to a 2^{nd} order polynomial using the least squares procedure to remove any trend in the baseline associated with the preceding M-wave. The 2^{nd} order polynomial was subtracted from the raw data, leaving the de-trended data with a mean of zero. RMS values were normalised to the maximum RMS (RMS_{max}) calculated over a 500 ms period centred on the peak soleus EMG during each participant's MVIC. Pilot work indicated that RMS calculations increased during increasing levels of voluntary plantar flexion contraction, were stable across NMES intensities, were not different between NMES sites and could be measured in every participant across NMES pattern and intensity. However, the asynchronous activity measure did not accurately reflect the voluntary contraction amplitude as a percentage of RMS_{max}. For example, a voluntary contraction of 5, 10 and 15% MVIC torque during the pilot work was measured as 4, 6 and 9% RMS_{max}, respectively, not 5, 10 and 15% RMS_{max} as one might expect. As such, RMS is reported here to provide a relative measure of the asynchronous activity during nNMES and mNMES and between $Time_1$ and $Time_2$.

Twenty M-wave, H-reflex, and asynchronous activity measurements were averaged at each $Time_1$ and $Time_2$ (6 to 7 s into the NMES) during a single

NMES pattern. For each participant, plantar flexion torque, M-waves, H-reflexes and asynchronous activity measured at $Time_1$ and $Time_2$ were averaged separately over the 5 repetitions of a NMES pattern in a single trial. Group means were calculated by pooling these mean data from each participant.

Statistical analyses were performed on group data using Statistica software (StatSoft, Tulsa, OK). Paired *t*-tests were used to test for differences in M_{max}, H_{max}-to-M_{max} ratios and peak twitch torques obtained from the M-H recruitment curves, produced at each NMES site. For data from trials with NMES, separate 3-factor repeated measures analyses of variance (ANOVA) tests were run on each dependent variable (torque, H-reflex, M-wave, and asynchronous activity) at both intensities (low and higher) to determine the influence of *NMES site* (nNMES versus mNMES), *NMES Pattern* (20 Hz constant frequency versus 20-100-20 Hz step frequency) and *Time* ($Time_1$ versus $Time_2$) on the evoked response. Significant main effects and interactions were tested post-hoc using Tukey's honestly significant difference tests when appropriate. An alpha level of $p < 0.05$ was used to evaluate statistical significance. All data are reported as mean ± standard error.

2.3 Results

2.3.1 M-H recruitment curve

There were no significant differences between M_{max} evoked by NMES at both sites ($t_{(9)} = 1.2$, $p = 0.3$). M_{max} was 6.9 ± 0.5 mV for nNMES and 6.4 ± 0.5 mV for mNMES. H_{max}-to-M_{max} ratios were significantly larger ($t_{(9)} = 6.7$, $p <$

0.001) for nNMES (0.6 ± 0.1) compared with mNMES (0.1 ± 0.01). There were no significant differences between peak twitch torques evoked by NMES at both sites ($t_{(9)} = 0.3$, $p = 0.79$) when M-wave amplitudes were not different ($t_{(9)} = 0.5$, $p = 0.61$). Twitch torques were 12.3 ± 1.6% MVIC for nNMES and 12.2 ± 1.8% MVIC for mNMES when M-waves were 80.1 ± 15.2% M_{max} and 79.7 ± 15.3% M_{max}, respectively.

2.3.2 Low intensity NMES

Figure 2-2 shows data recorded from one participant during nNMES (A, B) and mNMES (C, D). In this participant, during nNMES and mNMES, torque was stable during constant frequency NMES, but was augmented after the 100 Hz NMES during the step frequency pattern. During nNMES using the constant frequency pattern, H-reflexes were attenuated after the first response (see arrow; Figure 2-2A) and remained small, but relatively stable, throughout the NMES. When the step frequency pattern was delivered during nNMES (Figure 2-2B), a similar reflex depression was observed initially; however, H-reflexes and asynchronous activity were augmented following the 100 Hz NMES. M-waves were also depressed after the first response, but then remained small and stable for both patterns. During mNMES (Figure 2-2C and 2-2D), M-waves dominated the EMG for both patterns of NMES; however, during the step frequency pattern, M-waves, H-reflexes and asynchronous activity were larger after the 100 Hz NMES.

Figure 2-3 shows group data (n = 10) for all dependent variables (torque, M-waves, H-reflexes and asynchronous activity) during nNMES and mNMES using constant and step frequency patterns. For torque (Figure 2-3A), there was a

significant interaction between *NMES Pattern* and *Time* [$F_{(1, 9)} = 10.2$, $p = 0.01$]. There was no main effect of NMES site [$F_{(1, 9)} = 0.009$, $p = 0.9$], hence there was no difference in the torque generated by nNMES versus mNMES at either $Time_1$ or $Time_2$. As shown in the inset in Figure 2-3A, torque recorded at $Time_2$ was larger than torque at $Time_1$, only during the step pattern. For M-wave amplitude (Figure 2-3B), there was a significant interaction between *NMES site* and *Time* [$F_{(1, 9)} = 5.5$, $p = 0.04$] and there was no significant main effect of *NMES Pattern* [$F_{(1, 9)} = 1.1$, $p = 0.3$]. Thus, although M-wave amplitude was independent of *NMES Pattern*, M-waves were significantly larger (5 to 6 times) during mNMES at both $Time_1$ and $Time_2$ compared with nNMES and were larger at $Time_2$ compared with $Time_1$ during mNMES. H-reflex amplitude (Figure 2-3C) also showed a significant interaction between *NMES site* and *Time* [$F_{(1, 9)} = 6.88$, $p = 0.02$] and no main effect of *NMES Pattern* [$F_{(1, 9)} = 3.4$, $p = 0.1$]. H-reflex amplitude was also independent of *NMES Pattern*, however H-reflexes were larger (2 to 3 times) during nNMES at $Time_1$ and $Time_2$ compared with mNMES. For asynchronous activity (Figure 2-3D), there was a significant interaction between *NMES site* and *Time* [$F_{(1, 9)} = 5.1$, $p = 0.04$] and there was no significant main effect of *NMES Pattern* [$F_{(1, 9)} = 4.6$, $p = 0.09$]. Asynchronous activity during mNMES at $Time_2$ was significantly greater than it was during mNMES at $Time_1$, as well as at both time points during nNMES.

2.3.3 Higher intensity NMES

Figure 2-4 shows data recorded from the same participant as in Figure 2-2 during nNMES (A, B) and mNMES (C, D) at a NMES intensity to evoke ~20%

MVIC torque at $Time_1$. During NMES at both sites, torque remained relatively stable during constant frequency NMES, but was augmented after a period of 100 Hz NMES during the step frequency pattern. During nNMES using the constant frequency pattern, H-reflexes were attenuated compared with the first response and remained depressed throughout the NMES while M-waves were small and stable throughout. During the step frequency pattern, a similar reflex depression was observed during the initial 20 Hz NMES; however, H-reflexes and asynchronous activity were augmented after 100 Hz NMES, whereas M-waves were depressed. During mNMES, M-waves dominated the EMG for both patterns of NMES; however, during the step frequency pattern, M-waves, H-reflexes and asynchronous activity were larger after 100 Hz NMES.

Figure 2-5 shows data recorded from a single participant during nNMES (A, B) and mNMES (C, D) at a NMES intensity that evoked ~40% MVIC torque at $Time_1$. During NMES at both sites, torque remained stable during constant frequency NMES. Torque was also not augmented following 100 Hz NMES at either site. Interestingly, during the 100 Hz period of nNMES, torque decreased due to the activation of the common peroneal nerve in this participant, as indicated by tibialis anterior EMG activity (not shown). As such, these data were not included in the statistical analysis of group data. During nNMES using the constant frequency pattern, H-reflexes were attenuated after the first response, but recovered to an amplitude equal to the first response by the end of the NMES. During the step frequency pattern, similar reflex depression and recovery were observed during the initial 20 Hz NMES, and H-reflexes were large, but variable,

following 100 Hz NMES. Regardless of the *NMES Pattern*, M-waves were initially large, but decreased in size over the first 1 s of NMES and remained small and stable throughout the remaining NMES. Asynchronous activity was small and stable throughout and was unaffected by the 100 Hz NMES. During mNMES, only M-waves were evident in the EMG for both patterns of NMES.

Figure 2-6 shows group (n = 5) torque and EMG data for the higher intensity NMES trials. For torque amplitude (Figure 2-6A), there was a significant main effect of *Time* [$F_{(1, 4)} = 18.5$, $p = 0.01$] and no significant main effect of *NMES site* [$F_{(1, 4)} = 0.3$, $p = 0.63$] or *NMES Pattern* [$F_{(1, 4)} = 3.4$, $p = 0.14$]. Torque was significantly larger at $Time_2$ compared with $Time_1$, regardless of the NMES site or pattern. For M-wave amplitude (Figure 2-6B), there was a significant interaction between *NMES site* and *Time* [$F_{(1, 4)} = 26.3$, $p < 0.01$] and no significant main effect of *NMES Pattern* [$F_{(1, 4)} = 0.04$, $p = 0.8$]. M-waves were larger (5 to 6 times) for mNMES at both time points compared with nNMES and were larger at $Time_2$ compared with $Time_1$ during mNMES. For H-reflex amplitude (Figure 2-6C), there was a significant 2-way interaction between *NMES site* and *Time* [$F_{(1, 4)} = 10.9$, $p = 0.03$] and no significant main effect of *NMES Pattern* [$F_{(1, 4)} = 4.7$, $p = 0.1$]. H-reflexes were larger (2 to 3 times) during nNMES at $Time_1$ and $Time_2$ compared with mNMES at $Time_1$ and $Time_2$, respectively. Furthermore, following a period of 100 Hz NMES, H-reflexes were larger at $Time_2$ compared with $Time_1$ only during nNMES. For asynchronous activity (Figure 2-6D), there was a significant main effect of *NMES site* [$F_{(1, 4)} = 12.9$, $p = 0.02$] and *Time* [$F_{(1, 4)} = 12.6$, $p = 0.02$] and no significant main effect of *NMES*

Pattern [$F_{(1, 4)} = 5.0$, $p = 0.09$]. Asynchronous activity during NMES at both sites increased over time regardless of NMES site or pattern. Furthermore, asynchronous activity was larger for mNMES compared with nNMES, regardless of the NMES pattern or time.

2.4 Discussion

In this study we compared the contributions made by *central* and *peripheral* pathways to motor unit recruitment for contractions of similar amplitude generated by NMES applied over the tibial nerve (nNMES) and the triceps surae muscles (mNMES). As we anticipated, NMES at both sites recruited motor units through *peripheral* and *central* pathways, but the contributions made by these pathways for the two sites of NMES differed markedly. Specifically, during nNMES, contractions were generated primarily through *central* pathways (H-reflexes), while mNMES generated contractions primarily through *peripheral* pathways (M-waves). For NMES at both sites, the *central* contribution increased over time and could be augmented following a brief period of NMES at 100 Hz.

2.4.1 Torque

Torque was not significantly different during nNMES compared with mNMES for both NMES patterns and intensities. During low intensity constant frequency NMES, torque did not change from the beginning ($Time_1$) to the end ($Time_2$) of the NMES. The *extra torque* we did observe after brief periods of 100 Hz NMES during low intensity NMES, and over time during the high intensity

NMES has been attributed to multiple *central* mechanisms (see Section 2.4.3 below).

2.4.2 Pathways during nNMES versus mNMES

Although torque did not differ between NMES sites, different neural pathways contributed to contractions generated by mNMES and nNMES. Consistent with our first hypothesis and previous work in our laboratory (5), contractions evoked by nNMES of the plantar flexors had significantly smaller M-waves and significantly larger H-reflexes compared with mNMES. M-waves were 5 to 6 times larger during mNMES compared with nNMES. H-reflexes were evident in the EMG during NMES at both sites, but were 2 to 3 times larger during nNMES compared with mNMES. In line with our second hypothesis, mNMES produced more asynchronous activity than nNMES, regardless of the NMES pattern. Asynchronous activity was low at the beginning and increased over several seconds for NMES at both sites. Together, these results support previous assertions that mNMES can produce contractions with a significant *central* contribution (5, 17, 18, 40) and shows that this contribution is in the form of H-reflexes and asynchronous activity. The contribution of asynchronous activity to the evoked torque, however, may be less than that of the H-reflex. The *extra torque* generated by nNMES was accompanied by enhanced H-reflexes whereas equal levels of *extra torque* generated by mNMES were generated by enhanced asynchronous activity *and* enhanced M-waves. Thus, a portion of the *extra torque* during mNMES originated from a *peripheral* mechanism. In general, nNMES generated contractions with a greater contribution through *central*

pathways, while mNMES generated contractions with a greater *peripheral* contribution.

When NMES intensity was increased to produce contraction amplitudes of ~20 to 30% MVIC torque, H-reflexes and asynchronous activity were present during constant frequency NMES at both sites. During the step frequency pattern, H-reflex amplitudes increased after NMES at 100 Hz and reached ~24% M_{max} during nNMES and 5% M_{max} during mNMES. Although H-reflexes are initially depressed during repetitive NMES due to post-activation depression of neurotransmitter release from Ia afferents (31), we have previously reported large H-reflexes during nNMES of the plantar flexors (5, 37). In the present study, even at the higher NMES intensity, when anti-dromic transmission in motor axons (23) would be more pronounced, H-reflexes were present during NMES at both sites. In the participant who received NMES to generate ~40% MVIC torque (see Figure 2-5), H-reflexes were present only during nNMES, while only M-waves were evident in the EMG during mNMES; although, these data were not included in the group due to co-activation of tibialis anterior. Thus, at this highest NMES intensity studied, a *central* contribution was only present during nNMES, but further study at these higher intensities is required to substantiate this finding.

The significantly greater H_{max}-to-M_{max} ratio and predominance of H-reflexes during nNMES compared with mNMES is likely explained in part by the neuronal architecture beneath the NMES electrodes. nNMES, where sensory and motor axons are bundled close together beneath the NMES electrodes, likely recruited a relatively greater proportion of sensory axons than mNMES delivered

near the triceps surae motor points. At the level of the triceps surae muscles, axons of the tibial nerve branch diffusely (36). This branching, in combination with the increased inter-electrode distance and use of larger electrodes during mNMES, may have activated axons over a broader spatial distribution resulting in a less synchronous afferent volley arriving at the motor neuron during mNMES compared with nNMES. Thus, during mNMES the sensory volleys may not depolarise motor neurons synchronously and generate an H-reflex, rather, they may be more temporally dispersed and contribute to enhanced asynchronous activity. This effect of NMES site would be less for the M-wave, as the pathway to the muscle is shorter and circumvents *central* synapses compared with the pathway for the H-reflex.

During mNMES, M-waves were significantly enhanced over time during low and high intensity NMES. Some change in the amplitude of the M-wave can be expected due to changes in muscle architecture beneath the recording electrodes (20), but M-wave amplitude did not change overtime during nNMES. Since the recording site and contraction amplitudes were not different between NMES sites, a change in muscle architecture beneath the recording electrode does not explain the larger M-waves evoked during mNMES. However, muscle conformational changes beneath the stimulating electrodes may explain larger M-waves during mNMES. In isometric muscle contractions, the muscle fibres shorten and develop tension as the tendon stretches (26). This shortening would alter the position of muscle fibres beneath the NMES electrodes in such a way that more axons and possibly more motor points converge beneath the NMES

electrodes, resulting in greater numbers of activated axons, further enhancing the muscle contraction. Support for this rationale lies in the slow rise of M-wave amplitude in concert with the slow rise in torque during the first second of NMES when the muscle is shortening during mNMES.

2.4.3 *Central* mechanisms

Several *central* mechanisms may account for the enhanced H-reflexes and asynchronous activity that develop over time during NMES. Such mechanisms include: inadvertent or voluntary descending drive, post-tetanic potentiation at the Ia synapse, and activation of persistent inward currents in spinal neurons. Inadvertent voluntary activation of motor neurons could account for the increase in H-reflex amplitude (51) and asynchronous activity; however evidence suggests this is not what occurred. Similar levels of *extra torque* generated through *central* pathways, as occurred during the low intensity NMES in this study, can develop in people who are sleeping (18) or who have complete SCI (45). Furthermore, participants in this study did not find the NMES uncomfortable, and remained relaxed throughout the NMES and did not voluntarily contract the muscles of the ankle. Post-tetanic potentiation may also add to the enhanced *central* motor unit recruitment observed. Following repetitive NMES of Ia afferents, post-tetanic potentiation at the Ia synapse enhances excitatory post-synaptic potentials (27, 30). The development of persistent inward currents in spinal neurons have also been suggested as a mechanism underlying enhanced *central* motor unit recruitment (5, 17, 18, 37). Persistent inward currents have been demonstrated directly in spinal neurons in animals initiated by high frequency synaptic drive (7)

and indirectly in humans during periods of electrical NMES (17, 18) or vibration (21, 35).

2.4.4 Implications for NMES

NMES is used to generate contractions for maintaining muscle quality (therapeutic electrical stimulation; TES) and producing functional movements (functional electrical stimulation; FES) following SCI (22, 32, 33, 46). However, the non-physiological recruitment order of motor units during NMES limits the activation of low threshold motor units during TES and that, combined with synchronous motor unit activation, contributes to accelerated muscle fatigue during FES (46). The random recruitment order and synchronous discharge associated with recruitment through *peripheral* pathways (M-waves) is in sharp contrast to the asynchronous and orderly motor unit recruitment that occurs during a voluntary contraction. The synchronous discharge of motor units during NMES means that non-physiologically high firing rates are required to produce smooth contractions and these high firing rates increase the energy demand from each active motor unit, resulting in premature fatigue (1). Additionally, the random recruitment order enhances the susceptibility of low threshold motor units to disuse atrophy and fibre type transitions, leaving the muscle with a smaller proportion of fatigue-resistant motor units (46). The limited recruitment of low threshold motor units could be overcome by increasing the NMES intensity to depolarise all of the motor axons, but the disadvantage of synchronous motor unit recruitment would remain and such high intensities can be problematic for individuals with residual sensation (15) or compromised bone density (19). For

this reason, developing methods that recruit low threshold motor units at relatively low NMES intensities may have advantages for both TES and FES. Enhancing the extent to which NMES activates sensory axons and contributes to the evoked contractions through a *central* pathway in the form of H-reflexes or asynchronous activity may be one such method.

The data from the present experiments confirm previous indications that the contribution made by *central* and *peripheral* pathways to NMES-evoked contractions differ between nNMES compared with mNMES (5). Contractions produced by nNMES generated a larger *central* contribution (H-reflexes). mNMES evoked contractions with a greater contribution from direct motor axon activation (M-waves). Thus, nNMES may hold greater promise for maintaining muscle quality following SCI, as well as in the prevention of muscle fatigue during FES. Although, there may be issues around control for FES using nNMES as contractions evoked by nNMES of the plantar flexors have been shown to be less stable within a single contraction and less consistent between successive contractions compared with mNMES (5). The potential to reflexively activate a sufficiently large proportion of motor units to be useful for TES and FES may require a muscle with particularly strong reflex inputs, such as the triceps surae muscles. Whether recruitment during nNMES and mNMES differs for other muscle groups has not yet been tested. However, a *central* contribution to NMES-evoked contractions has been demonstrated for the triceps surae (5, 18, 37), tibialis anterior (37), quadriceps (Bergquist et al. *unpublished observation*) wrist extensors (5), biceps brachii (10, 42) and flexor pollicis longus (9).

Additionally, as NMES intensity is increased beyond what was tested in this study, for example, in response to fatigue during FES exercise, increased levels of antidromic collision will develop (51). This will progressively block H-reflex and asynchronous contributions to evoked contractions. Although, it has been estimated that 20-30% MVIC plantar flexion torque is required for walking (2), and the present results indicate that a *central* contribution to evoked contractions occurs over this range during NMES over the tibial nerve and, to a lesser extent, the triceps surae muscles. However, considerably greater levels of plantar flexion torque, as a percent of MVIC, may be required for walking in individuals with severely atrophied muscle and whether this can be achieved through *central* recruitment remains to be determined.

2.4.5 Summary

The contributions made by *central* and *peripheral* pathways to motor unit recruitment during NMES differed markedly for plantar flexion contractions of equal amplitude generated by nNMES compared mNMES of the plantar flexors. During nNMES, contractions were generated primarily through a *central* pathway while mNMES generated contractions predominately through a *peripheral* pathway. Thus, nNMES may be more advantageous for maintaining muscle quality and reducing muscle fatigue for rehabilitation compared with mNMES of the plantar flexors.

2.5 Figures

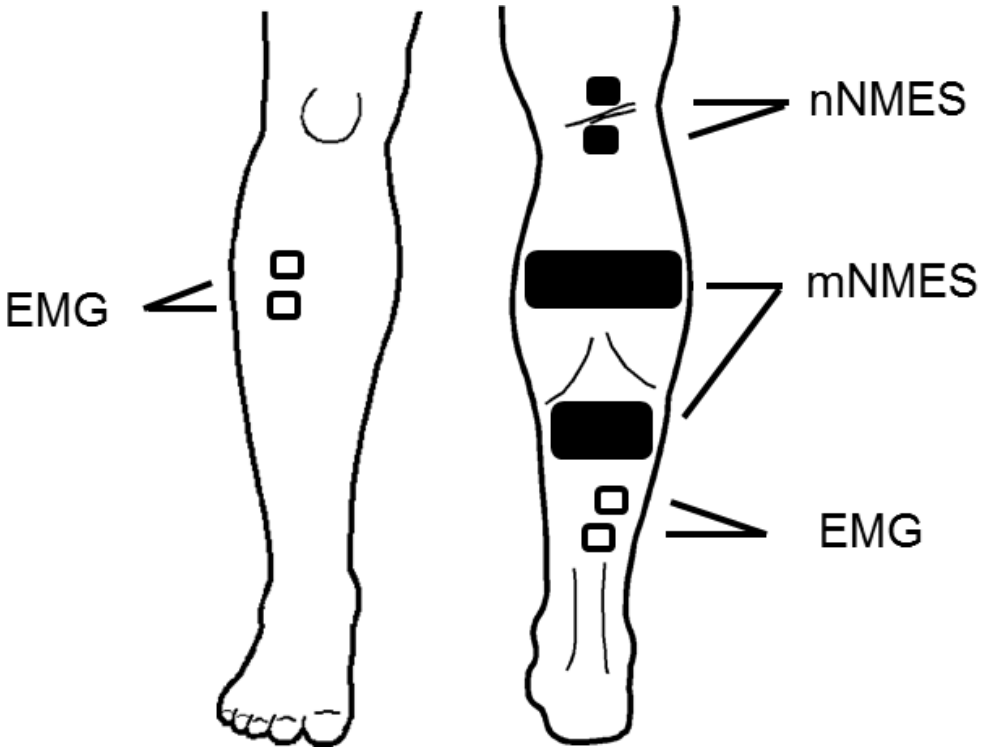

Figure 2-1 Schematic of the NMES and EMG sites.

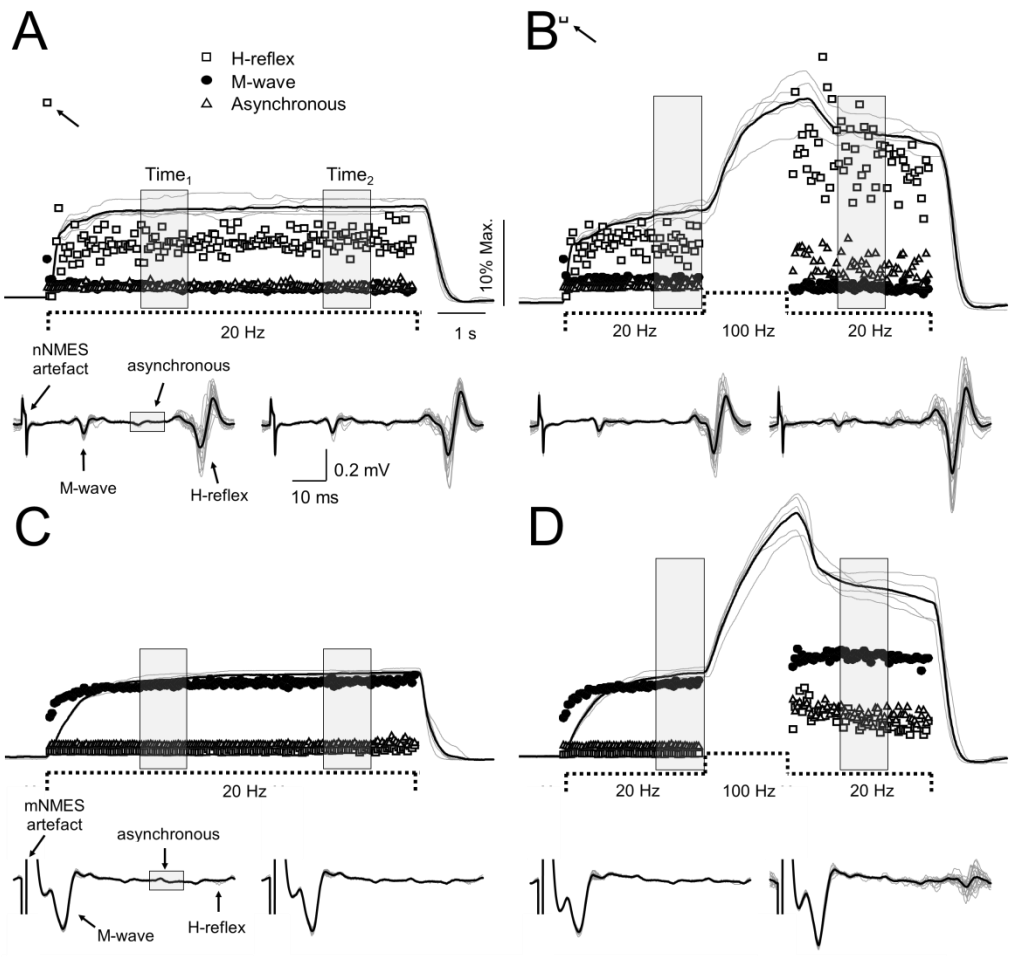

Figure 2-2 Torque and EMG responses evoked by nNMES (A and B) and mNMES (C and D) to evoke ~10% MVIC torque at $Time_1$ in a single participant. Responses to the 20 Hz constant frequency pattern are displayed in panels A and C while responses to the 20-100-20 Hz pattern are displayed in panels B and D. In the upper half of each panel, torque profiles represented by the bold black lines are averages of 5 grey lines in response to 5 trains of NMES and the symbols represent the average EMG data over 5 repetitions during a single trial. Vertical calibration represents 10% M_{max} for EMG and 10% MVIC for torque. The lower half of each panel shows EMG recorded at $Time_1$ (left trace) and $Time_2$ (right trace) during a single train of NMES. Bold black lines represent the average of 20 single responses (grey lines) to NMES. NMES artefacts for data recorded during mNMES have been truncated (C and D). All data are shown on the same scale, as indicated by the calibration bars in panel A. EMG during 100 Hz NMES was not quantified due to contamination by NMES artefacts.

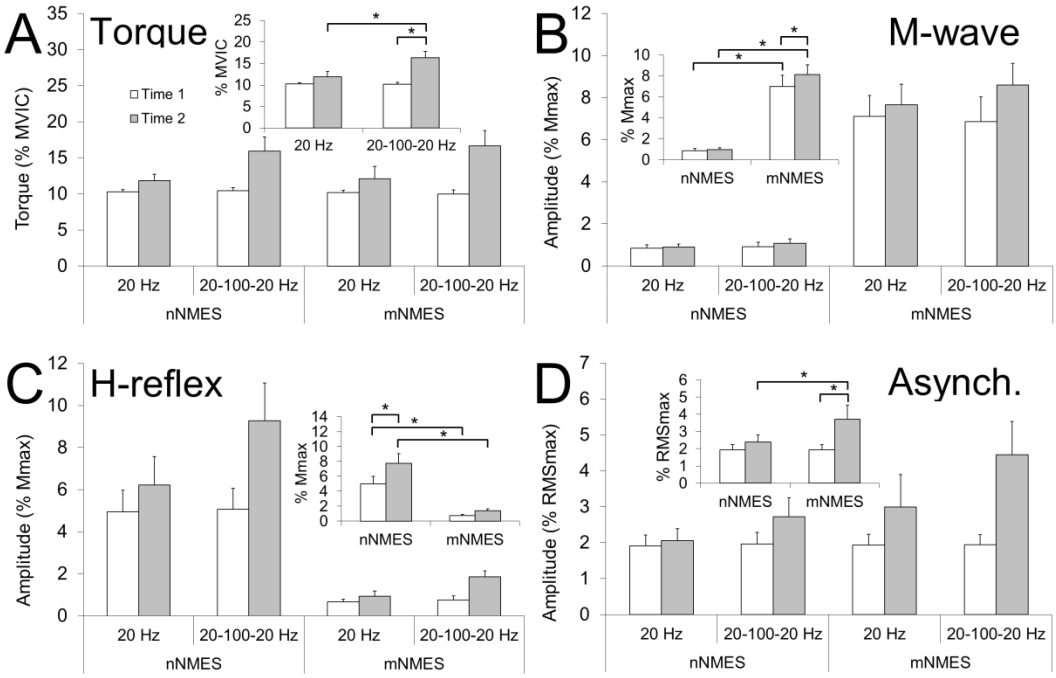

Figure 2-3 Normalised group data (n = 10) averaged at two time points ($Time_1$ and $Time_2$) during nNMES and mNMES at an intensity to evoke ~10% MVIC torque at $Time_1$. Significant 2-way interactions identified by statistical analyses are displayed within the insets. Asterisks indicate a significant difference at a level $p < 0.05$.

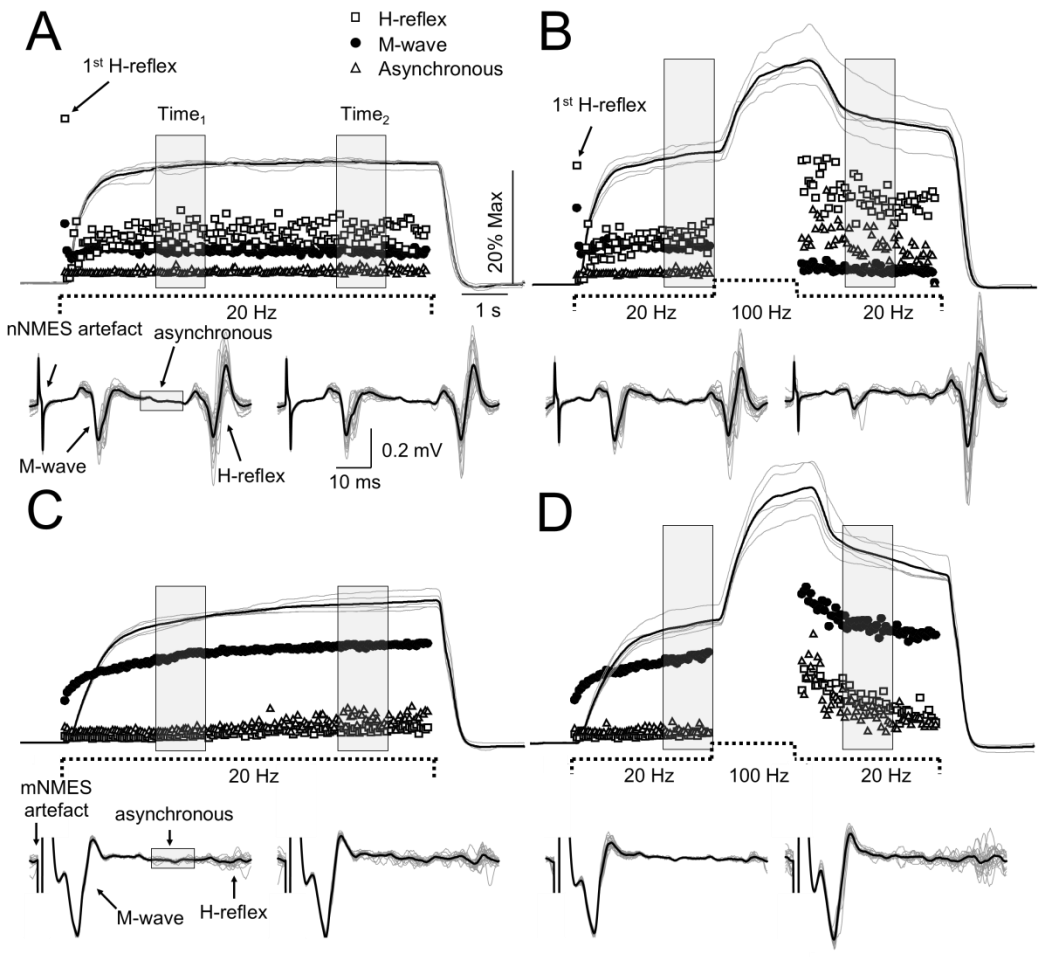

Figure 2-4 Torque and EMG responses evoked by nNMES (A and B) and mNMES (C and D) to evoke ~20% MVIC torque at $Time_1$ in a single participant. Organisation is equivalent to Figure 2-2. All data are shown on the same scale, as indicated by the calibration bars in panel A.

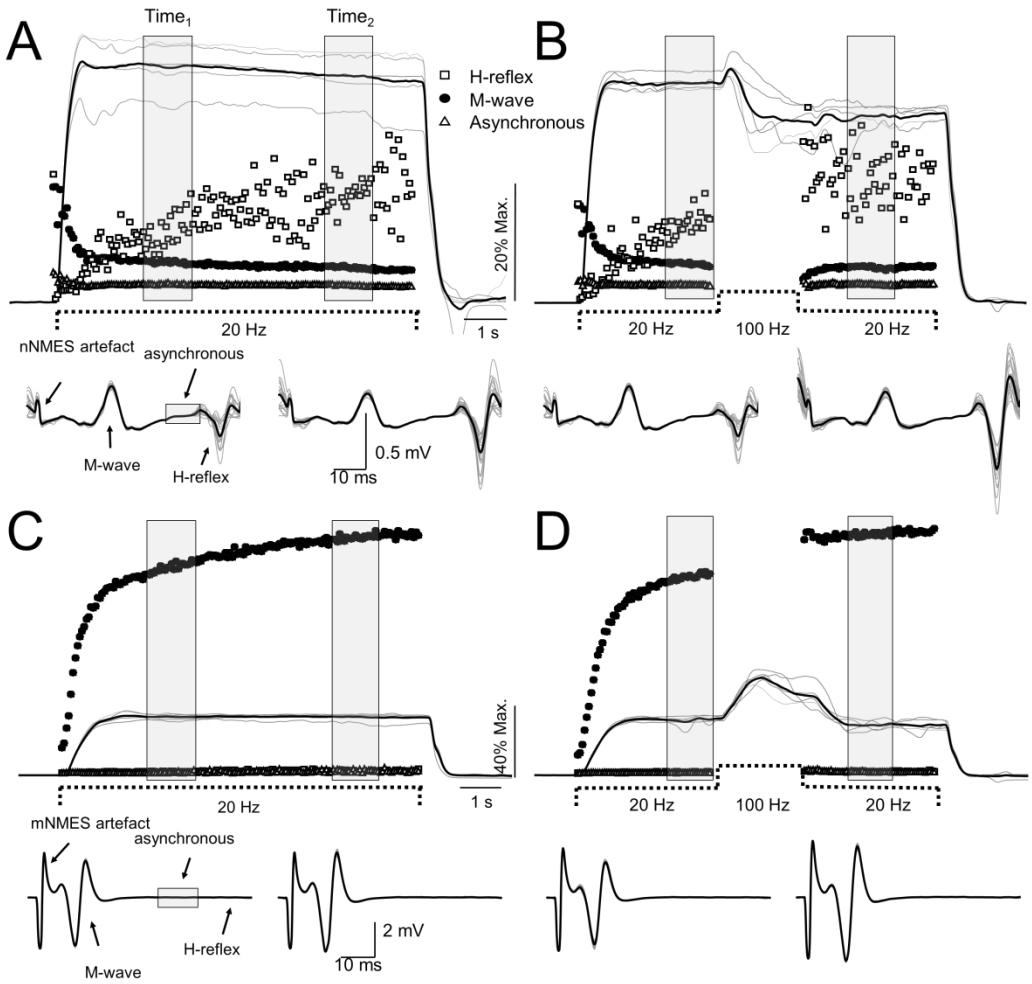

Figure 2-5 Torque and EMG responses evoked by nNMES (A and B) and mNMES (C and D) to evoke ~40% MVIC torque at $Time_1$ in a single participant. Organisation is equivalent to Figure 2-2 and 2-4. Panels A and B are shown on the same scale, as indicated by the calibration bars in panel A. Panels C and D are shown on the same scale, as indicated by the calibration bars in panel C.

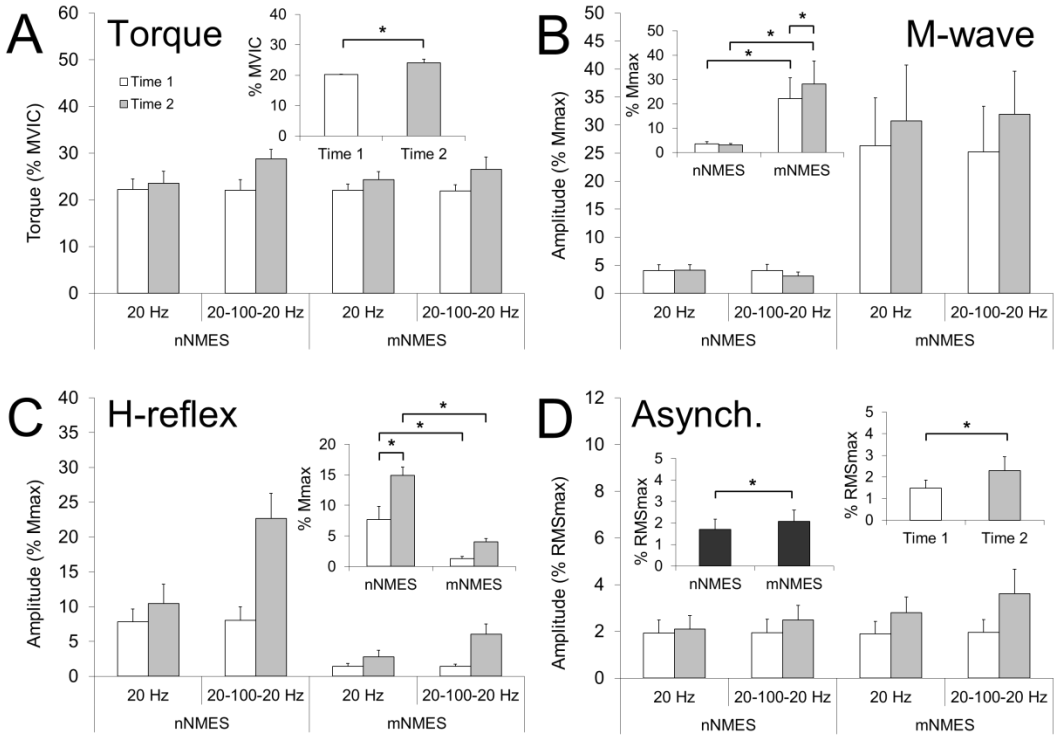

Figure 2-6 Normalised group data (n = 5) averaged at two time points ($Time_1$ and $Time_2$) during nNMES and mNMES at an intensity to evoke between 20% and 30% MVIC torque at $Time_1$. Significant main effects and 2-way interactions identified by statistical analyses are displayed within the insets. Asterisks indicate a significant difference at a level $p < 0.05$.

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CHAPTER 3: MOTOR UNIT RECRUITMENT WHEN NEUROMUSCULAR ELECTRICAL NMES IS APPLIED OVER A NERVE TRUNK COMPARED WITH A MUSCLE BELLY: QUADRICEPS FEMORIS[3]

3.1 Introduction

Neuromuscular electrical NMES (NMES) can be delivered using electrodes placed on the skin over either a nerve trunk (nNMES) or a muscle belly (mNMES). We have recently shown that when NMES is used to generate isometric plantar flexion contractions of the ankle, nNMES generated contractions through markedly different pathways than mNMES (4). During nNMES, contractions were generated primarily by the synaptic recruitment of motor neurons in the spinal cord (*central* pathways), while mNMES generated contractions predominantly through the activation of motor axons beneath the NMES electrodes (*peripheral* pathways). However, the ankle plantar flexors are not the most commonly stimulated muscles during NMES rehabilitation programs, and whether or not this effect of NMES site can be generalised to muscles more commonly used for NMES has not been tested. Thus, in the present experiments, we extend this line of investigation to the quadriceps femoris. The quadriceps muscle is the most often stimulated muscle for NMES rehabilitation (3) to reduce atrophy (3, 21, 23, 25), improve cardiovascular function (19, 30), mobility (46, 52) and glucose utilisation (32, 42) following damage to the central

[3] A version of this chapter has been published.
Bergquist AJ, Wiest MJ & Collins DF. *Journal of Applied Physiology*, 113(1):78-89, 2012.

nervous system. Whether transmission along *central* pathways contributes to NMES-evoked contractions of the quadriceps muscle is not known; in the present study we compare the extent to which transmission along *central* and *peripheral* pathways contributes to knee extension contractions evoked by nNMES (femoral nerve trunk) versus mNMES (quadriceps muscle belly).

Generating contractions through *peripheral* pathways by the depolarisation of motor axons beneath the NMES electrodes may limit the efficacy of NMES for maintaining muscle quality and for producing functional movement. The discharge of motor units recruited in this way is synchronous, time locked to each NMES pulse as represented by successive M-waves in the electromyographic (EMG) signal. The recruitment of motor units through *peripheral* pathways does not follow the *size principle* (12, 26, 53), and as a result, motor unit recruitment through this pathway during NMES leaves fatigue-resistant muscle fibres less active and consequently more vulnerable to disuse atrophy compared to contractions generated through synaptic recruitment (*central* pathways). Additionally, the non-physiological recruitment order and synchronous discharge of motor units contributes to the rapid fatigue associated with NMES-evoked contractions (50). In contrast, activating muscle through *central* pathways by depolarising sensory axons and recruiting motor units synaptically follows the *size principle* (28, 29), and motor unit discharge is either time-locked to each NMES pulse as an H-reflex or is temporally unrelated to the NMES and appears as *asynchronous activity* in the EMG signal (2, 4, 16, 17, 35, 38). Increasing the recruitment of fatigue-resistant muscle fibres by increasing

activity through *central* pathways during NMES may help reduce the atrophy and fibre type transitions associated with prolonged inactivity imposed by damage to the central nervous system.

The present experiments were designed to compare the contributions made by *peripheral* (M-wave) and *central* (H-reflex and asynchronous activity) pathways to motor unit recruitment for isometric knee extension contractions of the quadriceps muscle. Based on our experiments conducted on the triceps surae muscles (4), we hypothesised that: 1) contractions evoked by nNMES would have smaller M-waves, larger H-reflexes and less asynchronous activity compared to contractions of equal amplitude evoked by mNMES; 2) both sites of NMES would generate equivalent increases in torque following a brief period of high-frequency NMES (100 Hz), delivered during NMES at a lower frequency (15 or 25 Hz), which would be accompanied by enhanced H-reflexes during nNMES and enhanced asynchronous activity during mNMES. The results of the present study contribute to our understanding of how NMES generates contractions in the muscle most commonly used for NMES rehabilitation programs. We show for the first time that contractions of the quadriceps muscle can be generated through *central* pathways and that the effect of NMES site on the balance between motor unit recruitment through *peripheral* and *central* pathways is not unique to the triceps surae muscle, but that NMES site also affects motor unit recruitment of the quadriceps.

3.2 Methods

3.2.1 Participants

Thirteen human participants with no known neurological or musculoskeletal impairments volunteered for this study after providing informed written consent. Eleven participants [8 males and 3 females; age between 21 and 48 yr; 29.3 ± 8.33 (SD) yr] volunteered for the initial experiments (see Section 3.2.2 below). Each initial experiment lasted ~2.5 h. Seven participants [4 males and 3 females; age range: 25 to 48 yr; 32.0 ± 8.05 (SD) yr] volunteered for the additional experiments (see Section 3.2.3 below), 5 of whom had participated in the initial experiments. Each additional experiment lasted ~1 h. All experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta.

3.2.2 Initial experiments

All procedures were performed on the right thigh. To measure isometric knee extension torque, participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York) with the hip at 120° and the knee at 90°. The axis of the dynamometer was aligned with the axis of rotation of the participant's knee joint. The arm of the dynamometer was parallel to the anterior aspect of the tibia, with the lower edge of the pad positioned ~3 cm proximal to the lateral malleolus. The trunk, waist and thigh were stabilised using straps on the Biodex dynamometer chair.

3.2.2.1 Electromyography

Surface electromyography (EMG) was recorded from the vastus lateralis and vastus medialis using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT) in a bipolar configuration (Figure 3-1A). The electrodes were placed parallel to the predicted path of the muscle fibres with ~1 cm inter-electrode distance. For vastus lateralis, the distal electrode was positioned 8 to 12 cm from the patella while for vastus medialis the distal electrode was placed between 2 and 3 cm from the lateral boarder of the patella. A common reference electrode was placed over the patella. EMG signals were amplified 500 times and band-pass filtered at 10 to 1,000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

3.2.2.2 Maximum voluntary isometric contractions

Prior to trials involving NMES, participants performed maximum voluntary isometric contractions (MVICs) of the quadriceps, extending against the arm of the dynamometer for 3 to 5 s as forcefully as possible. Participants were provided with visual feedback of their torque production on a computer monitor and received verbal encouragement to promote maximal performance during each MVIC. Each participant completed 2 to 3 MVICs until peak isometric knee extension torque differed by less than 10% between trials. Each MVIC was separated by at least 3 min of rest to minimise fatigue.

3.2.2.3 Neuromuscular electrical stimulation (NMES)

NMES was delivered either over the femoral nerve trunk (nNMES) or over the quadriceps muscle belly (mNMES; Figure 3-1A) using 1 ms square-wave

pulses from a single channel constant-current stimulator (DS7A Digitimer, Welwyn Garden City, UK). NMES current was measured using a current probe (mA 2000 Noncontact Milliammeter; Bell Technologies, Orlando, Florida). nNMES was delivered through 2 adhesive gel electrodes in a monopolar arrangement. The anode (7.5 x 13 cm; model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) was positioned on the skin at the gluteal fold. The cathode (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) was placed on the skin of the femoral triangle at a position where a single pulse evoked a response (M-wave or H-reflex) in vastus lateralis at the lowest NMES intensity. mNMES was delivered in a bipolar configuration. The output of the single channel stimulator was divided between 2 pairs of flexible adhesive electrodes (7.5 x 13 cm; model CF7515, Axelgaard Manufacturing, Lystrup, Denmark). This configuration was found to maximise the activation of the quadriceps and reduce NMES discomfort in pilot experiments. The anodes were placed proximally over the muscle belly, while the cathodes were placed over the motor point of vastus lateralis and vastus medialis. Motor points were identified by the site on the surface of the skin in which an electrical pulse evoked a visible muscle twitch with the least current. If contractions of the adductors were observed, through visual inspection and palpation during NMES, the electrodes were re-positioned laterally and/or were cut smaller to more selectively activate the quadriceps muscle.

3.2.2.4 M-wave-H-reflex (M-H) recruitment curve

Separate M-H recruitment curves were constructed for nNMES and mNMES from responses to 50 NMES pulses delivered randomly every 8 to 10 s. For nNMES, current was delivered from below M-wave and H-reflex threshold to 1.2 times the minimum current required to evoke a maximal M-wave (M_{max}) in vastus lateralis. This intensity was also sufficient to evoke M_{max} in vastus medialis for all participants. To maintain similar levels of motor pool excitability during collection of the recruitment curve data (12), participants held a background contraction to produce ~5% MVIC torque using visual feedback displayed on a computer monitor. After collecting the data for the M-H recruitment curves, participants did not receive feedback of their torque production for the remainder of the experiment.

3.2.2.5 NMES patterns

In initial experiments, NMES was delivered in 2 patterns: 1) a constant frequency pattern of 15 Hz for 8 s and 2) a step frequency pattern of 15-100-15 Hz for 3-2-3 s for each phase, respectively (adapted from Refs 16 and 17). The 15 Hz frequency was chosen because in pilot experiments it was determined that 15 Hz was the highest frequency that allowed for quantifying asynchronous activity (see Section 3.2.4 below) and is just below a recommended frequency range (18 to 25 Hz) for NMES of the lower limb (50). The step frequency pattern was chosen because it allowed us to examine contractions evoked by NMES at 15 Hz before and after a period of 100 Hz NMES, which has been shown to enhance the *central* motor unit recruitment during NMES-evoked contractions (4, 35). The

constant frequency pattern then also acted as a control, allowing us to determine the effects of the 100 Hz step on motor unit recruitment.

A single trial of NMES consisted of 3 repetitions of a NMES pattern, with 60 s separating each pattern. For each NMES site, trials were collected using both NMES patterns and intensities. The order of trials was randomised for each participant. Throughout the NMES trials, participants were asked to remain relaxed and refrain from contributing voluntarily to the NMES-evoked contractions.

3.2.2.6 NMES intensity

To set the NMES intensity, 2 s of NMES was delivered every 5 to 10 s while the intensity was increased by ~2 mA increments during nNMES and ~5 mA increments during mNMES until the desired torque was achieved. If more than ~10 contractions were required to achieve the desired torque, participants were provided ~3 min of rest before continuing. NMES was delivered to evoke contractions of 10, 20 or 30% MVIC torque during the interval 2 to 3 s into the NMES ($Time_1$; see Figure 3-3A). For all trials, if the NMES was uncomfortable, the experimental session was concluded or trials at lower NMES intensities were collected. As a result, data were obtained for both nNMES and mNMES from 11 participants at 10% MVIC torque, 8 participants at 20% MVIC torque and 1 participant at 30% MVIC torque. For all participants, increases in NMES intensity were limited by discomfort during mNMES.

3.2.3 Additional experiments

During the initial experiments, the lower frequency of NMES was delivered at 15 Hz to enable the recording of asynchronous activity. However, this frequency is lower than the 20-25 Hz frequencies that we have used to stimulate the ankle musculature in previous experiments that have shown that torque, H-reflexes and asynchronous activity are augmented after a period of 100 Hz NMES (2, 4, 16, 17, 35). Thus, we conducted additional experiments in which the lower frequency in the NMES pattern was 25 Hz to provide a more valid comparison with the results of our previous studies.

These additional experiments followed the same protocol as the initial experiments except that the NMES was delivered at 25 Hz for 8 s (constant frequency pattern) or 25-100-25 Hz for 3-2-3 s for each phase, respectively (step frequency pattern) and was only delivered at 1 intensity, that which evoked 10% MVIC torque at $Time_1$. This contraction amplitude was chosen to maximise the chances of generating augmented torque and H-reflexes, as lower contraction amplitudes have produced the greatest levels of additional torque following 100 Hz NMES (2, 4). In these additional experiments, we collected M_{max} for each participant, but data for M-H recruitment curves were not collected and EMG data were not analysed during NMES patterns because H-reflex peak-to-peak measurements were contaminated by NMES artefacts during 25 Hz NMES.

3.2.4 Data acquisition and analysis

Data were sampled at 5 kHz using custom written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent

analysis that was conducted using custom written Matlab software (The Mathworks, Natick, MA). MVIC torque was calculated by averaging data over a 500 ms window centred on the peak isometric knee extensor torque during the MVIC. Torque generated during NMES was normalized to each participant's MVIC. The amplitudes of each M-wave and H-reflex recorded for the M-H recruitment curves and during 15 Hz NMES were measured peak-to-peak. Recruitment curves were generated by plotting M-wave and H-reflex amplitudes as a function of NMES intensity. For mNMES, we sometimes failed to observe a clear plateau in M-wave amplitude in the recruitment curves, even at maximal stimulator output (100 mA). Thus, for each participant, all M-waves and H-reflexes were normalized to the single largest M-wave (M_{max}) from the recruitment curve for nNMES. The single largest H-reflex (H_{max}) from the recruitment curves and M_{max} from the recruitment curve for nNMES were used to calculate H_{max}-to-M_{max} ratios. EMG during 25 Hz and 100 Hz NMES was not quantified due to contamination by NMES artefacts.

During mNMES, M-wave amplitude can be contaminated by the preceding NMES artefact due to the close proximity of the NMES and recording electrodes. Thus, to prevent over-estimation of the M-wave amplitude, we analysed the data post-hoc using a 2-step software based signal processing procedure (48). The algorithm removes the complete NMES artefact including both positive and negative spikes as well as any exponentially decaying tail. Likewise, during nNMES, H-reflex amplitude can be contaminated by the preceding M-wave due to the proximity of the NMES site to the spinal cord. In

other words, the H-reflex may begin on the tail of the M-wave. To calculate the H-reflex amplitude, we adopted a 4-step software based signal processing procedure (39). This process isolated the tail of the M_{max} signal from the M-H recruitment curve, when no reflex was present, and scaled the tail using a template according to the amplitude of the M-wave to be removed. The scaled M-wave tail was then subtracted, leaving the uncontaminated H-reflex for peak-to-peak analysis. All data were analysed using both signal processing algorithms, regardless of NMES site.

To quantify asynchronous activity during 15 Hz NMES, we calculated the root mean square (RMS) of the EMG activity over a 10 ms interval between 55 and 65 ms after each NMES artefact (see Figure 3-1B). As in a previous study (4), a duration of 10 ms was chosen as this time window was not contaminated by the NMES artefact, M-wave or H-reflex and for the present study it minimised the effect of the H-reflex silent period on the asynchronous measurement. To prevent over-estimation of the RMS calculation, all data in the intervals over which asynchronous activity was quantified were fit to a 2^{nd} order polynomial using the least squares procedure to remove any trend in the baseline associated with the preceding M-wave or H-reflex. The 2^{nd} order polynomial was subtracted from the raw data before the RMS was calculated, leaving the de-trended data with a mean of zero. RMS values were normalised to the maximum RMS (RMS_{max}) calculated separately over a 500 ms period centred on the peak vastus lateralis and vastus medialis EMG during each participant's MVIC. Pilot experiments, in which we delivered 8 s of 15 Hz NMES to evoke 10% MVIC torque of the quadriceps while

participants were at rest or held voluntary isometric contractions to generate 5 to 20% MVIC, additional to the NMES-evoked contraction, confirmed that we could measure asynchronous activity in the quadriceps and that our measure of RMS activity increased during increasing levels of voluntary contraction. Further, at a given background contraction amplitude, asynchronous activity was not different between NMES sites and could be measured in every participant. However, the pilot experiments showed that the asynchronous activity measure did not accurately reflect the voluntary contraction amplitude as a percentage of RMS_{max}. For example, a voluntary contraction of 5, 10 and 20% MVIC torque was measured as 2, 4 and 7% RMS_{max}, respectively. As such, RMS is reported here to provide a relative measure of the involuntary asynchronous activity generated by the sensory volley during nNMES and muscle belly and between $Time_1$ and $Time_2$.

Fifteen M-wave, H-reflex and asynchronous activity measurements were averaged over each of 2 time periods ($Time_1$: 2 to 3 s into the NMES; $Time_2$: 6 to 7 s into the NMES; see also Figure 3-2A) during a single NMES pattern. For each participant, isometric knee extension torque, M-waves, H-reflexes and asynchronous activity measured at $Time_1$ and $Time_2$ were averaged separately over the 3 repetitions of a NMES pattern in a single trial. Group means were calculated by pooling these mean data from each participant. The consistency of isometric knee extension torque, M-waves and H-reflexes between successive contractions was measured by calculating the coefficient of variation (CV =

[SD/mean] x100) between the mean values calculated for the 3 consecutive contractions at $Time_2$.

Statistical analyses were performed using Statistica software (StatSoft, Tulsa, OK). Kolmogorov-Smirnov and Lillefors tests for normality showed that group data were normally distributed. For the initial experiments, analyses were performed on group torque, vastus lateralis and vastus medialis data from trials in which NMES was delivered to evoke 10% and 20% MVIC torque. Paired *t*-tests were used to test for differences in M_{max} and H_{max}-to-M_{max} ratios, obtained from the M-H recruitment curves produced at each NMES site. Paired *t*-tests were also used to compare NMES current between the 2 sites separately for each intensity.

For data from trials with repetative NMES, separate 3-factor repeated measures analyses of variance (rmANOVA) tests were run on each dependent variable (Torque, H-reflex, M-wave, and asynchronous activity) at both NMES intensities (10% and 20% MVIC) to determine the influence of *NMES Site*, *NMES Pattern* (constant frequency versus step frequency) and *Time* ($Time_1$ versus $Time_2$) on the evoked response. To determine whether asynchronous activity was present during NMES, we calculated the RMS of the baseline EMG prior to delivery of NMES, when participants were relaxed and we knew no asynchronous activity would be present, and included these data as a third level of *Time* in the rmANOVA analyses for asynchronous activity ($Time_{pre}$ versus $Time_1$ versus $Time_2$). Two-factor rmANOVA tests were run on Torque, M-wave, and H-reflex CV data to determine the influence of *NMES Site* and *NMES Pattern* on the consistency of the evoked response. Due to the similarity of data recorded from

vastus lateralis and vastus medialis, and to avoid excessive repetition, we describe in detail only data collected from vastus lateralis in RESULTS, as the results of the rmANOVA tests for vastus lateralis and vastus medialis data did not differ.

For the additional experiments, a 3-factor rmANOVA was run on Torque data to determine the influence of *NMES Site*, *NMES Pattern* (constant frequency versus step frequency) and *Time* ($Time_1$ versus $Time_2$) on the amplitude of the evoked response. A 2-factor rmANOVA test was run on torque CV data to determine the influence of *NMES Site* and *NMES Pattern* on the consistency of the evoked torque at $Time_2$.

An alpha level of 0.05 was used to evaluate statistical significance. All data are reported as mean ± standard deviation.

3.3 Results

3.3.1 Recruitment curve

M-H recruitment curve data recorded from vastus lateralis for 1 participant during nNMES (A) and mNMES (B) are shown in Figure 3-2. The right side of each panel shows single EMG traces from the corresponding numerical site in the recruitment curve. In this participant, the H_{max}-to-M_{max} ratio was 0.22 for nNMES and 0.04 for mNMES. For the group (n = 11), there was no significant difference between M_{max} evoked by NMES at both sites ($t_{(10)} = 1.05$, $p = 0.32$). M_{max} was 10.4 ± 3.8 mV for nNMES and 9.7 ± 2.7 mV for mNMES. H_{max}-to-M_{max} ratios were significantly larger ($t_{(10)} = 3.8$, $p < 0.001$) for nNMES (0.21 ± 0.10; range: 0.09 to 0.37) compared to mNMES (0.02 ± 0.01; range: 0.01 to 0.03). Robust H-

reflexes could be evoked in all 11 participants during nNMES. Conversely, H-reflexes were rare and very small when present during mNMES.

3.3.2 NMES – single participant data

Data recorded from 1 participant during nNMES (A, C and E) and mNMES (B, D and F) during the constant frequency and step frequency pattern are shown in Figures 3-3 and 3-4, respectively. In the top half of each panel, the solid lines show torque and the symbols represent the amplitude of the EMG measures from vastus lateralis during NMES at 15 Hz. There was no asynchronous activity present during NMES at either site or amplitude and thus, these data are not shown in Figures 3-3 or 3-4. During constant frequency NMES at both sites (Figure 3-3), mean torque remained stable throughout the NMES (i.e. was similar at $Time_1$ and $Time_2$). However, there were periods of time when torque oscillated rapidly (~7 to 8 Hz) during nNMES, as can be seen in the individual traces (grey lines) in panels A, C and E. During these periods, and throughout the NMES, H-reflexes alternated between large and small (see the open squares, which are an average of 3 H-reflex measurements across 3 consecutive contractions, and the grey lines in the EMG traces in panels A, C, and E) while M-waves were relatively consistent. During nNMES, H-reflexes dominated the EMG at all 3 contraction amplitudes (10, 20 and 30% MVIC torque) while M-waves were small and relatively stable. In contrast, during mNMES, M-waves dominated the EMG. When the step frequency pattern was delivered in this participant (Figure 3-4), torque, M-waves and H-reflexes were not augmented after 100 Hz NMES.

3.3.3 NMES – group data

3.3.3.1 Initial experiments

Statistical analyses were performed on data recorded when 15 Hz NMES was delivered to evoke 10% and 20% MVIC torque at $Time_1$ for the group. There was no asynchronous activity, significantly greater than measured at baseline, during NMES at either site or intensity and thus, these data are not shown in Figures 3-5 or 3-6. For both contraction amplitudes, significantly less current was required for nNMES than mNMES. The mean current required to produce 10% MVIC torque at $Time_1$ was 12.6 ± 4.7 mA for nNMES and 46.0 ± 13.1 mA for mNMES ($t_{(10)} = 8.1$, $p < 0.001$). The mean current to produce 20% MVIC torque was 14.4 ± 6.5 mA for nNMES and 65.3 ± 22.8 mA for mNMES ($t_{(7)} = 7.1$, $p < 0.001$). The current required for 1 participant who received NMES to produce 30% MVIC torque was 12 mA for nNMES and 54 mA for mNMES.

Figure 3-5 shows group (n = 11) torque and vastus lateralis EMG data for trials in which the NMES intensity was adjusted to evoke 10% MVIC torque at $Time_1$. There were no significant differences in torque across all factors (Figure 3-5A). However, the CV for torque (Figure 3-5B), showed a significant main effect of *NMES Site* [$F_{(1,10)} = 9.79$, $p = 0.01$]. Torque was more consistent between contractions during mNMES compared to nNMES, regardless of NMES pattern. For M-wave amplitude (Figure 3-5C), there was a significant main effect of *NMES Site* [$F_{(1,10)} = 17.19$, $p = 0.001$]. M-waves were ~10 times larger during mNMES compared to nNMES, regardless of NMES pattern or time. Additionally, the CV for M-waves (Figure 3-5D) showed a significant main effect of *NMES*

Site [$F_{(1,10)} = 7.29$, $p = 0.02$]. M-waves were more consistent between contractions during mNMES compared to nNMES, regardless of NMES pattern. For H-reflex amplitude (Figure 3-5E), there was a significant main effect of *NMES Site* [$F_{(1,10)} = 19.55$, $p < 0.01$]. H-reflexes were ~9 times larger during nNMES compared to mNMES, regardless of NMES pattern or time. Additionally, the CV for H-reflexes (Figure 3-5F) showed a significant main effect of *NMES Site* [$F_{(1,10)} = 19.55$, $p < 0.01$]. H-reflexes were more consistent between contractions during mNMES compared to nNMES, regardless of NMES pattern. However, we acknowledge that since H-reflexes were very small, when present, during mNMES, this may not be a relevant comparison.

Group data (n = 8) for torque and vastus lateralis EMG are shown in Figure 3-6 for trials in which the NMES intensity was adjusted to evoke 20% MVIC torque. There were no significant differences in the amplitude of torque across all factors (Figure 3-6A). Additionally, there were no significant differences in the CV for torque (Figure 3-6B). Thus, at this higher level of NMES, there were no differences in the consistency of torque between contractions across both factors. For M-wave amplitude (Figure 3-6C), there was a significant main effect of *NMES Site* [$F_{(1,7)} = 22.94$, $p = 0.003$]. M-waves were ~7 times larger for mNMES compared to nNMES, regardless of the NMES pattern or time. The CV for M-waves (Figure 3-6D) showed a significant main effect of *NMES Site* [$F_{(1,7)}=6.17$, $p=0.04$]. M-waves were more consistent between contractions during mNMES compared to nNMES, regardless of NMES pattern. For H-reflex amplitude (Figure 3-6E), there was a significant main effect of

NMES Site [$F_{(1,7)} = 13.79$, $p = 0.009$]. H-reflexes were ~8 times larger for nNMES compared to mNMES, regardless of NMES pattern or time. However, there were no significant differences in the CV for H-reflexes (Figure 3-6F). Thus, at this higher level of NMES, there were no differences in the consistency of H-reflexes between contractions across both factors.

3.3.3.2 Additional experiments

Statistical analyses were performed on group torque data (n = 7) recorded when 25 Hz NMES was delivered to evoke 10% MVIC torque at $Time_1$. There were no significant differences in torque across all factors (Figure 3-7A). However, the CV for torque (Figure 3-7B) showed a significant main effect of *NMES Site* [$F_{(1,10)} = 12.13$, $p = 0.01$]. Torque was more consistent between contractions during mNMES compared to nNMES, regardless of NMES pattern.

3.4 Discussion

In this study, we compared the contributions made by *peripheral* (M-wave) and *central* (H-reflex, asynchronous activity) pathways to motor unit recruitment during isometric contractions of similar amplitude generated by NMES applied over the femoral nerve trunk (nNMES) and the quadriceps muscle (mNMES). We found that, similar to the results obtained from experiments on the triceps surae (4), NMES site (nNMES versus mNMES) largely determined the pathways by which motor units were recruited when NMES was delivered to activate the quadriceps muscle and generate knee extension torque. However, unlike the triceps surae and other muscles studied previously, neither torque nor

activity through *central* pathways were augmented following 100 Hz NMES, nor was any asynchronous activity evoked during NMES at either site.

3.4.1 Torque

3.4.1.1 Contraction amplitude

NMES intensity was adjusted to generate similar torque at $Time_1$ for both sites. Accordingly, torque was not significantly different during nNMES compared to mNMES for any of the relevant comparisons in the present study. Additionally, torque at $Time_2$ was not different from that at $Time_1$ for all relevant comparisons. Thus, torque did not increase over time during constant frequency NMES or, contrary to our hypothesis, following the brief periods of 100 Hz NMES during the step frequency pattern. In a recent study, Thompson et al. (2011) delivered mNMES using a step frequency pattern, similar to that used presently, in 9 neurologically-intact participants and reported an increase in torque of 21% from before to after a period of 100 Hz NMES; whether this increase was statistically significant was not tested (51). This apparent increase in torque is in marked contrast to the present results in which there were no differences in torque for the same comparison. Regardless, the results of Thompson et al. (2011) and those reported presently indicate that the effect of a brief period of high frequency NMES on increasing torque is less for the quadriceps than has been reported previously for other muscles (triceps surae: ~50-412%, Refs 2, 4, 16, 17 and 35; tibialis anterior: ~140%, Refs 17 and 35; wrist extensors: 46-62%, Ref 2; biceps brachii: 42-116%, Refs 9 and 41; flexor pollicus longus: 47-54%, Ref 9). The reasons for the discrepancy between the

results of Thompson et al. (2011) and those reported presently are unclear; however, we do not believe that the lack of an increase in torque in the present study was the result of a sampling bias related to the recruitment of *non-responders*. Of the 13 participants in the present study, 6 participated in our previous study investigating similar effects in the triceps surae (4). In the previous study, these 6 participants generated on average a ~48% increase in plantar flexion torque and presently, these same participants generated on average only a ~9% increase in knee extension torque. The present study was not designed to distinguish between *responders* and *non-responders*, although such a study may shed light on neural mechanisms that distinguish these two groups, some of which are discussed below (see Section 3.4.2 below).

3.4.1.2 Contraction consistency

Although torque did not differ between NMES sites, the amplitude of consecutive contractions was more consistent during mNMES (CV: ~10%) compared to nNMES (CV: ~20%), regardless of NMES pattern or frequency (15 Hz vs 25 Hz) when NMES was delivered to evoke 10% MVIC torque at $Time_1$. When 15 Hz NMES was delivered to evoke 20% MVIC torque, no significant difference in contraction consistency was found. These differences in contraction consistency at lower contraction amplitudes are consistent with data from the ankle plantar flexors (2). When NMES was delivered to evoke ~5% MVIC torque in the plantar flexors, Baldwin et al. (2006), found that mNMES of the plantar flexors was more consistent between consecutive contractions (CV: ~10%) compared to nNMES (CV: ~20%)(2). The variability in torque in the present

study during nNMES at $Time_2$ may be due to the variability in H-reflex amplitude observed during this same period of time, as described in the following section.

3.4.2 Pathways during nNMES versus mNMES

The relative contributions made by *peripheral* and *central* pathways to motor unit recruitment were markedly different between NMES sites. As hypothesised, nNMES generated contractions with smaller M-waves (7 to 10 times) and larger H-reflexes (8 to 9 times) compared to contractions of equal amplitude generated by mNMES, regardless of NMES pattern or intensity. Thus, nNMES generated contractions predominantly through *central* pathways, while mNMES generated contractions predominantly through *peripheral* pathways. This effect of NMES site is consistent with the larger H_{max}-to-M_{max} ratios obtained with nNMES compared to mNMES. Similar to nNMES of the triceps surae (4), much of the motor unit recruitment during nNMES of the quadriceps was via *central* pathways in the form of H-reflexes; for both muscle groups, contractions of up to 30% MVIC torque could be produced almost exclusively though this pathway in some participants. For both the triceps surae (4) and quadriceps muscle (present study), nNMES, where all the sensory and motor axons are located in close proximity to the NMES electrodes, likely recruited a relatively greater proportion of sensory axons compared to NMES delivered mNMES near the motor points, where sensory axons are more widely dispersed throughout the muscle.

Our hypothesis that there would be more asynchronous activity during mNMES compared to nNMES was not supported. Unlike the triceps surae (4), we

recorded no asynchronous activity during NMES at either site. This is despite the fact that in pilot experiments, we were able to measure asynchronous activity during NMES that was generated voluntarily (see Section 3.2.4 above) and measured increases in this activity when voluntary contraction amplitude increased. Thus, we do not believe that the lack of asynchronous activity recorded in the present study was due to an inability to measure it. Rather, we believe that there was no asynchronous activity generated during NMES of the quadriceps muscle. We have previously proposed that asynchronous activity is due, at least in part, to the activation of persistent inward currents in spinal neurons (16, 17). The lack of asynchronous activity in the quadriceps EMG may indicate that neurons in circuits controlling the quadriceps are less likely to exhibit this behaviour.

Our second hypothesis was not supported by the present data, as neither torque, H-reflex nor asynchronous activity increased following 100 Hz NMES during the step frequency pattern. Increases in torque, H-reflexes and asynchronous activity following 100 Hz NMES have been attributed to mechanisms in *central* circuits (4, 35), such as increased probability of neurotransmitter release from pre-synaptic terminals, associated with post-tetanic potentiation, and/or increased motor neuron excitability, due to the activation of persistent inward currents in spinal neurons. Thus, the lack of such increases in the present study indicates that there may be differences in the frequency dependant changes in sensorimotor integration in *central* circuits controlling the quadriceps muscle, compared to muscles studied previously. However, small increases in torque, which are not accompanied by increases in EMG activity,

may also be due to an intrinsic muscle property (8) that is dependent upon muscle length (22).

3.4.3 Implications for NMES

An interesting feature of NMES is the unique pattern of motor unit recruitment underlying the evoked contractions (5, 6, 41). Unlike voluntary contractions, when motor unit recruitment is temporally asynchronous, spatially diffuse (1) and orderly from slow-fatigue-resistant to fast-fatigable with increasing contraction amplitude (28, 29, 33), it is generally accepted that motor unit recruitment during NMES, at least during mNMES, is temporally synchronous (1, 41), mainly, but not exclusively (1), superficial (41, 45, 49, 54), and occurs randomly without obvious sequencing related to motor unit type (1, 26, 33, 41). As a consequence, the capacity to produce repeated contractions that do not fatigue rapidly with mNMES is compromised compared to voluntary exercise (6, 24, 34, 41). This may be particularly relevant for the quadriceps where fatigue-resistant motor units are mainly in deeper compartments of the muscle (36, 40) and thus are more difficult to activate during mNMES compared to voluntary contractions, even at rather high NMES intensities (41, 49). Despite this, hypertrophy of fatigue-resistant muscle fibres in the quadriceps has been reported, but such adaptation requires a high volume (4 h per day, 7 days per week; Ref 47) or intensity of training (~60% MVIC; Ref 24), the former of which may not be practical to achieve as part of a long term exercise program and the latter of which can be problematic for individuals with residual sensation (50) or compromised bone density (21).

Generating contractions by nNMES may alleviate some of these issues. Firstly, nNMES required significantly less current than mNMES. Secondly, increases in NMES intensity in the present study were limited, in every case, by discomfort during mNMES. Thus, nNMES produces contractions that require less battery power and generate less discomfort for the participant; however, there is evidence that nNMES generates more discomfort for the participant (44, 49), and thus this line of inquiry requires further investigation. Thirdly, both human *in vivo* (20) and computational modelling (43) data support the idea that NMES recruits motor units randomly in relation to axon diameter, in which case motor units recruited as M-waves during nNMES would be expected to be randomly distributed throughout the muscle. Finally, motor unit recruitment through *central* pathways follows Henneman's *size principle* (28, 29) and thus recruits fatigue-resistant motor units first. These fatigue-resistant muscle fibres are located deep in the quadriceps muscle (36, 40) and may therefore be less accessible during mNMES (45, 49, 51). Thus, contractions mediated through *central* pathways should minimise the non-physiological recruitment order commonly reported for mNMES (26, 41) and, for the quadriceps, recruit a relatively greater proportion of fatigue-resistant motor units with a relatively lower NMES intensity. This may help protect these vulnerable units from atrophy and transformation to fast-fatigable units; a common occurrence after periods of inactivity as the result of spinal cord injury (7, 14, 21, 27). Consequently, the greater recruitment through *central* pathways evoked by nNMES, and general lack of activity through *central* pathways contributing to the evoked contractions during mNMES, in the present

study, suggests that nNMES holds promise for maintaining muscle quality (therapeutic electrical stimulation; TES) and possibly for producing functional movements (functional electrical stimulation; FES) following damage to the central nervous system compared with mNMES.

Despite these promising theoretical advantages of delivering nNMES, there are potential practical limitations to stimulating the quadriceps for FES. Firstly, the position of the cathode in the femoral triangle is highly susceptible to movement as a result of the contraction itself, due to the nearby tendon, and as a result of limb movements, making it difficult to deliver consistent current. Secondly, even if movement of the nNMES electrode can be minimised, contractions generated through *central* pathways are less consistent between successive contractions compared to contractions generated through *peripheral* pathways (2), however this may only be the case at lower NMES intensities. Although contraction stability within a contraction was not quantified in the present study, we did observe instances in which torque oscillated during nNMES. These oscillations in torque occurred simultaneously with oscillations in H-reflex amplitude, similar to that which we have observed for soleus H-reflexes (15). Thirdly, it is unclear whether contractions with a significant contribution through *central* pathways will be of sufficient amplitude for FES applications, although presently we show such contractions up to 30% MVIC torque. Fourthly, during FES-assisted movements, it may be that motor unit recruitment through *central* pathways would diminish, as it is well known that H-reflexes reduce in size during passive and voluntary movement (10, 11, 18, 31); however, such H-

reflex modulation is reduced or absent in people with spinal cord injury (37). Finally, as NMES intensity is increased beyond what was tested in the present study, for example, in response to fatigue during FES-assisted exercise, increased levels of antidromic transmission in motor axons will develop (55), which will progressively block motor unit recruitment through *central* pathways. Thus, overall, it may be that nNMES would be most immediately beneficial for therapeutic purposes (TES), such as muscle conditioning which would require less precise control of evoked contraction amplitudes, until some of the anticipated limitations associated with nNMES for functional movement applications (FES) can be addressed.

3.4.4 Conclusion

This study is the first to demonstrate motor unit recruitment through *central* pathways during NMES-evoked contractions of the quadriceps femoris, one of the most utilised muscle groups for NMES rehabilitation. During mNMES, contractions were generated predominately through *peripheral* pathways (M-waves), while nNMES generated contractions with a greater contribution through *central* pathways (H-reflexes). However, unlike other muscles studied previously, neither torque nor activity through *central* pathways were augmented following 100 Hz NMES, nor was any asynchronous activity evoked during NMES at either site. Bearing in mind the aforementioned limitations of nNMES with regards to the consistency of evoked contractions, nNMES may be considered a good compliment to, as opposed to a replacement for, mNMES for maintaining muscle quality and reducing muscle fatigue for NMES rehabilitation programs.

3.5 Figures

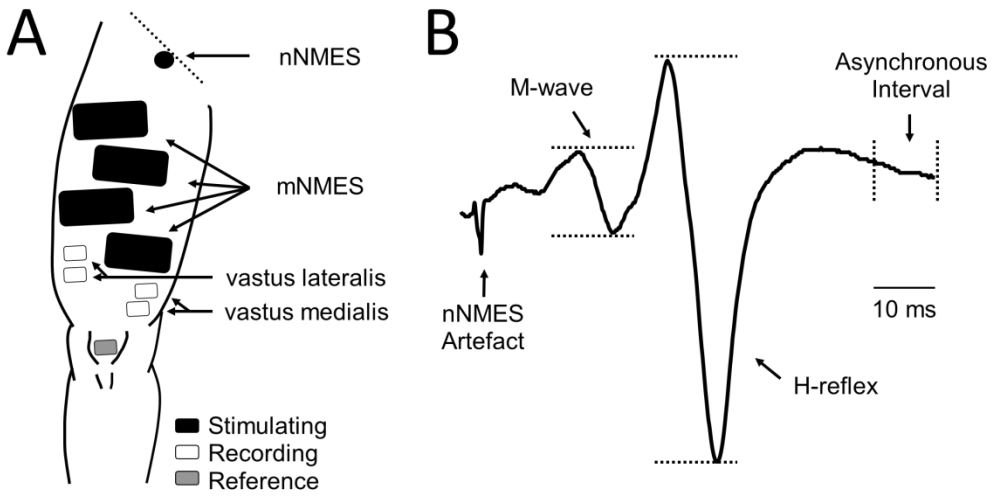

Figure 3-1 A) Schematic of the NMES, recording and reference electrode sites on the right leg. The NMES electrode placed over the gluteal fold is not shown. B) An EMG waveform recorded from vastus lateralis, elicited by nNMES, showing peak-to-peak M-wave and H-reflex sites as well as the interval over which RMS was calculated for the measurement of asynchronous activity.

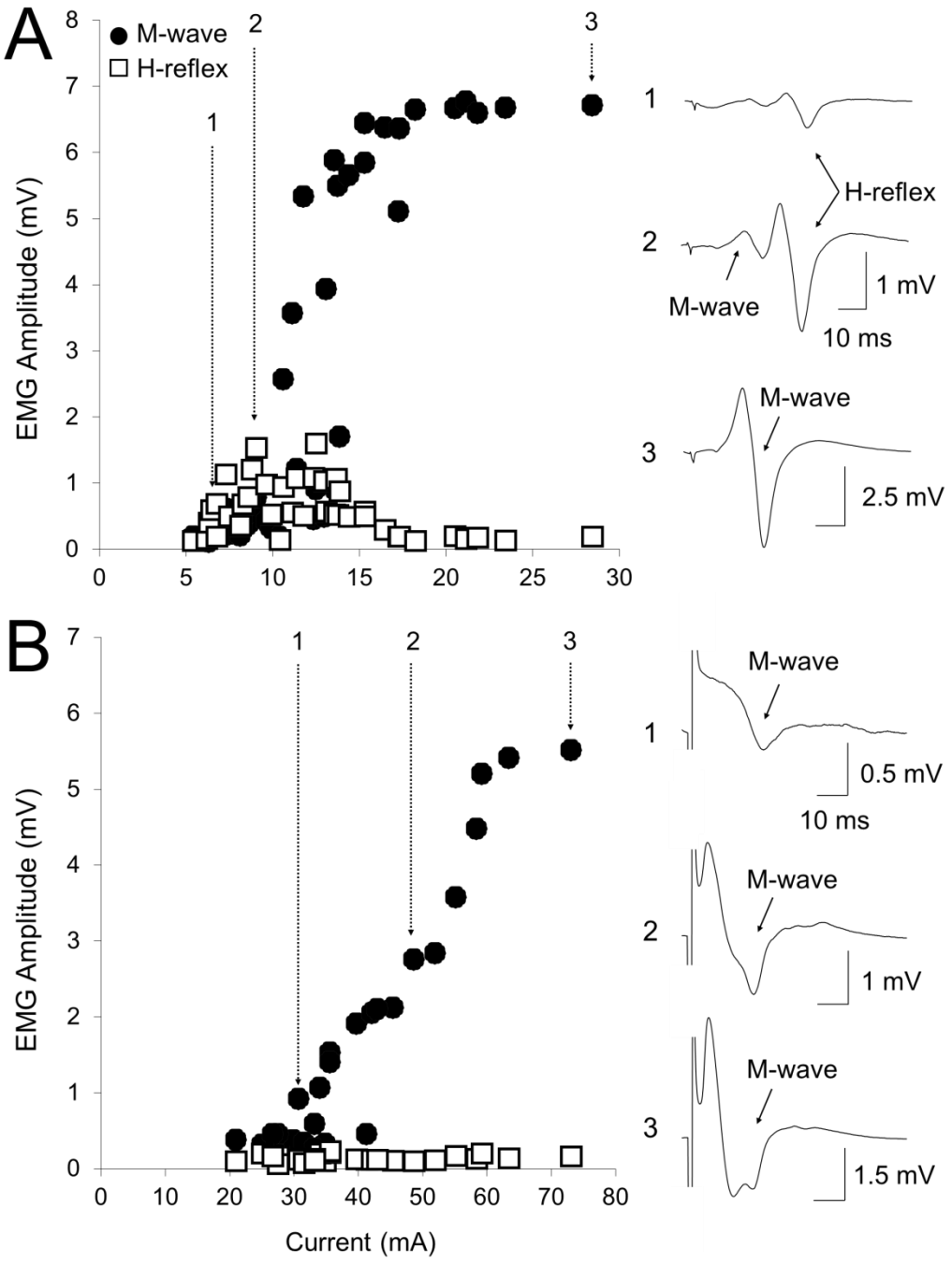

Figure 3-2 M-H recruitment curves for vastus lateralis produced by nNMES (A) and mNMES (B) in a single participant. The right side of each panel shows single raw EMG traces recorded at the corresponding numerical site in the recruitment curve. These raw EMG traces shown have not been processed post-hoc.

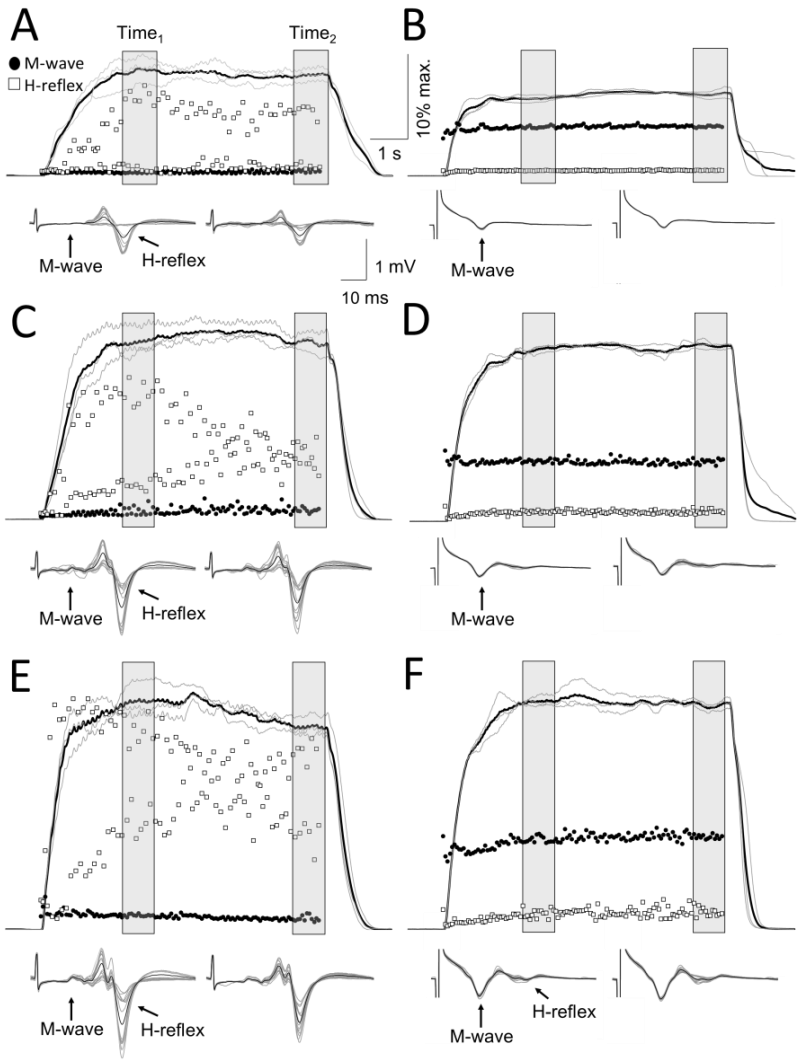

Figure 3-3 Torque and vastus lateralis EMG responses evoked by constant frequency (15 Hz for 8 s) nNMES (A, C and E) and mNMES (B, D and F) to evoke ~10% (A and B), 20% (C and D) and 30% (E and F) MVIC torque at $Time_1$ in the same participant as shown in Figure 3-2. The shaded areas highlight the time periods ($Time_1$ and $Time_2$) over which data were quantified for statistical analyses. In the upper half of each panel, torque represented by the black lines are average responses to 3 trains of NMES (grey lines) and the symbols represent the average EMG data over 3 repetitions during a single trial. Vertical calibration represents 10% M_{max} for EMG and 10% MVIC for torque. The lower half of each panel shows raw EMG recorded at $Time_1$ (left trace) and $Time_2$ (right trace) during a single train of NMES. These raw EMG traces shown have not been processed post-hoc. Bold black lines represent the average of 15 single responses (grey lines) during the NMES. All data are shown on the same scale, as indicated by the calibration bars in panel A.

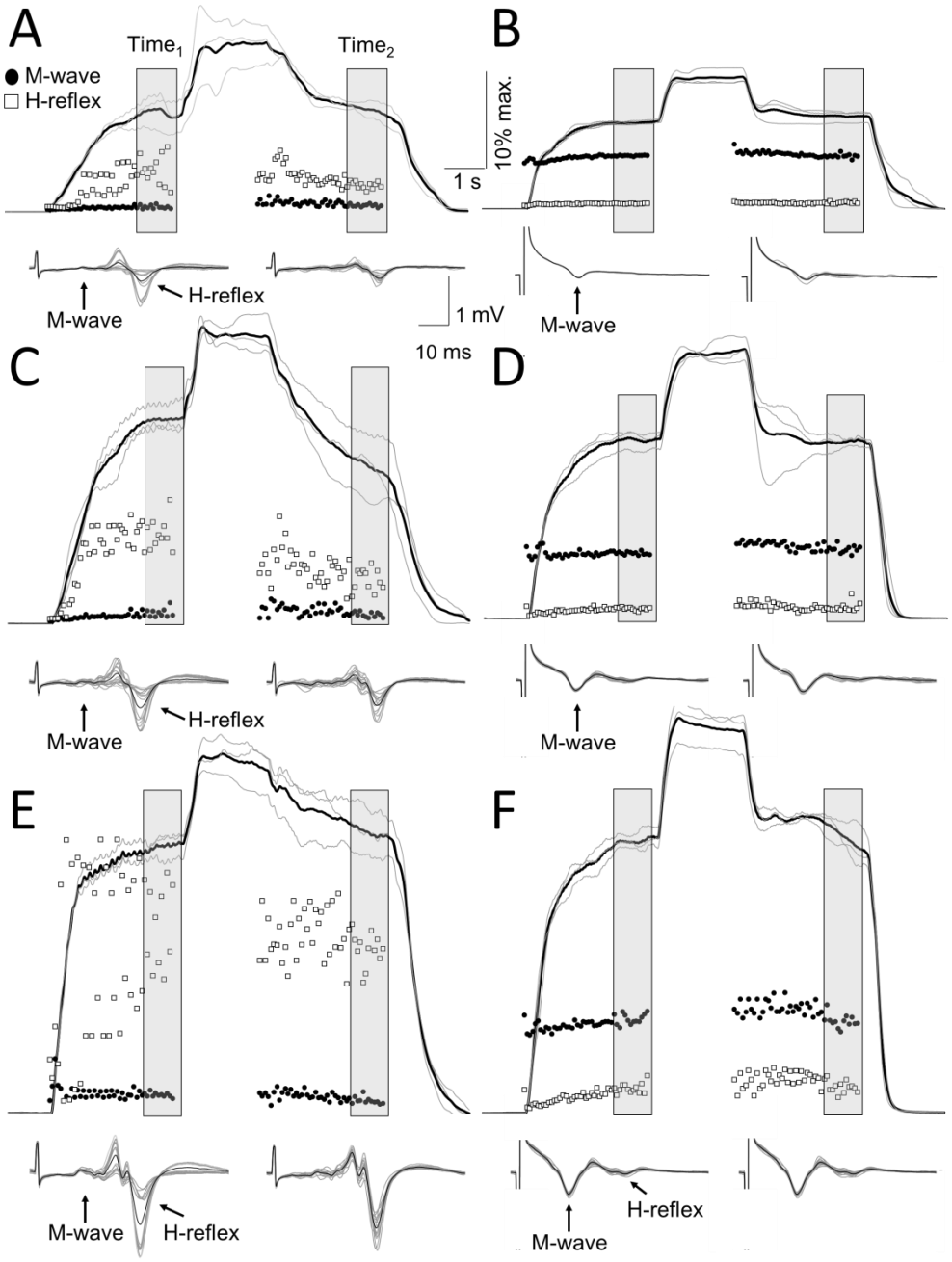

Figure 3-4 Torque and vastus lateralis EMG responses evoked by step frequency (15-100-15 Hz for 3-2-3 s, respectively) nNMES (A, C and E) and mNMES (B, D and F) to evoke ~10% (A and B), 20% (C and D) and 30% (E and F) MVIC torque at $Time_1$ in the same participant as Figures 3-2 and 3-3. Data are presented in the same way as in Figure 3-3. EMG during 100 Hz NMES was not quantified due to contamination by NMES artefacts.

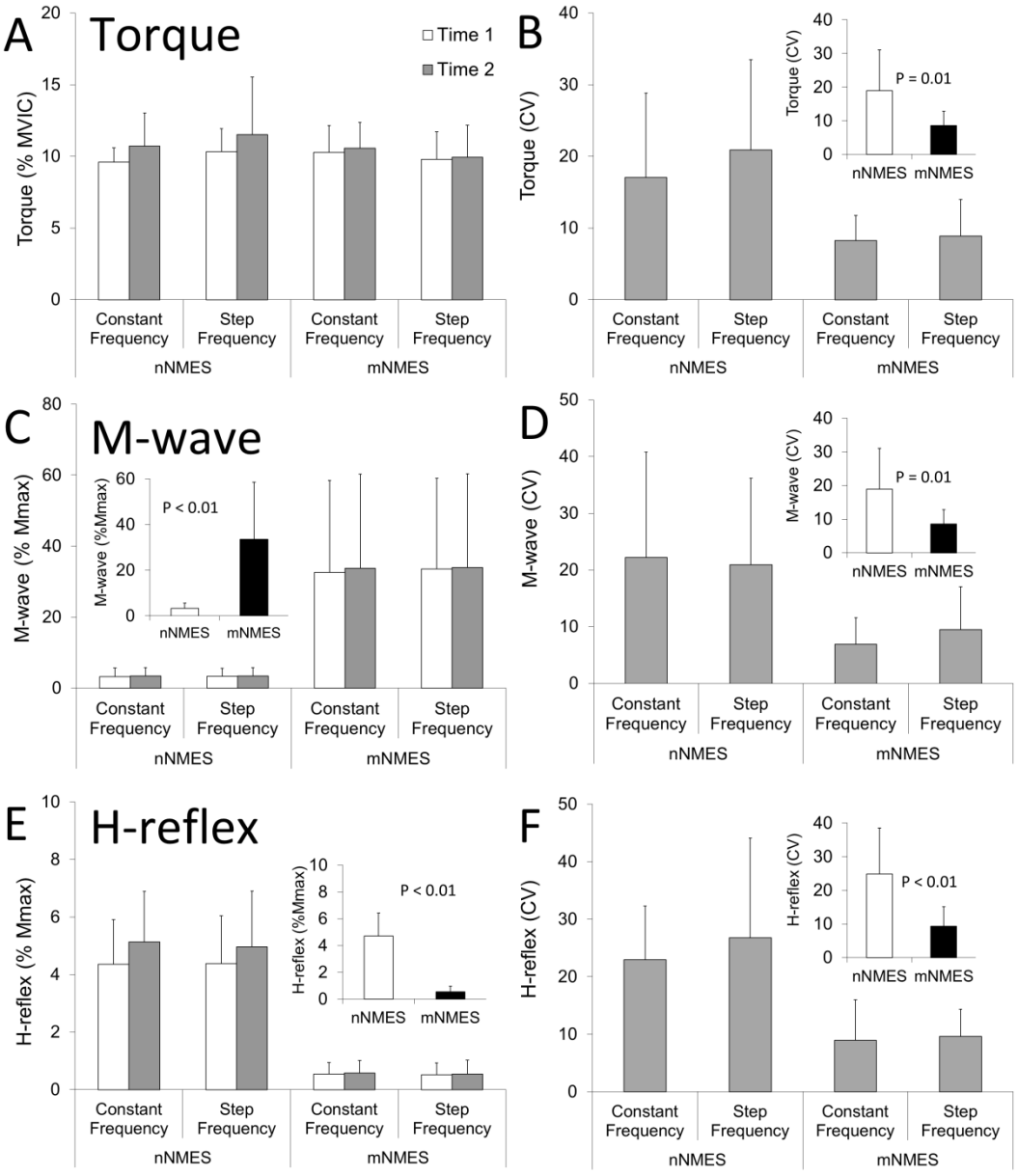

Figure 3-5 Group torque and EMG data (n = 11) during NMES (15 Hz for 8 s and 15-100-15 Hz for 3-2-3 s, respectively) over the femoral nerve trunk (nNMES) and quadriceps muscle belly (mNMES) at an intensity to evoke 10% MVIC torque at $Time_1$. Normalised data averaged at $Time_1$ and $Time_2$ are shown in panels A, C, and E. Coefficient of variation data averaged at $Time_2$ are shown in panels B, D and F. Significant main effects identified by the rmANOVA analyses are displayed within the insets.

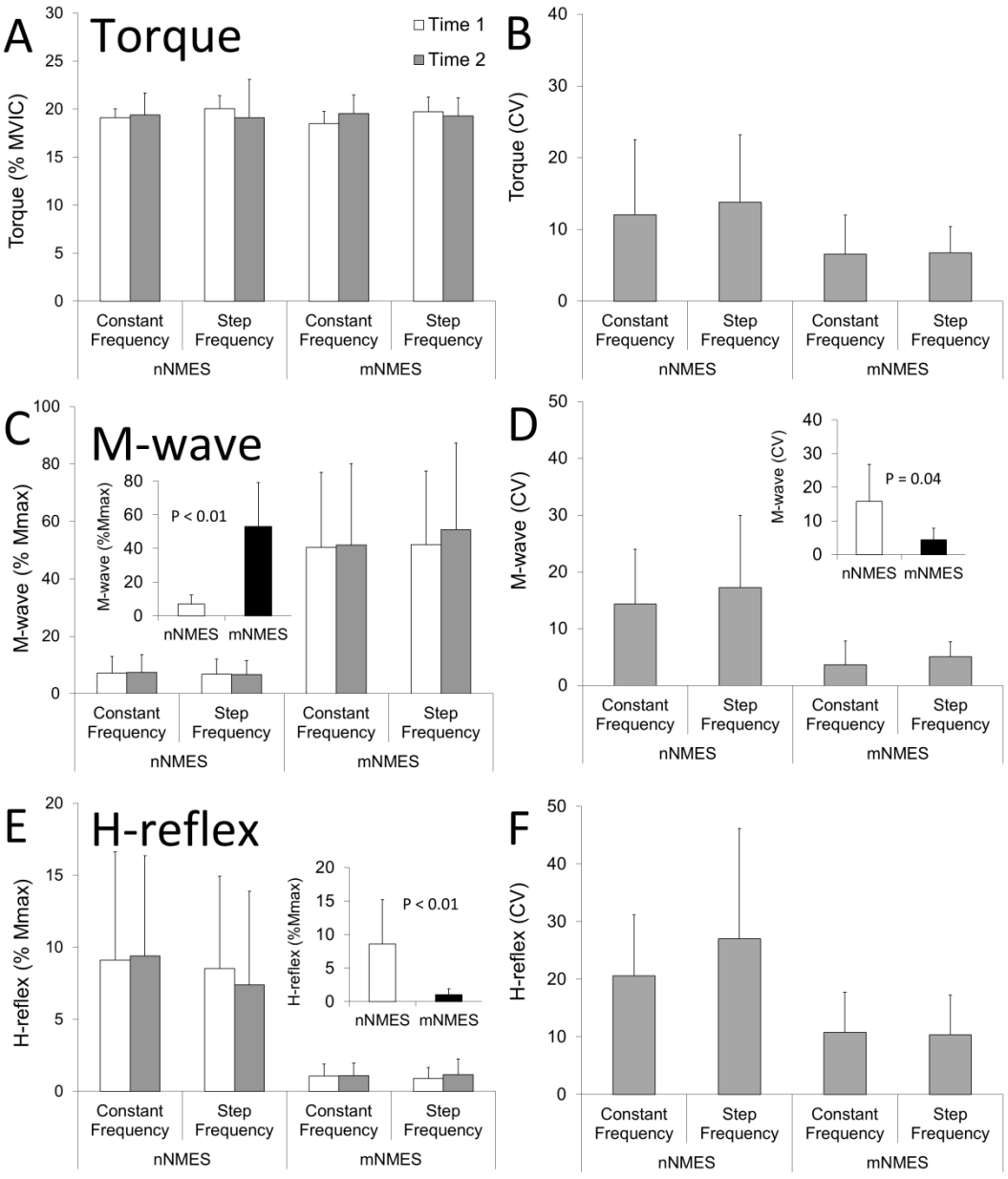

Figure 3-6 Group torque and EMG data (n = 8) during NMES (15 Hz for 8 s and 15-100-15 Hz for 3-2-3 s, respectively) over the femoral nerve trunk (nNMES) and quadriceps muscle belly (mNMES) at an intensity to evoke 20% MVIC torque at $Time_1$. Normalised data averaged at $Time_1$ and $Time_2$ are shown in panels A, C, and E. Coefficient of variation data averaged only at $Time_2$ are shown in panels B, D and F. Significant main effects identified by the rmANOVA analyses are displayed within the insets.

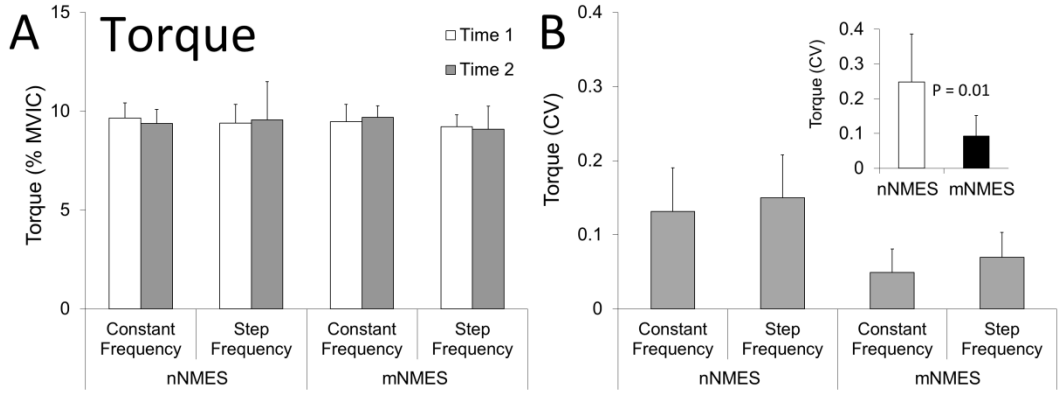

Figure 3-7 Group torque data (n = 7) during NMES (25 Hz for 8 s and 25-100-25 Hz for 3-2-3 s, respectively) over the femoral nerve trunk (nNMES) and quadriceps muscle belly (mNMES) at an intensity to evoke 10% MVIC torque at $Time_1$. Normalised data averaged at $Time_1$ and $Time_2$ are displayed in panel A. Coefficient of variation data averaged at $Time_2$ are displayed in panel B. A significant main effect identified by the rmANOVA analysis is displayed within the inset.

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CHAPTER 4: H-REFLEXES IMPROVE FATIGUE-RESISTANCE OF ELECTRICALLY-EVOKED CONTRACTIONS IN PEOPLE WITH CHRONIC MOTOR-COMPLETE SPINAL CORD INJURY: EFFECT OF STIMULATION SITE[4]

4.1 Introduction

Neuromuscular electrical stimulation (NMES) can generate contractions for people who have had a spinal cord injury (SCI; Ref 34). Such contractions can reduce muscle and bone atrophy (21), assist activities of daily living and provide opportunities for exercise (18). Unfortunately, premature contraction fatigue limits the effectiveness of NMES for these applications (43). Much of this fatigue is thought to be due to the non-physiological way in which motor units are recruited during NMES (8, 40).

During voluntary contractions, motor units are recruited synaptically according to Henneman's *size principle*, with fatigue-resistant motor units recruited first (42). In contrast, during NMES-evoked contractions, it is generally accepted that motor units are recruited in random order (1, 20, 31, 41) by the activation of motor axons beneath the NMES electrodes (8, 40). Accordingly, during NMES, signals travel along a *peripheral* pathway from the NMES site to the muscle, which generates a motor- or M-wave in the electromyographic (EMG) signal recorded from innervated muscles (6). This difference in recruitment order

[4] The individuals contributing to the work presented in this chapter were:
Bergquist AJ, Okuma Y, Wiest MJ and Collins DF.

between voluntary and NMES-evoked contractions indicates that NMES-evoked contractions recruit relatively fewer fatigue-resistant motor units than voluntary contractions of similar amplitude. This is thought to be one of the main reasons that NMES-evoked contractions fatigue rapidly (8, 40).

We have suggested that one way to improve the fatigue-resistance of NMES-evoked contractions is to maximise the depolarisation of sensory axons beneath the stimulating electrodes, thereby enhancing the synaptic recruitment of motor units (3, 5, 37). Depolarising sensory axons recruits motor units by signals travelling along reflex pathways through the spinal cord and recruitment order follows the *size principle* (10, 56, 57). The discharge of motor units recruited through these *central* pathways can be synchronous with each stimulus pulse, in which case it is measured as a Hoffmann- or H-reflex in the EMG signal (6). The *central* contribution to NMES-evoked contractions can also be measured as motor unit discharge that is not synchronised to each NMES pulse (asynchronous activity; Refs 5, 6, 13 and 38). In people without SCI, the extent to which contractions are generated through *peripheral* (M-waves) and *central* (H-reflexes, asynchronous activity) pathways depends on *where* NMES is delivered. For both the ankle plantar flexors (soleus; Ref 5) and knee extensors (vastus lateralis and medialis; Ref 7), NMES over the muscle belly (mNMES) generated contractions predominantly through *peripheral* pathways (M-waves), while NMES over the nerve trunk (nNMES) generated contractions with robust contributions through *central*, predominantly H-reflex, pathways.

The present experiments build on this previous work (5, 7) and were designed to determine whether fatigue is reduced during nNMES, which can recruit motor units according to the *size principle*, compared with a more traditional approach using mNMES, which tends to recruit motor units randomly with respect to type. We hypothesised that fatigue, defined as a significant reduction in torque over repeated contractions (27), would occur sooner (after fewer contractions into a fatigue protocol) and would be greater (generate less torque by the end of a fatigue protocol) during mNMES than nNMES. The ankle plantar flexors were studied because these muscles are important for standing and walking, and there is interest in stimulating them for restoring gait in people with SCI (2, 25, 34, 44). Further, we have established that mNMES and nNMES can generate contractions of the ankle plantar flexors through markedly different pathways in people without SCI (5).

4.2 Methods

4.2.1 Participants

Eleven participants with chronic (> 2 y) motor-complete SCI volunteered for this study after providing informed written consent (Table 4-1). It was not possible to elicit a muscle contraction within the range of our stimulator output during either mNMES or nNMES in 3 of 11 participants (participants 9 to 11 in Table 4-1). Thus, herein we report data collected from the 8 participants in whom we were able to generate contractions. All participants took part in 2 experimental sessions, each lasting ~2 h and separated by at least 5 d. A fatigue protocol (see

Section 4.2.6 below) delivered using mNMES or nNMES was tested in different sessions. The order of testing the NMES sites was randomised for each participant. All procedures were performed on the right leg. Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York) to measure isometric plantar flexion torque. The right foot was secured to the Biodex footplate with the hip at ~110°, the knee at ~90° and the ankle at ~90° with the lateral malleolus aligned with the axis of the dynamometer. With the knee at ~90°, the soleus muscle, the muscle from which we recorded, generates the majority of plantar flexion torque (14, 47). This study was approved by the Health Research Ethics Board at the University of Alberta.

4.2.2 Electromyography (EMG)

Surface EMG was recorded from soleus using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT) arranged in a bipolar configuration. The electrodes were placed parallel to the predicted path of the muscle fibres with ~1 cm inter-electrode distance (Figure 4-1). A reference electrode was placed over the tibia of the right leg. EMG signals were amplified between 500 and 1000 times and band-pass filtered at 10 to 1000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

4.2.3 Neuromuscular electrical stimulation (NMES)

NMES was delivered using a constant-current stimulator (200 μs pulse duration; DS7AH Digitimer, Welwyn Garden City, UK) and current was measured using a current probe (mA 2000 Noncontact Milliammeter; Bell Technologies, Orlando, Florida). mNMES was delivered over the triceps surae

muscles through 2 flexible adhesive gel electrodes (7.5 x 13 cm; model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) trimmed to fit (Figure 4-1). The anode was placed over the lateral and medial gastrocnemii at the point of the largest circumference. The cathode was placed over the soleus, just distal to the gastrocnemii. nNMES was delivered over the tibial nerve trunk through 2 flexible adhesive gel electrodes (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) placed on the skin of the popliteal fossa with an inter-electrode distance of ~1 cm (Figure 4-1). If contractions of the tibialis anterior or peroneus muscles were observed through visual inspection and palpation during NMES, the electrodes were re-positioned medially and, in the case of mNMES, were sometimes cut smaller to more selectively activate triceps surae.

4.2.4 Peak twitch torque (PTT) and the maximal evocable M-wave (M_{max})

At the beginning of each session, PTT and M_{max} were determined using single pulses delivered over the tibial nerve trunk (nNMES). Current amplitude was increased incrementally every 8 to 10 s to ~1.5 times the current required to evoke $M_{max.}$ This NMES intensity was sufficient to generate maximal PTT and M-waves in all participants. The number of pulses used for this assessment was consistent between sessions and was always less than 10.

4.2.5 Setting NMES intensity

To set the NMES intensity for the fatigue protocol, 2 s trains of 20 Hz NMES were delivered 20 s apart while the NMES intensity was adjusted until the peak torque generated was equivalent to the PTT for that participant. Approximately 5 NMES trains were required to set the NMES intensity for each

session. Once the NMES intensity was set, it was not altered for the remainder of the session. In people with chronic SCI, PTT of the plantar flexors is equivalent to ~27% of the torque generated during maximal tetanic (40 Hz) NMES (52). Thus, PTT provides a convenient sub-maximal normalisation value. We chose to set the NMES for the fatigue protocol at this sub-maximal intensity for a few reasons: 1) NMES is often delivered at sub-maximal intensities in rehabilitation settings, 2) sub-maximal intensities minimise the risk of fracturing osteoporotic bones in people with chronic SCI (21) and 3) sub-maximal intensities minimise antidromic collisions in motor axons (30), allowing for a contribution through *central* pathways.

4.2.6 Fatigue protocol

Five min after setting the NMES intensity, a fatigue protocol consisting of intermittent 20 Hz trains, 2-s-on-2-s-off for 5 min (75 contractions) was delivered. The 20 Hz frequency was chosen because: 1) it is the highest frequency that allows for accurate soleus H-reflex analysis uncontaminated by the NMES artefacts (50 ms inter-pulse interval), 2) it minimises the incidence of muscle spasms compared with higher frequencies (51), 3) it is within a recommended frequency range (18 to 25 Hz) for NMES of the lower limbs (50).

4.2.7 Data collection and analysis

Data were sampled at 10 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analysis that was conducted using custom written Matlab software (The Mathworks, Natick, MA). PTT was measured as the mean peak torque generated

by 3 supra-maximal nNMES pulses delivered at the beginning of each session. Torque generated during the fatigue protocol was normalised to each participant's PTT recorded during each respective session. The amplitude of each M-wave and H-reflex recorded during the fatigue protocol was measured peak-to-peak and normalized to each participant's M_{max} recorded at the beginning of each session. To prevent over-estimation of M-wave amplitude, due to contamination of the EMG signal by the NMES artefact, all data were analysed post-hoc using a 2-step software based signal processing procedure that removes the exponentially decaying tail of the NMES artefact (45). To quantify asynchronous activity, the root mean squared of baseline EMG activity over a 10 ms interval between the M-wave and H-reflex was calculated. As in a previous study (5), a duration of 10 ms was chosen since this time window was not contaminated by the NMES artefact, M-wave or H-reflex. To prevent over-estimation of the root mean squared calculation, all data in the intervals over which asynchronous activity was quantified were fit to a 2^{nd} order polynomial using the least squares procedure to remove any trend in the baseline associated with the preceding M-wave. The 2^{nd} order polynomial was subtracted from the raw data before the root mean squared was calculated, leaving the de-trended data with a mean of zero.

The amplitude of torque, M-waves, H-reflexes and asynchronous activity during each fatigue protocol were calculated for each 2 s contraction (40 EMG measurements / contraction). For each participant, torque, M-waves, H-reflexes and asynchronous activity were averaged over 5 successive contractions (20 s intervals) throughout the fatigue protocol to generate 15 data bins (i.e. bin 1 =

mean of contractions 1 to 5, bin 2 = mean of contractions 6 to 10, etc.) for each NMES site. Group means were calculated by pooling these mean data. A fatigue index was calculated for each fatigue protocol by dividing the mean torque for bin 15 by the mean torque for bin 1 and multiplying by 100 (i.e. mean $torque_{bin15}$ / mean $torque_{bin\ 1}$ x 100).

Statistical analyses were performed on group and individual data using Statistica software (StatSoft, Tulsa, OK). Shapiro-Wilk's tests for normality showed that all data were normally distributed. Dependent (paired) *t*-tests were used to test for differences in PTT and fatigue index between NMES sites. Separate 2-factor repeated measures analysis of variance (rmANOVA) tests (2 x 15) were used to determine the influence of *NMES site* (mNMES x nNMES) and *Time* (bin 1 to 15) on torque, M-waves, H-reflexes and asynchronous activity during the 5 min fatigue protocol. For analysis of asynchronous activity, we calculated the root mean squared of the baseline EMG prior to delivery of NMES, when no NMES-evoked asynchronous activity would be present, and included these data as a 16th level of *Time* in the rmANOVA test (4 x 16), to determine whether asynchronous activity developed during NMES. Univariate correlational analyses (Pearson product-moment correlations) were used to determine whether changes in M-wave amplitude correlated significantly with changes in torque in each participant during the fatigue protocol. The mean correlation coefficient was qualified as either *very weak* (r = 0.0 to 0.19), *weak* (r = 0.2 to 0.39), *moderate* (r = 0.4 to 0.59), *strong* (r = 0.60 to 0.79) or *very strong* (r = 0.8 to 1.0)(48).

The above analyses permitted comparisons based on *where* NMES was delivered (mNMES versus nNMES), independent of *how* (M-waves versus H-reflexes) contractions were generated. However, 4 of 8 participants generated contractions without any measureable activity through *central* pathways, regardless of NMES site. Therefore, to permit comparisons of torque based on *how* contractions were generated, we divided our participants into 2 groups based on whether H-reflexes contributed to contractions during nNMES (Group 1; n = 4) or not (Group 2; n = 4) and used a 2-factor mixed between-within participants rmANOVA test (also known as a split-pot rmANOVA test) to test for differences in fatigue index between NMES sites both between and within groups. H-reflexes were considered absent if: 1) no obvious waveform was present at an appropriate H-reflex latency, and 2) if the mean peak-to-peak measurement over this period was less than 2% M_{max}. Significant main effects and interactions identified by the ANOVA tests were tested post-hoc using Tukey's honestly significant difference tests when appropriate. An alpha level of 0.05 was used to evaluate statistical significance. All data are reported as mean ± standard error.

4.3 Results

The results of these experiments have been divided into 2 sections. The first section provides a comparison of contraction fatigue based on *where* NMES was delivered (mNMES versus nNMES), independent of *how* (M-waves versus H-reflexes) contractions were generated. This analysis was conducted to test the hypothesis that fatigue would occur sooner and would be greater during mNMES

than nNMES and was undertaken based on the expectation that *how* the contractions were generated would be markedly different between NMES sites in all participants (5, 7). Unexpectedly, however, only half of the participants generated contractions through *central* pathways (H-reflexes) during nNMES. As such, the second section describes the results of analyses designed to compare fatigue between participants who generated contractions with (Group 1; n = 4) and without (Group 2; n = 4) H-reflexes during nNMES.

There was no asynchronous activity greater than measured at baseline during NMES at either site and thus these data are not presented in this Results section.

4.3.1 Comparing fatigue based on *where* NMES was delivered (mNMES versus nNMES)

There were no significant differences in PTT between mNMES (8.2 ± 1.9 Nm) and nNMES (8.2 ± 2.0 Nm) sessions [$t_{(7)} = 0.44$, $p = 0.67$]. The current used for the fatigue protocols was 162.6 ± 12.1 mA for mNMES and 46.0 ± 5.8 mA for nNMES.

Figure 4-2 shows mean torque (A), M-wave (B) and H-reflex (C) amplitudes during the mNMES and nNMES fatigue protocols. Each bin represents data averaged over 5 successive contractions for each participant and then averaged across the group. For torque, there was a significant interaction between *NMES site* and *Time* [$F_{(14, 98)} = 1.80$, $p = 0.04$]. Compared to the first data bin (time 0 to 20 s), torque declined significantly (*dagger* †) starting between 41-60 s into the fatigue protocol (bin 1 > bins 3-15) for both mNMES and nNMES.

However, nNMES generated more torque than mNMES during the last 1/3 of the fatigue protocol (bins 11 to 15; *asterisk* *****). By the end of the fatigue protocols (bin 15), torque had dropped by ~73% (compared to bin 1) for mNMES and ~55% for nNMES. Accordingly, Figure 4-3 shows that the fatigue index for the group (n = 8) was significantly smaller during mNMES than nNMES ($t_{(7)} = 2.39$; $p = 0.04$).

For M-waves, there was a significant interaction between *NMES site* and *Time* [$F_{(14, 98)} = 5.90$, $p < 0.01$]. Throughout the entire fatigue protocol, M-waves were significantly larger during mNMES than nNMES. Compared to the first data bin (time 0 to 20 s), M-waves declined significantly (*double dagger* ‡) starting between 61 to 80 s into the fatigue protocol (bin 1 > bins 4 to 15) during mNMES. For H-reflexes, there was also a significant interaction between *NMES site* and *Time* [$F_{(14, 98)} = 4.45$, $p < 0.01$]. H-reflexes were significantly larger during nNMES than mNMES throughout the entire fatigue protocol. Compared to the first bin, H-reflexes increased significantly (*number sign* #) starting between 201 to 220 s into the fatigue protocol (bin 1 < bins 11 to 15) during nNMES. Across the group of 8 participants, M-waves were 6 to 7 times smaller and H-reflexes were 10 to 15 times larger during nNMES than mNMES, when averaged over the entire fatigue protocol (Figure 4-2B and 4-2C).

During the fatigue protocol, torque was *strongly* and *moderately* correlated with M-waves for mNMES (mean correlation: r = 0.66, participant range: -0.55 to 0.99, significant correlations: 7 out of 8) and nNMES (mean correlation: r = 0.55, participant range: -0.19 to 0.98, significant correlations: 6 out of 8), respectively.

However, changes in torque were not tightly coupled to changes in M-waves in each participant (Figure 4-4). The length of each line represents the magnitude of the decrease in torque, while the slope of each line represents the magnitude of the increases or decreases in M-wave amplitude.

4.3.2 Comparing fatigue based on *how* contractions were generated (M-waves versus H-reflexes)

Unexpectedly, nNMES generated H-reflexes in only 4 of 8 participants. When averaged across the entire nNMES fatigue protocol, M-waves were 3.8 ± 0.1 $\%M_{max}$ while H-reflexes were 22.1 ± 11.7 $\%M_{max}$ in the 4 participants who generated contractions through H-reflex pathways. For the 4 participants who did not have H-reflexes during nNMES, M-waves were 5.3 ± 0.2 $\%M_{max}$, averaged across the entire fatigue protocol.

Figure 4-5 shows torque and EMG recorded from a participant in whom H-reflexes contributed sporadically to contractions during mNMES (Figure 4-5A) and in whom contractions were generated almost exclusively through H-reflexes (Figure 4-5B). During the initial 5 contractions, torque was similar (~15 Nm) between NMES sites. However, by the end of the fatigue protocol, mNMES generated ~6 Nm of torque, while nNMES generated ~10 Nm of torque. During mNMES, contractions were evoked by successive M-waves with H-reflexes appearing in relatively few contractions. This was the only participant in whom H-reflexes were generated during mNMES. Interestingly, H-reflexes were largest during the 9 contractions in which torque spiked during mNMES (contractions 28, 46, 48, 57, 64, 65, 68, 69, 74; Figure 4-5A). The large H-reflex during mNMES

that is shown in the EMG in Figure 4-5A corresponds with the last NMES pulse from contraction 74. This is the only participant in whom contractions were generated almost exclusively through H-reflexes during nNMES (Figure 4-5B).

In contrast to the data shown in Figure 4-5, Figure 4-6 shows data recorded from a participant in whom, regardless of NMES site, contractions were evoked by successive M-waves with no measurable H-reflex. In this participant, torque was similar during NMES at both sites and decreased from ~8 Nm at the beginning to ~1 Nm by the end of each NMES fatigue protocol.

To determine whether generating contractions through H-reflex pathways influenced the fatigue-resistance of evoked contractions, fatigue indices were compared between and within groups of participants who generated contractions with (Group 1; n = 4) and without (Group 2; n = 4) H-reflexes during nNMES. As mentioned previously, H-reflexes were generated, but only sporadically, in 1 of 8 participants during mNMES (Figure 4-5A). Figure 4-7 shows the fatigue index for Groups 1 and 2 during NMES at both sites. There was a significant interaction between *NMES site* and *Group* ($F_{(1,6)}$ = 26.0; $p < 0.01$). During mNMES, there was no difference in fatigue index between groups, both of which generated contractions mainly through successive M-waves ($p = 0.98$). During nNMES, there was less fatigue when H-reflexes contributed to contractions as the fatigue index was significantly larger for Group 1, all of whom generated contractions through H-reflex pathways, than Group 2, who generated contractions only through successive M-waves ($p = 0.047$). Within Group 1, the fatigue index during mNMES (mainly M-waves) was significantly smaller than during nNMES

(when H-reflexes contributed; $p < 0.01$). Within Group 2, there was no difference in fatigue index between NMES sites, both of which generated contractions through M-waves only ($p = 0.96$). When taking into consideration *how* contractions were generated, torque decreased the least when nNMES generated contractions through H-reflexes (Group 1, n = 4, nNMES, ~39% decrease) and torque decreased the most when nNMES and mNMES generated contractions only through M-waves (Group 2, n = 4, nNMES, ~71% decrease; Group 1 and 2, n = 8, mNMES, ~73% decrease).

4.4 Discussion

The present experiments were designed to investigate whether fatigue can be reduced using nNMES, which can recruit motor units according to the *size principle*, compared with a more traditional approach using mNMES, which tends to recruit motor units randomly with respect to type. To test our hypothesis that fatigue would occur sooner and would be greater during mNMES than nNMES, we compared torque based on *where* NMES was delivered (mNMES versus nNMES), independent of *how* contractions were generated (M-waves versus H-reflexes). Our hypothesis was based on the expectation that mNMES and nNMES would generate contractions through markedly different pathways in each participant (5, 7). However, unexpectedly, this was not the case, as nNMES generated contractions through H-reflex pathways in only 4 of 8 participants. As such, we divided our participants into 2 groups based on whether H-reflexes contributed to contractions during nNMES (Group 1; n = 4) or not (Group 2; n =

4). This second way of analysing the data compared fatigue based on *how* contractions were generated and tested more specifically whether contractions generated through H-reflex pathways were more fatigue-resistant than contractions generated only by successive M-waves.

4.4.1 Comparing fatigue based on *where* NMES was delivered (mNMES versus nNMES)

Contrary to our hypothesis, mNMES did not fatigue sooner than nNMES. Consistent with our hypothesis, however, mNMES generated significantly less torque than nNMES over the last 1/3 of the 5 min fatigue protocol and the fatigue index was significantly smaller for mNMES than nNMES. Thus, for isometric contractions of the chronically paralysed plantar flexors, mNMES generated contractions that fatigued more than nNMES. This is the first demonstration that *where* NMES is delivered can influence the fatigue-resistance of the evoked contractions.

As expected, based on experiments on people without SCI (5, 7), the extent to which transmission along *central* and *peripheral* pathways contributed to evoked contractions differed between NMES sites in the present study. Across the group of 8 participants, M-waves were 6 to 7 times smaller and H-reflexes were 10 to 15 times larger during nNMES than mNMES, when averaged over the entire fatigue protocol (Figure 4-2B and 4-2C). This is the first demonstration that *where* NMES is delivered affects *how* contractions are generated in people who have had a SCI. We believe that this effect of NMES site reflects differences in

how motor and sensory axons are recruited beneath the electrodes between sites (see Ref 6 for discussion).

The progressive decline in M-wave amplitude that often occurs when fatigue develops during NMES-evoked contractions (53) is thought to reflect failure of neuromuscular propagation between the stimulation and recording sites (22). Propagation failure can occur beneath the stimulating electrodes (33), at axonal branch points (12, 35), at the neuromuscular junction (35), or at the sarcolemma (39). Presently, M-wave amplitude decreased significantly during mNMES, but not during nNMES. The reason that M-waves did not change during nNMES is not clear, however, M-waves were much smaller during nNMES than mNMES. It may be that, if the nNMES intensity was increased to generate larger M-waves, a decline in M-wave amplitude would have been observed.

Although torque was *strongly* and *moderately* correlated with M-wave amplitude during the fatigue protocol for mNMES and nNMES, respectively, the changes in torque were not tightly coupled to the changes in M-wave in each participant. For example, people whose contractions fatigued the most did not necessarily have M-waves that decreased the most and, in others, torque declined substantially while M-waves were relatively unaffected or even increased. This dissociation between torque and M-wave amplitude indicates that the fatigue observed presently is not only due to neuromuscular propagation failure. This is consistent with the finding that in the chronically paralysed plantar flexors, M-wave amplitude fully recovered 5 min after a fatigue protocol, while torque remained depressed by 50% (53). Thus, the primary contributor to the fatigue

presently observed is likely distal to the sarcolemma and related to impaired excitation-contraction coupling. Accordingly, NMES approaches that recruit motor units that are relatively resistant to developing fatigue due to failure of excitation-contraction coupling, such as nNMES in the present study, hold promise for reducing fatigue during NMES (see Section 4.4.3 below).

In contrast to the progressive decline in M-waves during mNMES, H-reflexes increased during nNMES. At the same time, M-waves did not change, suggesting that the changes in H-reflexes were not due to changes at the NMES site. Instead, the enhanced H-reflexes may be due to post-activation potentiation of synaptic transmission (29) or to the activation of persistent inward currents in spinal neurons (26, 32).

Unlike previous work conducted on the plantar flexors in people without SCI (5), we recorded no asynchronous activity in any of our participants with SCI. In this previous work (5), NMES was delivered using relatively long pulse durations (1000 μs) with brief periods of high frequencies (100 Hz) and/or with long on-times (7 to 8 s-on). Thus, the lack of asynchronous activity presently may be due to our NMES parameters that generated a relatively small afferent volley, which may have been insufficient for generating asynchronous activity (19). Alternatively, asynchronous activity and the mechanisms that generate it may be less prevalent in people with SCI than in people without. In line with this latter idea, it may be that previous recordings of asynchronous activity in participants without SCI (5) were the result of involuntary descending drive (5, 24) which would not be present in our participants with chronic motor-complete SCI.

4.4.2 Comparing fatigue based on *how* contractions were generated (M-waves versus H-reflexes)

Although nNMES generated contractions that fatigued the least, unexpectedly, H-reflexes contributed to evoked contractions in only 4 of 8 participants. This, however, provided a unique opportunity to test more specifically the effect of H-reflexes on the fatigue-resistance of NMES-evoked contractions. During nNMES, there was a significantly larger fatigue index (~61%) for the 4 participants in Group 1 in whom H-reflexes contributed to contractions, compared with the 4 participants in Group 2 in whom H-reflexes did not contribute (~29%), supporting the idea that generating contractions through H-reflex pathways improves the fatigue-resistance of NMES-evoked contractions of the paralysed plantar flexors in people with chronic motor-complete SCI. Importantly, this difference in fatigue index was not because participants in Group 1 had plantar flexors that were more fatigue-resistant than those in Group 2, since there was no difference in fatigue index between groups during mNMES when contractions were generated predominantly by M-waves in both groups. Further, within the participants in Group 1, the fatigue index for nNMES (when H-reflexes contributed to contractions) was significantly larger than the fatigue index for mNMES (when H-reflexes were mainly absent), providing clear evidence that the differences in fatigue are not due to differences in muscle quality, but rather reflect the fact that H-reflexes reduced fatigue. Although this is not the first demonstration that H-reflexes can contribute to NMES-evoked contractions of muscle paralysed by SCI (11), this is the first demonstration that generating

contractions through this pathway improves the fatigue-resistance of NMES-evoked contractions of the paralysed plantar flexors in people with chronic motor-complete SCI.

The prevalence of soleus H-reflexes in people with SCI is often under-reported, however our finding that H-reflexes contributed to evoked contractions in only 4 of 8 participants is inconsistent with 2 previous studies in which H-reflexes were evoked in 10 of 12 (11) and 7 of 9 (16) participants with motor-complete SCI. The discrepancy in H-reflex prevalence between these studies and the present study may be explained by differences in NMES pulse duration. These previous studies utilised a 1000 μs pulse duration that is optimal for generating soleus H-reflexes (36, 46), whereas presently, a 200 μs pulse duration was utilised due to limitations of our stimulator (DS7AH). Regardless of pulse duration, in people with chronic SCI who are free from lower motor neuron damage, complete absence of soleus H-reflexes during nNMES is surprising since inhibition of the H-reflex pathway is reduced after SCI. People with chronic SCI show reduced post-activation depression (49), reduced Ia presynaptic (23) and reciprocal (9, 15) inhibition of neural circuits that control the soleus muscle. Of note, 4 of 8 participants tested presently were taking baclophen ($GABA_B$ receptor agonist) to minimise muscle spasms (17), however, this did not seem to influence the prevalence of H-reflexes since 2 participants who were taking baclophen (80 mg / day) generated contractions through H-reflexes, while 2 other participants who were not taking baclophen did not (Table 4-1).

4.4.3 Clinical Significance

Contraction fatigue during NMES limits its effectiveness for clinical applications (43). Although it has not been tested directly, such fatigue is thought to be due, in part, to the random order in which motor units are recruited during NMES (1, 20, 31, 41). We propose that the presently observed improvements in fatigue-resistance, when contractions were generated through H-reflexes, were the result of recruiting motor units in their physiological recruitment order, since slow fatigue-resistant motor units (type I) dominate the soleus H-reflex (10, 57). However, in people with chronic SCI, soleus is made up of predominantly type IIB fibres (28) and fatigue is consistent with a complete transition from slow fatigue-resistant to fast fatigable motor units (51). The finding that H-reflexes improve fatigue-resistance in people who have chronic motor-complete SCI might indicate that a more natural recruitment order reduces fatigue even in a muscle that is reportedly made up of predominantly type II muscle fibres. Regardless, we would expect the fatigue-resistance of contractions driven through H-reflexes to be even better in people with SCI who have regular experience with NMES, as they typically have better muscle quality than their sedentary counterparts (54, 55). Alternatively, H-reflexes may recruit different motor unit populations, of similar threshold, pulse by pulse, or may recruit motor units with *rotation* (4, 58), whereby motor units that have been discharging for long periods of time, stop firing and are replaced by previously inactive motor units of similar threshold. Such alternation of motor unit activity may improve the fatigue-resistance of NMES-evoked contractions, regardless of recruitment order, by providing time

for contractile elements in previously active motor units to recover, while still maintaining torque output.

Despite the observed improvements in fatigue-resistance during nNMES, there are a number of practical issues to consider before taking this work from the laboratory to applications for rehabilitation. Firstly, the torque generated by contractions generated by H-reflexes is less consistent both within (3) and between (3, 7) successive contractions. Thus, it may prove difficult to adequately control contractions for fine motor tasks. Secondly, it is unclear whether contractions driven by H-reflexes will be of sufficient amplitude to restore function for applications such as standing and walking, although presently contractions of 100% PTT, which is equivalent to ~27% of torque generated during maximal tetanic (40 Hz) NMES (52), were evoked, at least in part, through H-reflexes in 4 participants. Whether contractions of sufficient amplitude can be generated through H-reflex pathways with increasing NMES intensities, in which case motor unit recruitment through H-reflex pathways will progressively decline through increases in antidromic collision in motor axons (59), has yet to be determined. Lastly, only half of the present participants had H-reflexes during nNMES. Thus, nNMES may not reduce fatigue in everyone. However, the NMES parameters were not optimal for generating H-reflexes in the present study (see Section 4.4.2 above) and it may be possible to generate robust contractions through H-reflexes in more participants when NMES parameters are optimised.

4.4.4 Summary

Presently we report 4 novel findings: 1) We show for the first time that *where* NMES is delivered (mNMES versus nNMES) can affect how contractions are generated in people with chronic motor-complete SCI. 2) We demonstrate marked differences in the fatigue-resistance of contractions evoked by mNMES and nNMES in these people. 3) We go on to show that this fatigue-resistance is dependent upon *how* contractions are generated; contractions generated by H-reflexes fatigued less than those generated only by M-waves. 4) Asynchronous motor unit activity did not contribute to NMES-evoked contractions of muscle paralysed by SCI. In conclusion, nNMES generates contractions that are more fatigue-resistant than mNMES, but only when H-reflexes contribute to the evoked contractions. Generating contractions through H-reflex pathways may be advantageous when fatigue limits the benefits of NMES-based rehabilitation programs. Whether contractions generated by transmission along H-reflex pathways will be effective for restoring functional movement remains to be determined.

4.5 Tables

Table 4-1 Participant demographics

Code/Sex	Age	Years after SCI	Level of SCI	AIS	Baclophen (mg/day)	H-reflex
1F	33	10	C 4-5	B	0	Yes
2M	58	5	C 6-7	B	0	Yes
3M	42	24	C 5-6	B	0	No
4M	29	11	C 5-7	B	80	Yes
5M	35	7	C 5	A	80	No
6M	45	4	T 4	A	40	No
7M	62	18	C 4-5	B	0	No
8M	25	3	C 5-6	B	80	Yes
9F	48	16	T 1-2	A	80	N/a
10M	57	22	C 5-6	B	0	N/a
11M	34	6	T 6-7	B	60	N/a

AIS, American spinal injury association impairment scale

4.6 Figures

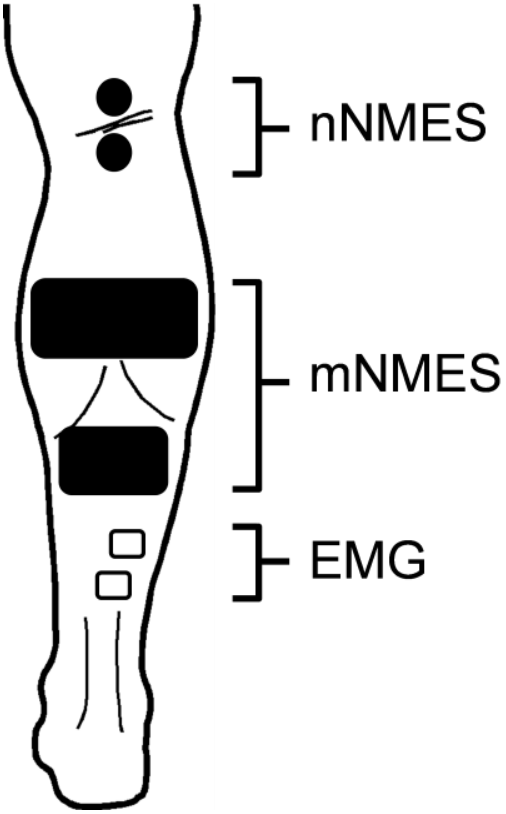

Figure 4-1 Schematic of the NMES and EMG sites on the right leg.

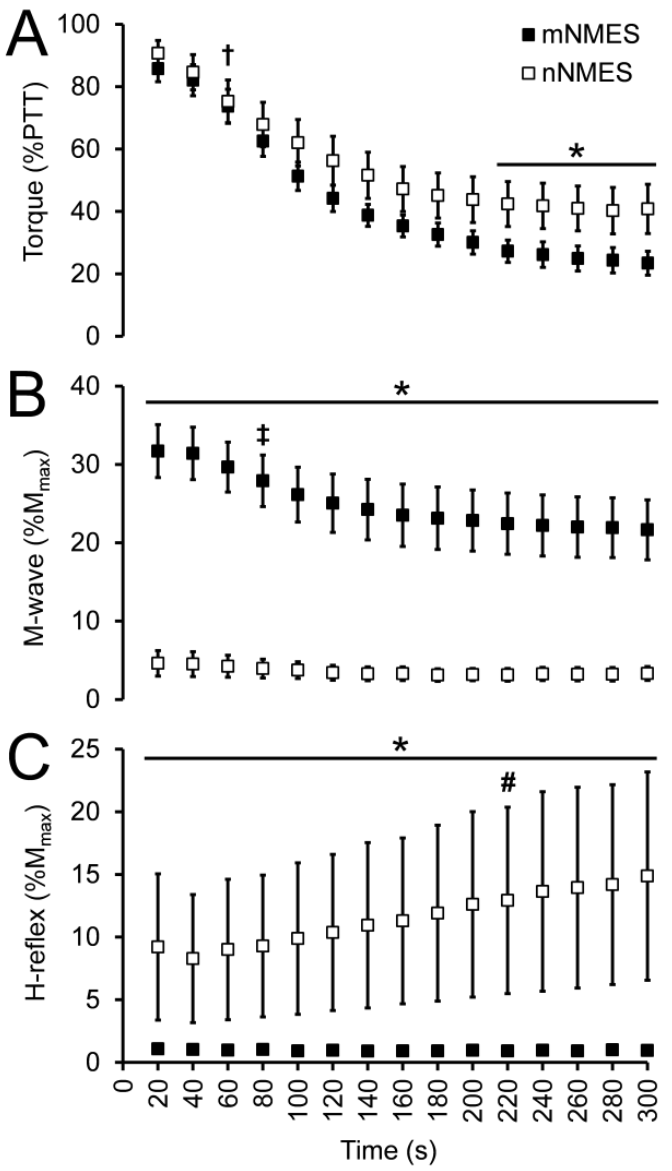

Figure 4-2 Torque (A), M-waves (B) and H-reflexes (C) during the mNMES and nNMES fatigue protocols (n = 8). Each symbol represents data averaged over 20 s (5 consecutive contractions; 1 bin). The dagger (†) indicates a significant decrease in torque from the initial 20 s bin for both mNMES and nNMES. The asterisk (*) indicates a significant difference between mNMES and nNMES. The double dagger (‡) indicates a significant decrease in M-waves from the initial 20 s bin for mNMES. The number sign (#) indicates a significant increase in H-reflexes from the initial 20 s bin for nNMES. Error bars represent 1 standard error.

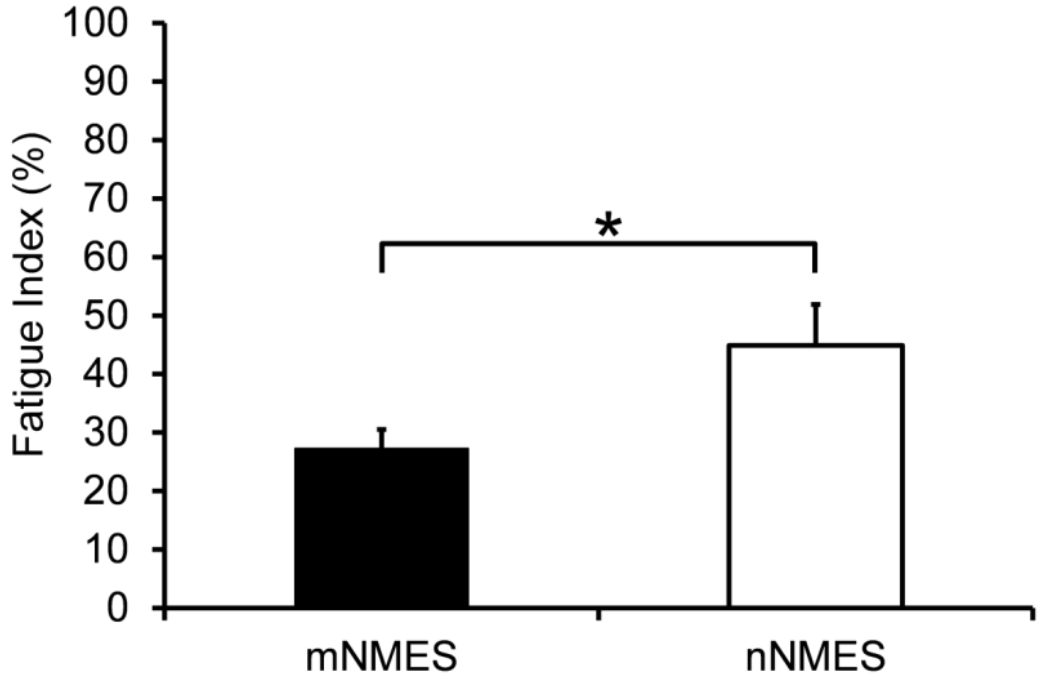

Figure 4-3 Fatigue indices (mean torquebin15 / mean torquebin 1 x 100) for the mNMES and nNMES fatigue protocols for the group (n = 8). Error bars represent 1 standard error.

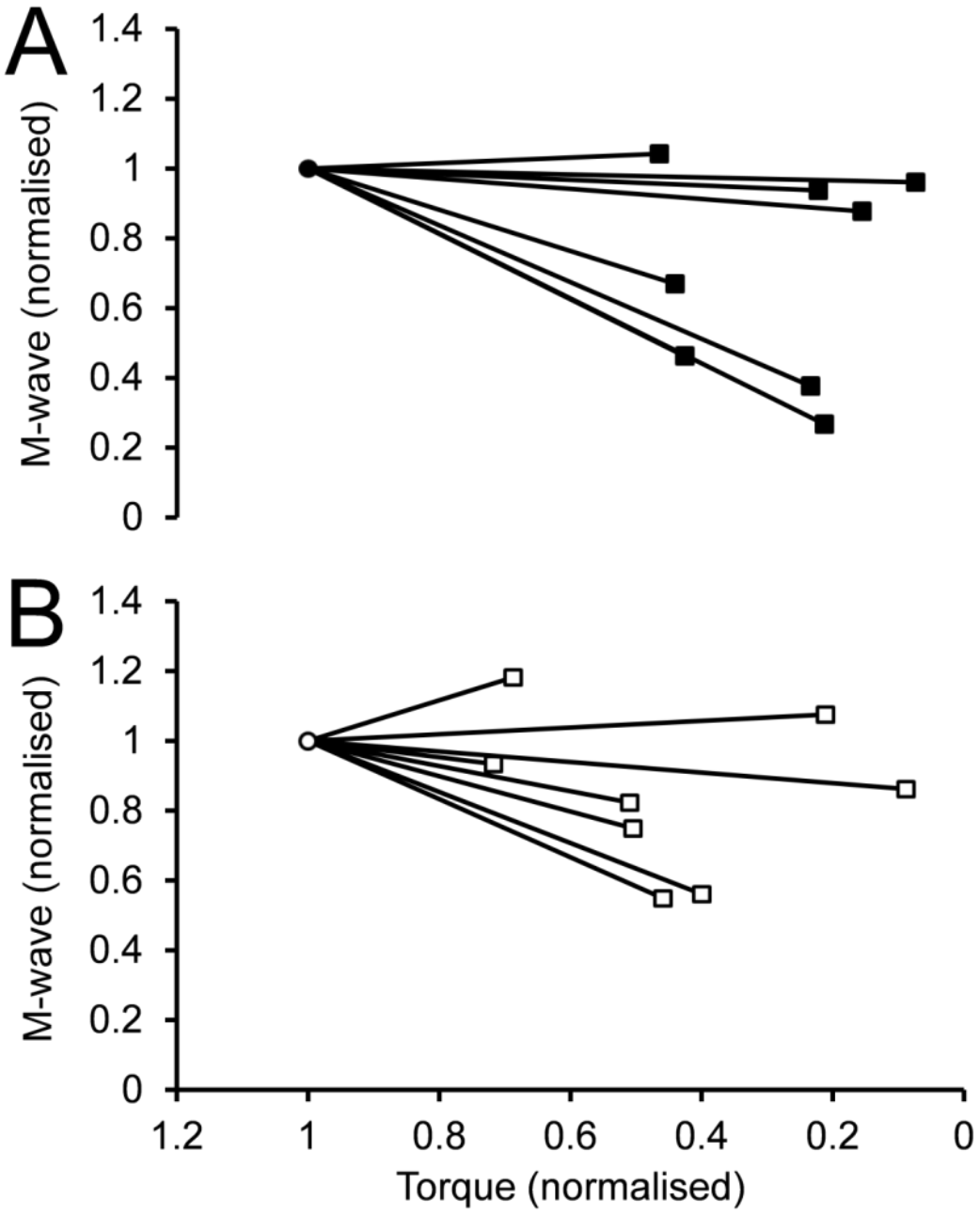

Figure 4-4 Normalised torque plotted against normalised M-waves for each participant (separate lines) at the beginning (bin 1; ● and ○) and end (bin 15; ■ and □) of the fatigue protocol during mNMES (A) and nNMES (B).

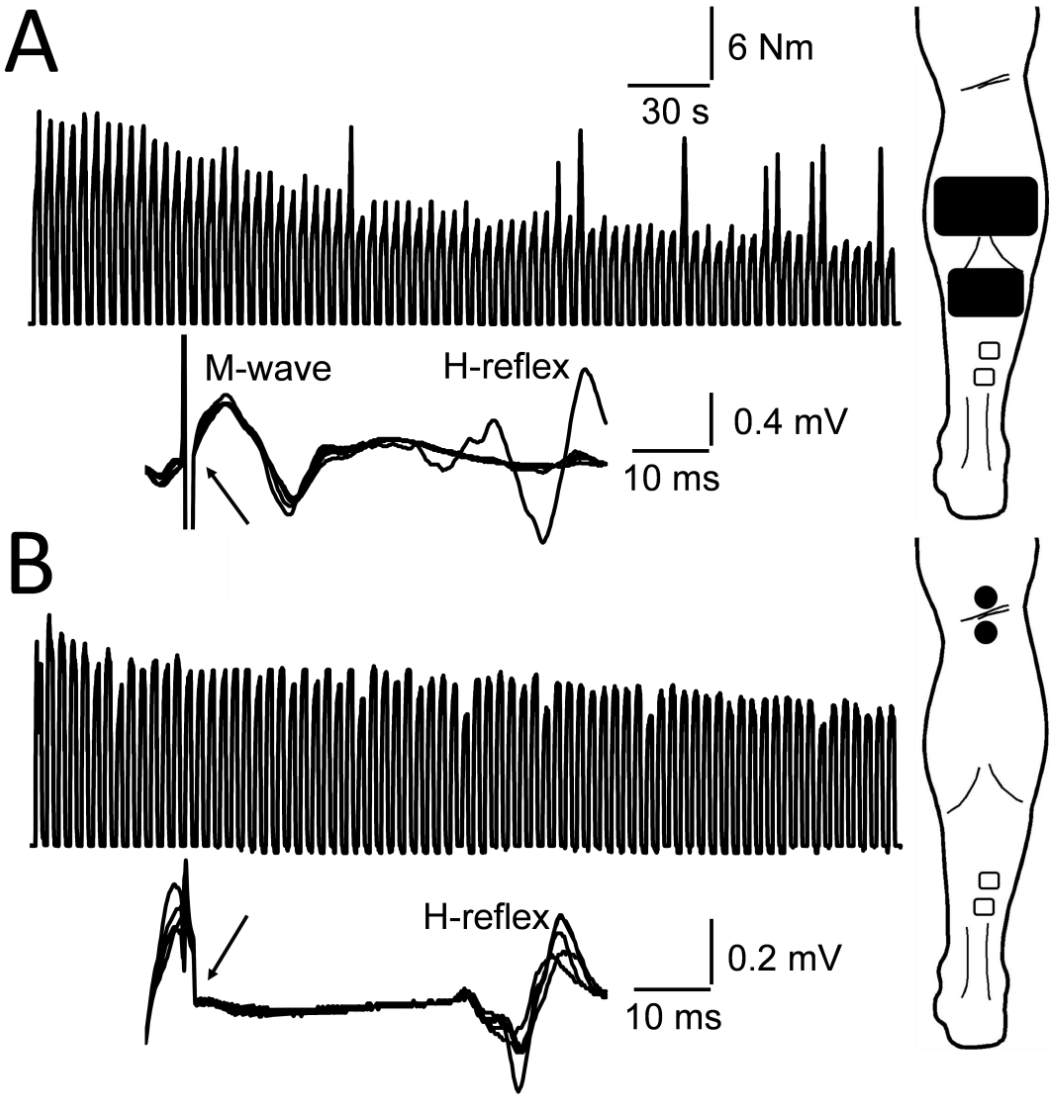

Figure 4-5 Torque and EMG evoked by mNMES (A) and nNMES (B) in a single participant who generated contractions with H-reflexes during nNMES (Group 1). In the top of each panel, the solid line represents torque in response to the 5 min, 2-s-on-2-s-off, fatigue protocol (75 contractions). The bottom of panels A and B show EMG in response to the last NMES pulse for each of the last 5 contractions. The arrows point to where the tails of the preceding NMES artefact (A) or H-reflex (B) were removed. All torque data are shown on the same scale, as indicated in panel A. EMG data are shown on different scales.

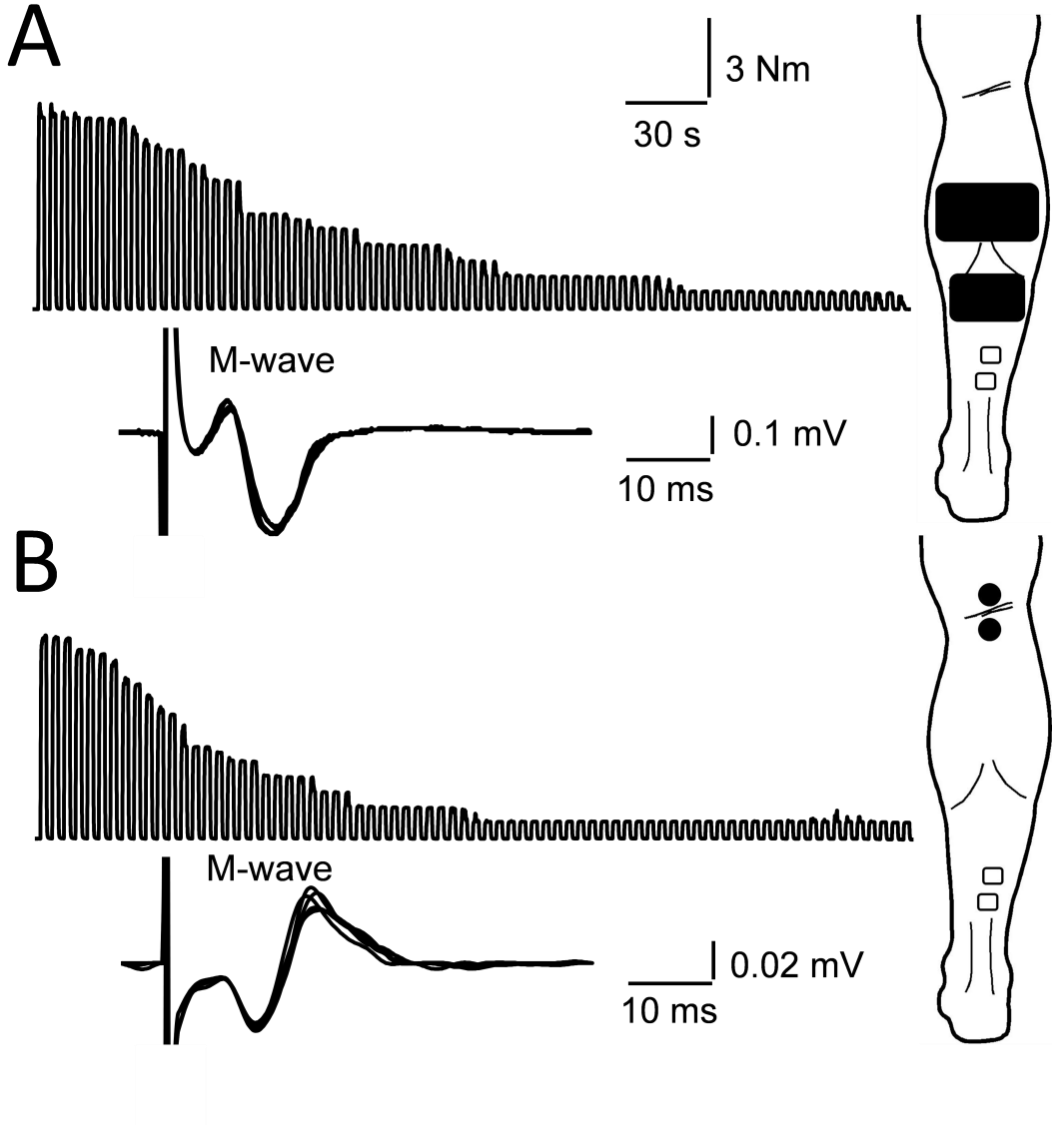

Figure 4-6 Torque and soleus EMG evoked by mNMES (A) and nNMES (B) in a single participant who generated contractions only through successive M-waves (Group 2). The organisation is equivalent to Figure 4-5. Torque data are shown on the same scale, as indicated in panel A. EMG data are shown on different scales.

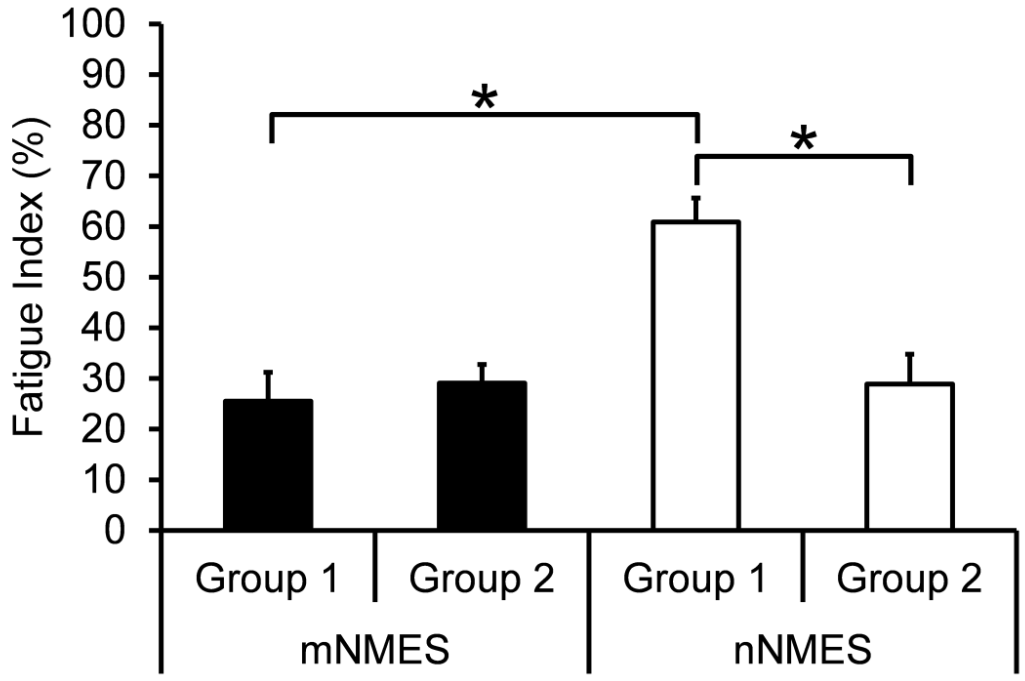

Figure 4-7 Fatigue indices (mean $torque_{bin15}$ / mean $torque_{bin1}$ x 100) for the mNMES and nNMES fatigue protocols for participants who generated contractions with (Group 1; n = 4) and without (Group 2; n = 4) H-reflexes during nNMES. H-reflexes were generated in only 1 of 8 participants during mNMES (see Figure 4-5A). Error bars represent 1 standard error.

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CHAPTER 5: H-REFLEXES IMPROVE FATIGUE-RESISTANCE OF ELECTRICALLY-EVOKED CONTRACTIONS IN PEOPLE WITH CHRONIC MOTOR-COMPLETE SPINAL CORD INJURY: EFFECT OF STIMULATION PULSE DURATION[5]

5.1 Introduction

Contractions generated by neuromuscular electrical stimulation (NMES) fatigue rapidly (3, 4, 23), limiting the effectiveness of NMES for restoring movement for people with spinal cord injury (SCI; Refs 4, 26 and 33). Much of this fatigue is thought to be due to the non-physiological way in which motor units are recruited during NMES (3, 4, 23). NMES typically recruits motor units by the activation of motor axons beneath the NMES electrodes, which generates a motor- or M-wave in the electromyographic (EMG) signal recorded from an innervated muscle (3). Motor unit recruitment in this way, through M-waves (*peripheral* pathway), occurs randomly with respect to motor unit type (1, 9, 17, 24). This random motor unit recruitment order is in stark contrast to recruitment during voluntary contractions, whereby fatigue-resistant motor units are recruited first according to Henneman's *size principle* (25). Therefore, contractions evoked by NMES are generated by relatively fewer fatigue-resistant motor units than voluntary contractions of similar amplitude. This kind of motor unit recruitment

[5] The individuals contributing to the work presented in this chapter were:
Bergquist AJ, Okuma Y, Wiest MJ and Collins DF.

may be particularly problematic for people with *chronic* SCI, whose motor units below the level of the lesion may be compromised to begin with (7, 15, 31, 34).

During NMES, motor units can also be recruited through the activation of sensory axons, which generates a Hoffmann- or H-reflex in the EMG signal (3). Motor unit recruitment in this way, through H-reflexes, follows the *size principle* (5, 36, 38). Recently, we have demonstrated that, in people with chronic motor-complete SCI, NMES-evoked contractions are more fatigue-resistant when motor units are recruited through H-reflexes (*central* pathways) compared with M-waves (*peripheral* pathway; Chapter 4 of the present thesis). Therefore, maximising motor unit recruitment through *central* pathways may be beneficial for further improving the fatigue-resistance of NMES contractions.

In people who are neurologically-intact, the extent to which contractions are generated through *central* pathways depends on the duration of the NMES pulses (21). Specifically, longer pulse durations (500 to 1000 μs) generate a larger H-reflex relative to the M-wave (20, 29, 30) because longer pulse durations preferentially activate sensory over motor axons (22, 39). Such differential recruitment is thought to be due to the fact that sensory axons have a longer strength duration time constant and lower rheobase than motor axons (22, 39). Since H-reflexes improve fatigue-resistance of NMES-evoked contractions in people with SCI (Chapter 4), and long pulse durations more effectively elicit H-reflexes when NMES is delivered in single pulses (20, 29, 30), and repetitively (21), then long pulse durations may be most effective for generating fatigue-resistant contractions. The effect of pulse duration on the recruitment of H-

reflexes and the fatigue-resistance of NMES-evoked contractions has not been tested in people with SCI.

The present experiments were designed to compare: 1) the recruitment of motor units through H-reflexes using a short (50 μs; $NMES_{50}$) and long (1000 μs; $NMES_{1000}$) pulse duration over a full range of NMES intensities to generate M-wave and H-reflex recruitment curves in people with SCI and 2) the fatigue-resistance of contractions evoked by $NMES_{50}$ (20 Hz, 2 s-on 2 s-off, 75 contractions), in which H-reflexes were predicted to be smallest, and $NMES_{1000}$, in which H-reflexes were predicted to be largest, in people with SCI. Based on a previous study in people who were neurologically-intact (20), we hypothesised that H-reflex versus M-wave (H vs M) recruitment curves would be shifted to the left when using $NMES_{1000}$ compared with $NMES_{50}$. We also hypothesised that fatigue, defined as a significant reduction in torque over repeated contractions (13), would occur sooner (after fewer contractions into a fatigue protocol) and would be greater (generate less torque by the end of a fatigue protocol) during $NMES_{50}$ compared with $NMES_{1000}$. The ankle plantar flexors were studied because these muscles are important for standing and walking and there is interest in stimulating them for people with SCI (2, 12, 19, 27). Additionally, we have demonstrated that the contributions of *peripheral* and *central* pathways to plantar flexor contractions generated by $NMES_{50}$ and $NMES_{1000}$ can be markedly different in people without SCI (21).

Herein we report findings from initial experiments with 4 participants. Sample size calculations (see Section 5.2.6.3 below) indicate that 12 *additional*

participants (24 additional experimental sessions) are required to detect significant differences for each dependent variable, assuming that effect sizes are unchanged upon further data collection. We plan to renew our ethics and continue data collection until we have satisfied our sample size requirements. We expect we will be collecting data for this project until September 2014.

5.2 Methods

5.2.1 Participants

Five participants with chronic (> 2 y) motor-complete SCI volunteered for this study after providing informed written consent (Table 5-1). Each of these participants had volunteered for a previous study involving NMES of the plantar flexors (Chapter 4). NMES generated considerable muscle spasms in one participant (participant 5M in Table 5-1). Thus, herein we report data collected from the 4 participants in whom we were able to generate contractions without spasms. All participants took part in 2 experimental sessions, each lasting ~2 h and separated by at least 5 d. In each session, NMES was delivered using either a 50 μs ($NMES_{50}$) or 1000 μs ($NMES_{1000}$) pulse duration. All procedures were performed on the left leg. Participants were comfortably secured in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York) to measure isometric plantar flexion torque. The left foot was strapped to the Biodex footplate with the hip at ~110°, the knee at ~90° and the ankle at ~90° with the lateral malleolus aligned with the axis of the dynamometer. With the knee at ~90°, the soleus muscle, the muscle from which we recorded, generates the

majority of plantar flexion torque (8, 32). This study was approved by the Health Research Ethics Board at the University of Alberta.

5.2.2 Electromyography (EMG)

Surface EMG was recorded from soleus using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT) arranged in a bipolar configuration. The electrodes were placed parallel to the predicted path of the muscle fibres with ~1 cm inter-electrode distance. A reference electrode was placed over the tibia of the left leg. EMG signals were amplified between 500 and 1000 times and band-pass filtered at 10 to 1000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

5.2.3 Neuromuscular electrical stimulation (NMES)

NMES was delivered using a constant-current stimulator (DS7A Digitimer, Welwyn Garden City, UK). NMES was delivered over the tibial nerve trunk in separate sessions using 2 flexible adhesive gel electrodes (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) placed on the skin of the popliteal fossa, with an inter-electrode distance of ~1 cm. If contractions of the tibialis anterior or peroneus muscles were observed through visual inspection and palpation during NMES, the electrodes were re-positioned medially to more selectively activate triceps surae.

5.2.4 H-reflex versus M-wave (H vs M) recruitment curves

At the beginning of each session, data were collected to construct an H vs M recruitment curve from responses to 50 single pulses delivered randomly every 8 to 10 s using either $NMES_{50}$ or $NMES_{1000}$. Current was delivered from below

M-wave and H-reflex threshold to ~1.5 times the minimum current required to evoke a maximal M-wave (M_{max}) or to maximal stimulator output, whichever was reached first.

5.2.5 Fatigue protocol

5.2.5.1 Peak twitch torque (PTT)

The NMES intensity for the fatigue protocol was adjusted relative to each participants peak twitch torque (PTT). Thus, after collecting data for the H vs M recruitment curve, PTT was determined using $NMES_{1000}$ pulses. Current amplitude was increased incrementally every 8 to 10 s up to ~1.5 times the current required to evoke M_{max}. This NMES intensity was sufficient to generate maximal PTT in all participants. The number of pulses used for this assessment was consistent between sessions and was always less than 10.

5.2.5.2 Setting NMES intensity

To set the NMES intensity for the fatigue protocol, 2 s trains of 20 Hz NMES were delivered 20 s apart while the current was adjusted until the peak torque generated during the 2 s train was equivalent to 100% of each participants PTT. Approximately 5 NMES trains were required to set the NMES intensity for each session. Once this intensity was set, the current was not altered for the remainder of the session. In people with chronic SCI, PTT of the plantar flexors is equivalent to ~27% of the torque generated during maximal tetanic (40 Hz) NMES (35). Thus, PTT provides a convenient sub-maximal normalisation value. We chose to set the NMES for the fatigue protocol at this sub-maximal amplitude for three reasons: 1) NMES is typically delivered at sub-maximal amplitudes in

rehabilitation settings, 2) sub-maximal amplitudes minimise the risk of fracturing osteoporotic bones in people with chronic SCI (10) and 3) sub-maximal amplitudes minimise antidromic collisions in motor axons (16), allowing for a contribution through H-reflexes.

5.2.5.3 Fatiguing NMES

After 5 min of rest following setting of the NMES intensity, intermittent 20 Hz NMES trains, 2-s-on-2-s-off for 5 min (75 contractions), were delivered. The 20 Hz frequency was chosen because: 1) it is the highest frequency that allows for accurate soleus H-reflex analysis uncontaminated by the NMES artefacts (50 ms inter-pulse interval), 2) it minimises the incidence of muscle spasms compared with higher frequencies (34), 3) it is within a recommended frequency range (18 to 25 Hz) for NMES of the lower limbs (33).

5.2.6 Data collection and analysis

Data were sampled at 10 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analysis that was conducted using custom written Matlab software (The Mathworks, Natick, MA). The amplitude of each M-wave and H-reflex was measured peak-to-peak. To prevent over-estimation of M-wave amplitude, due to contamination of the EMG signal by the NMES artefact, all EMG data were analysed post-hoc using a 2-step software based signal processing procedure that removes the exponentially decaying tail of the NMES artefact (28) as done previously (Chapter 4).

5.2.6.1 H vs M recruitment curves

In some participants, there was no plateau in M-wave amplitude during the recruitment curves using $NMES_{50}$, even at maximal stimulator output (100 mA). Thus, for each participant, all M-waves and H-reflexes were normalised to the single largest M-wave (M_{max}) from the trials used to identify each participants PTT in which NMES was delivered using $NMES_{1000}$ (1000 μs pulse duration). Three characteristics of each H vs M recruitment curve were quantified: 1) H_{max}-to-M_{max} ratio (H_{max}/M_{max}), 2) H-reflex amplitude when the M-wave was 5% M_{max} ($H_{5\%Mmax}$) and 3) M-wave amplitude at H_{max} (M_{Hmax}). H_{max} was calculated as the average of the 3 largest H-reflexes from the H vs M recruitment curves. $H_{5\%Mmax}$ was calculated as the mean amplitude of the H-reflexes that were accompanied by M-waves that fell between 2% and 8% of M_{max}. Between 6 and 13 H-reflexes fell within this range for a given participant and were included in the average. M_{Hmax} was calculated as the average amplitude of the 3 M-waves that accompanied the 3 largest H-reflexes used for the H_{max} calculation.

5.2.6.2 Fatigue protocol

PTT was measured as the mean peak torque generated by 3 supra-maximal $NMES_{1000}$ pulses, delivered after identifying the NMES intensity required to achieve PTT. Torque generated during the fatigue protocol was normalized to each participant's PTT. The amplitude of torque, M-waves and H-reflexes during the fatigue protocols were calculated for each 2 s contraction (40 EMG measurements / contraction). For each participant, torque, M-waves and H-reflexes were averaged separately over 5 successive contractions (20 s intervals)

throughout the fatigue protocol to generate 15 data bins (i.e. bin 1 = mean of contractions 1 to 5, bin 2 = mean of contractions 6 to 10, etc.) for each protocol. Group means were calculated by pooling these mean data. A fatigue index was calculated for each fatigue protocol by dividing the mean torque for bin 15 by the mean torque for bin 1 and multiplying by 100 (mean $torque_{bin15}$ / mean $torque_{bin\ 1}$ x 100).

5.2.6.*3 Statistical a*nalyses

Given the small sample size of the present data set (n = 4), no statistical analyses were conducted to test our hypotheses. Rather, herein we report the results of sample size calculations and descriptive statistics for the data collected thus far. Sample size calculations were performed using G*Power 3.1 software (11) and were based on achieving a power of 0.80 and the assumption that effect sizes will remain the same upon further data collection. Upon further data collection, statistical analyses will be performed using Statistica software (StatSoft, Tulsa, OK).

Dependent (paired) *t*-tests will be conducted on group data to test for differences in H_{max}/M_{max}, $H_{5\%Mmax}$, M_{Hmax}, PTTs and fatigue indices between NMES pulse durations. Sample size calculations for dependent *t*-test analyses indicate that statistically significant differences between NMES pulse durations will be identified for H_{max}/M_{max}, $H_{5\%Mmax}$ and M_{Hmax} with 13 (d = 0.85), 4 (d = 2.26) and 3 (d = 4.19) participants who generate H-reflexes, respectively. A sample size calculation was not done on PTT because it is not expected that PTT will differ between sessions since PTT is generated with $NMES_{1000}$ during both

sessions. Sample size calculations for the dependent *t*-test analysis of fatigue indices indicate that statistically significant differences between NMES pulse durations will be identified with 16 (d = 0.76) participants.

Separate 2-factor repeated measures analysis of variance (rmANOVA) tests (2 x 15) will be conducted on group data to determine the influence of *NMES Pulse Duration* ($NMES_{50}$ x $NMES_{1000}$) and *Time* (bin 1 to 15) on torque, M-waves and H-reflexes during the 5 min fatigue protocol. Sample size calculations for the rmANOVA *interaction* for torque, M-waves and H-reflexes indicate that statistically significant differences between NMES pulse durations will be identified with 8 (η^2 = 0.037), 7 (η^2 = 0.046) and 5 (η^2 = 0.057) participants, respectively. Significant main effects and interactions identified by the rmANOVA tests will be tested post-hoc using Tukey's honestly significant difference tests when appropriate. An alpha level of 0.05 will be used to evaluate statistical significance. All data are reported as mean ± standard error.

5.3 Results

5.3.1 H vs M recruitment curves

Figure 5-1A displays H vs M recruitment curves collected using $NMES_{50}$ (50 μs) and $NMES_{1000}$ (1000 μs) from a single participant. In this participant, M-waves during $NMES_{50}$ did not plateau with increases in current and reached only 83% of the M_{max} recorded during the PTT trial using $NMES_{1000}$. In this participant, H_{max}/M_{max} was largest during $NMES_{50}$ and M_{Hmax} was largest during $NMES_{50}$. Figure 5-1B displays data from Figure 5-1A when M-waves were less

than 10% M_{max}. From this figure, it is evident that $H_{5\%Mmax}$ (average size of the H-reflex when the M-wave was between 2% and 8% M_{max}) was larger using $NMES_{1000}$ (~39% M_{max}) compared with $NMES_{50}$ (~14% M_{max}).

In 2 of the 4 participants tested presently, it was not possible to evoke measureable H-reflexes during either $NMES_{50}$ or $NMES_{1000}$. Figure 5-2 shows the mean values for the 3 measures from the recruitment curves for the 2 participants in whom H-reflexes were generated. For these 2 participants, H_{max}/M_{max} was similar between pulse durations, however, $H_{5\%Mmax}$ was ~3.5 times smaller and M_{Hmax} was ~3.2 times larger during $NMES_{50}$ compared with $NMES_{1000}$.

5.3.2 Fatigue protocol

PTT, which was determined at the beginning of both sessions using $NMES_{1000}$, was similar between the $NMES_{50}$ (9.4 ± 2.3 Nm) and $NMES_{1000}$ (9.7 ± 3.1 Nm) sessions for the group (n = 4). Figure 5-3 shows torque and EMG recorded from 1 participant during the fatigue protocol using $NMES_{50}$ (Figure 5-3A) and $NMES_{1000}$ (Figure 5-3B). During the initial 5 contractions, torque was similar (~9 Nm) between NMES pulse durations. However, by the last five contractions of the fatigue protocol, $NMES_{50}$ generated ~2 Nm of torque, while $NMES_{1000}$ generated ~4 Nm of torque. During $NMES_{50}$, contractions were generated mainly through successive M-waves (Figure 5-3A inset), while during $NMES_{1000}$, contractions were generated mainly through successive H-reflexes (Figure 5-3B inset).

Figure 5-4 shows mean torque (A), M-wave (B) and H-reflex (C) amplitudes during the 5 min fatigue protocol using $NMES_{50}$ (50 μs) and

$NMES_{1000}$ (1000 μs). Each bin represents data averaged over 5 successive contractions for each participant and then averaged across the group of 4. Compared with the first data bin (time 0 to 20 s), torque declined similarly over the first half of the fatigue protocol for both $NMES_{50}$ and $NMES_{1000}$. However, $NMES_{1000}$ generated ~2 times more torque than $NMES_{50}$ over the last half of the fatigue protocol. By the end of the fatigue protocol (bin 15), torque had dropped by 79% (compared with bin 1) for $NMES_{50}$ and by 64% for $NMES_{1000}$. Figure 5-5 shows that the fatigue index for the group (n = 4) was ~1.8 times larger during $NMES_{1000}$ than $NMES_{50}$.

When averaged across the entire fatigue protocol, M-waves (Figure 5-4B) were 2 times larger and H-reflexes (Figure 5-4C) were 3 times smaller during $NMES_{50}$ compared with $NMES_{1000}$.

5.4 Discussion

The present experiments were designed to compare M-wave and H-reflex recruitment curves for data collected using $NMES_{50}$ and $NMES_{1000}$, as well as to test the fatigue-resistance between contractions evoked using $NMES_{50}$, in which H-reflexes were predicted to be smallest, and $NMES_{1000}$, in which H-reflexes were predicted to be largest, in people with chronic SCI. The following discussion is based on qualitative interpretation of the data, as statistical analyses have not been conducted.

5.4.1 Effect of pulse duration on H vs M recruitment curves after SCI

Consistent with our first hypothesis and a previous study conducted in people who are neurologically-intact (20), the H vs M recruitment curve was shifted to the left when using a long (1000 μs; $NMES_{1000}$), as opposed to a short (50 μs; $NMES_{50}$), pulse duration. When the NMES intensity was adjusted to evoke an M-wave that was 5% M_{max}, H-reflexes were smaller using $NMES_{50}$ (8% M_{max}) compared with using $NMES_{1000}$ (28% M_{max}). When the NMES intensity was adjusted to evoke H_{max}, M-waves were larger using $NMES_{50}$ (28% M_{max}) compared with $NMES_{1000}$ (9% M_{max}). Lastly, NMES pulse duration did not markedly affect the H_{max}-to-M_{max} ratio. Together, these results support the idea that a long pulse duration ($NMES_{1000}$) recruits more sensory axons relative to motor axons at lower NMES intensities compared with a short pulse duration ($NMES_{50}$). The fact that H_{max}-to-M_{max} ratio was not different between pulse durations even though the size of the M-wave, and therefore the size of the antidromic volley (14), at H_{max} was larger with $NMES_{50}$ compared with $NMES_{1000}$ indicates that factors other than antidromic collision limit the size of the H-reflex in people with SCI, as has been suggested previously in people who are neurologically-intact (20). Other factors that may influence the size of the H_{max} include presynaptic inhibition of Ia afferent terminals (40) and oligosynaptic inhibitory post-synaptic potentials in motor neurons (6).

5.4.2 Effect of pulse duration on fatigue-resistance after SCI

Consistent with our second hypothesis, $NMES_{1000}$ generated contractions that were more fatigue-resistant than $NMES_{50}$. $NMES_{1000}$ generated more torque

over the last 80% of the 5 min fatigue protocol and had a larger fatigue index (generated more torque by the end of the fatigue protocol) compared with $NMES_{50}$.

Based on experiments with people who were neurologically-intact (21), we expected that contributions of *peripheral* and *central* pathways to NMES-evoked contractions would differ between pulse durations. When averaged over the entire fatigue protocol, M-waves were 2 times larger and H-reflexes were 3 times smaller during $NMES_{50}$ compared with $NMES_{1000}$. Thus, consistent with the H vs M recruitment curve data, $NMES_{1000}$ generated contractions with qualitatively greater activity through *central* pathways (H-reflexes) compared with $NMES_{50}$. This dependence upon pulse duration likely reflects the fact that longer pulse durations preferentially activate sensory over motor axons due to sensory axons having a longer strength duration time constant and lower rheobase than motor axons (22, 39).

To date, this is only the second demonstration that activity through *central* pathways (H-reflexes) can improve the fatigue-resistance of NMES-evoked contractions in people with SCI. In a previous study (Chapter 4), we compared the effect of NMES site (using a 200 μs pulse duration) on the fatigue-resistance of plantar flexor contractions in people with chronic motor-complete SCI. During NMES over the muscle belly, contractions were generated mainly by successive M-waves with little activity through H-reflexes. During NMES over the tibial nerve trunk, however, robust H-reflexes were evoked in half of the participants. For the group of participants in whom H-reflexes contributed to the evoked

contractions, the fatigue index was significantly larger during NMES over the tibial nerve trunk (~61%), when H-reflexes were prominent, compared with NMES over the muscle belly (~26%), when contractions were generated mainly be successive M-waves. Upon further data collection, we may divide our participants into 2 groups based on the presence of H-reflexes, as in this previous study. Together, these two studies provide evidence that maximising motor unit recruitment through *central* pathways can improve the fatigue-resistance of NMES-evoked contractions.

When the effect of NMES pulse duration on fatigue-resistance has been tested previously, it has had little to no effect on the fatigue-resistance of NMES-evoked contractions (13, 18, 37). Some researchers have even considered modulating the pulse duration *within* an NMES session to potentially activate different populations of *motor axons,* according to differences in their size and depth, however this had no effect on fatigue-resistance (18, 37). In general, contributions through *central* pathways have not been considered when testing the effect of pulse duration on fatigue-resistance. This may be because, in most cases, pulse duration has been tested during NMES over a muscle belly or at relatively high NMES intensities (>50% maximum), both conditions in which contributions through *central* pathways would be minimised.

5.4.3 Clinical implications

Contraction fatigue during NMES is thought to be due in part to the random order in which motor units are recruited during NMES (1, 9, 17, 24). Since slow fatigue-resistant motor units dominate the soleus H-reflex (5, 38), it is

likely that the presently observed improvements in fatigue-resistance for contractions generated by larger H-reflexes were the result of recruiting a greater proportion of fatigue-resistant motor units, according to the *size principle*. Thus, as in a previous study (Chapter 4), a more natural recruitment order can improve fatigue-resistance of NMES-evoked contractions in people with chronic SCI, whose muscle is reportedly made up of predominantly fast fatigable fibre types (15). Figure 5-3 of the present study provides a clear example of how differences in the contributions of *peripheral* and *central* pathways can influence the fatigue-resistance of evoked contractions within the same participant. This is an important finding, since it cannot be argued that differences in fatigue-resistance are due to differences in muscle quality (distribution of motor unit types within the muscle), because the same muscle is being stimulated in both cases. Rather, we would argue that differences in fatigue-resistance between mNMES and nNMES are due to differences in the *order* in which individual motor units are recruited within the muscle.

The most notable limitation of the present study is the low sample size. Thus, our interpretation of the results should be considered with caution, at least until an appropriate sample size is obtained. As in a previous study (Chapter 4), recruitment of participants with chronic motor-complete SCI has been our main challenge. It took nearly 2 years to recruit 11 participants for the experiments described in Chapters 4 and 5 (3 participants of whom were not appropriate candidates for NMES; see Section 4.2.1 of Chapter 4). Despite the low sample size, we believe these findings are important, given the relatively large effect

sizes. Thus, we are likely to find significant differences with relatively low sample sizes (between 5 and 16 participants), depending on the effect and on whether we continue to recruit participants in whom H-reflexes do *not* contribute to evoked contractions. We plan to have data collection complete for this project by September 2014.

Interpretations of the present results are also limited to the NMES intensity chosen. Although $NMES_{1000}$ shifted the H vs M recruitment curve to the left, H_{max}-to-M_{max} ratio was unaffected by pulse duration. In other words, the present data indicate that short pulse durations can generate equally robust H-reflexes, albeit at a higher NMES intensity and accompanied by larger M-waves, compared with longer pulse durations. As such, the present effect of pulse duration on the size of the H-reflex would be minimal if NMES were delivered at or above H_{max} for the short pulse duration. Whether a difference in fatigue-resistance would exist between pulse durations without a difference in *central* contribution is not known. Presently, $NMES_{1000}$ generated contractions of ~100% PTT, equivalent to ~27% of the torque that can be generated during maximal tetanic NMES (35), that were more fatigue-resistant and were associated with greater activity through *central* pathways (H-reflexes) compared with similarly sized contractions evoked by $NMES_{50}$. Before incorporating long pulses into clinical practice, it will be important to quantify fatigue-resistance across a range of NMES intensities.

5.4.4 Summary

Presently we report 2 novel findings: 1) We show that NMES pulse duration ($NMES_{50}$ versus $NMES_{1000}$) affects the relative recruitment of motor and sensory axons during NMES over the tibial nerve trunk in people with chronic motor-complete SCI. 2) We demonstrate marked differences in the fatigue-resistance of contractions evoked by $NMES_{50}$ and $NMES_{1000}$ in these people, which we suggest are due to differences in the contributions of *peripheral* and *central* pathways to evoked contractions between pulse durations; contractions generated with large H-reflexes fatigued less than similarly sized contractions generated predominantly by M-waves. In conclusion, $NMES_{1000}$ generates contractions that are more fatigue-resistant and are associated with greater activity through *central* pathways (H-reflexes) compared with $NMES_{50}$.

5.5 Tables

Table 5-1 Participant demographics

Code/Sex	Age	Years after SCI	Level of SCI	AIS	Baclophen (mg/day)	H-reflex
1F	33	10	C 4-5	B	0	Yes
2M	58	5	C 6-7	B	0	Yes
3M	42	24	C 5-6	B	0	No
4M	62	18	C 4-5	B	0	No
5M	29	11	C 5-7	B	80	n/a

AIS, American spinal injury association impairment scale

5.6 Figures

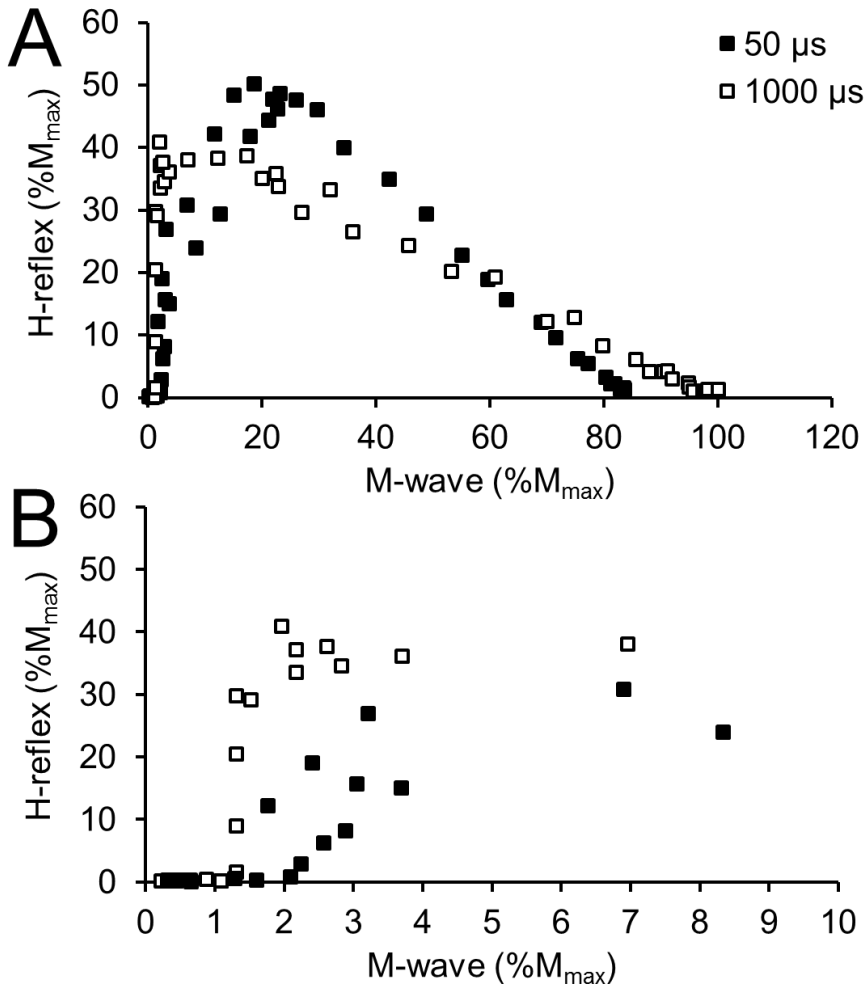

Figure 5-1 H vs M recruitment curves collected from a single participant using $NMES_{50}$ (50 µs pulse duration) and $NMES_{1000}$ (1000 µs pulse duration). (A) Data collected over a full range of NMES intensities. (B) Data from panel A on an expanded scale showing data when M-waves were less than 10% M_{max}.

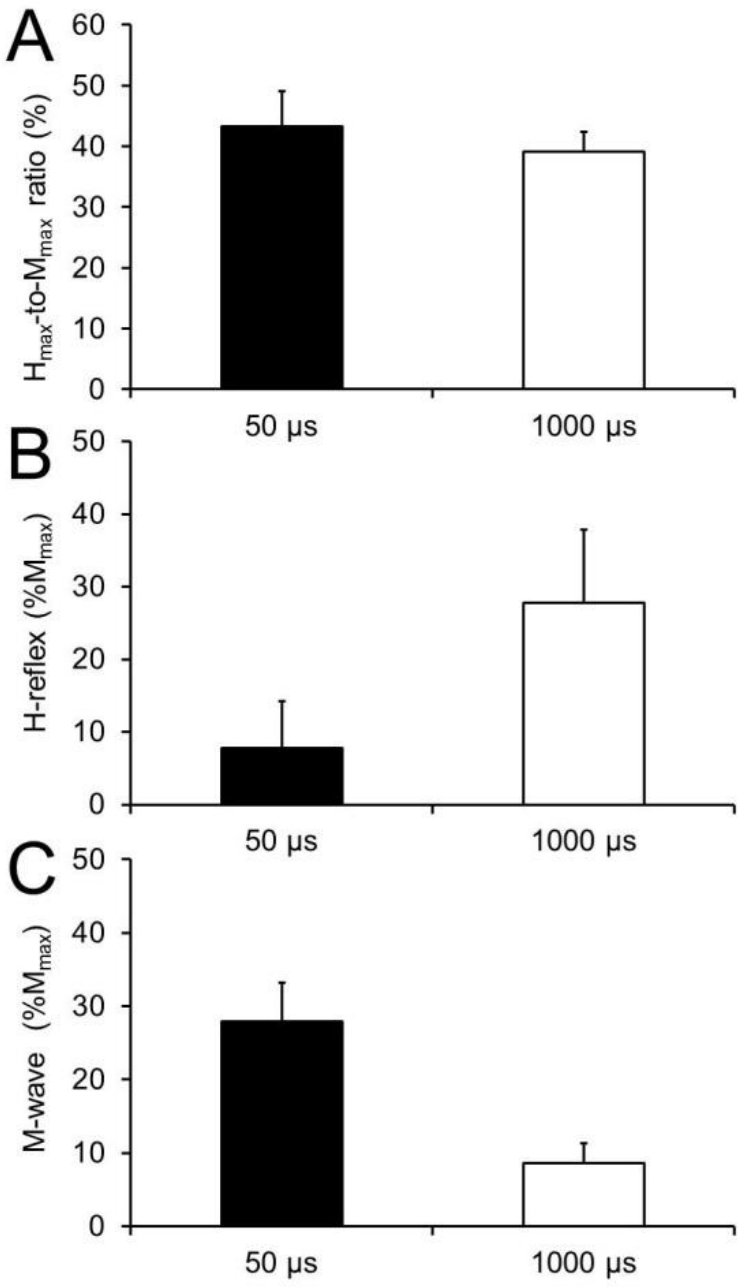

Figure 5-2 Group data (n = 2) showing differences in the recruitment of H-reflexes between $NMES_{50}$ (50 µs pulse duration) and $NMES_{1000}$ (1000 µs pulse duration). (A) H_{max}-to-M_{max} ratios. (B) H-reflex when the M-wave was ~5% M_{max} ($H_{5\%Mmax}$). (C) The size of the M-wave at H_{max} (M_{Hmax}). Error bars represent 1 standard error.

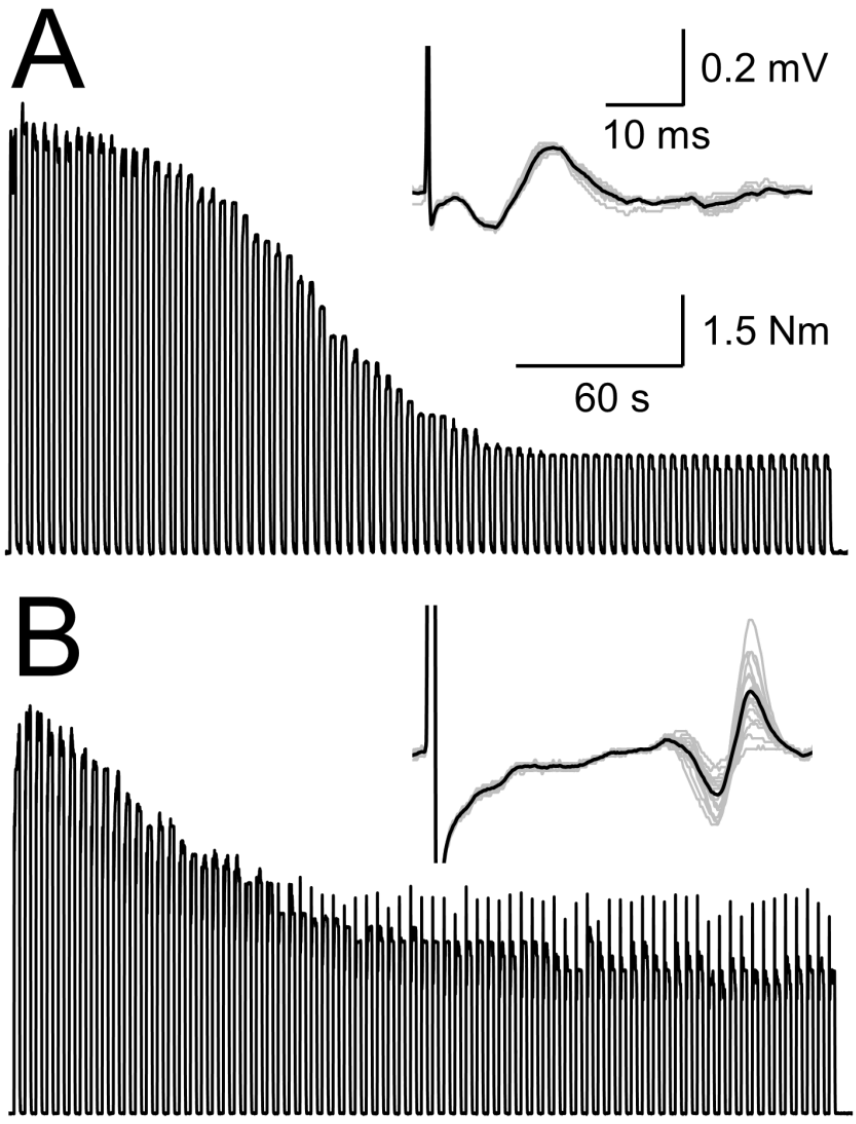

Figure 5-3 Torque and EMG evoked by $NMES_{50}$ (50 μs pulse duration; panel A) and $NMES_{1000}$ (1000 μs pulse duration; panel B) in a single participant. The solid line represents torque during the 5 min, 2-s-on-2-s-off, fatigue protocol. The inset in the top right corner of each panel shows EMG in response to the last 20 NMES pulses of the last contraction in the fatigue protocol. These raw EMG traces shown have not been processed post hoc. Bold black lines represent the average of the 20 single responses (grey lines). All data are shown on the same scale, as indicated by the calibration bars in panel A.

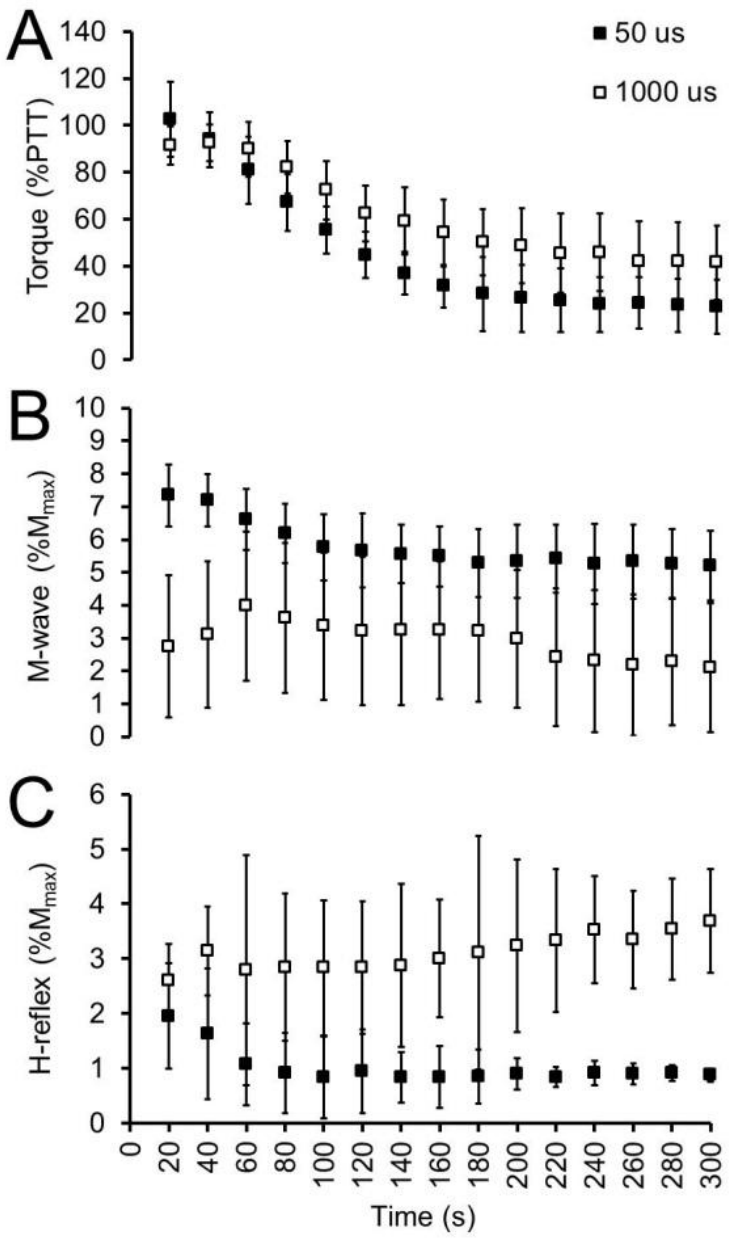

Figure 5-4 Group data (n = 4) showing torque (A), M-wave (B) and H-reflex (C) amplitudes during the fatigue protocol using $NMES_{50}$ (50 μs pulse duration) and $NMES_{1000}$ (1000 μs pulse duration). Error bars represent 1 standard error.

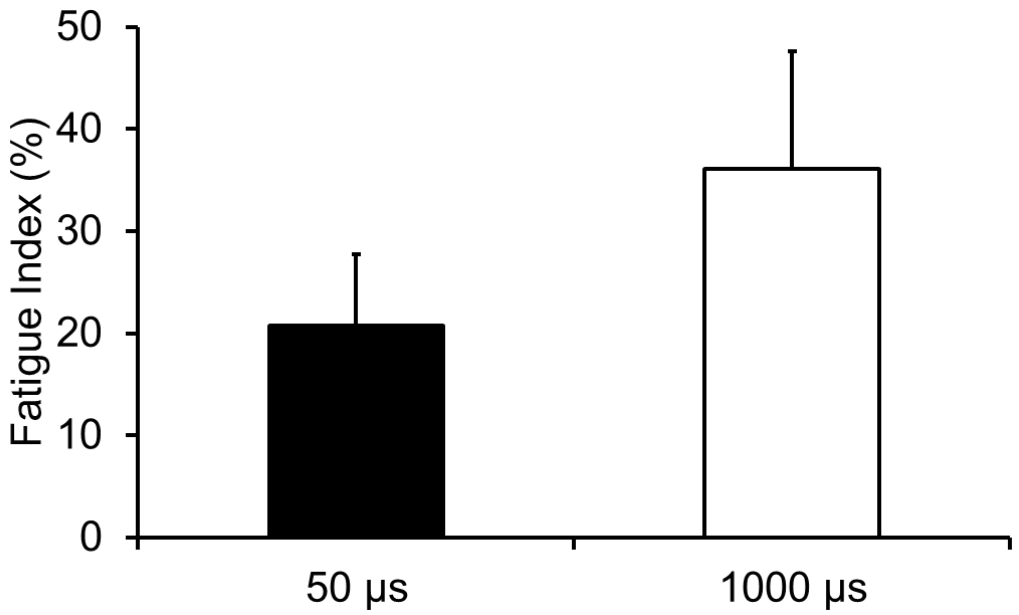

Figure 5-5 Group data (n = 4) showing fatigue indices for the fatigue protocols that used $NMES_{50}$ (50 µs pulse duration) and $NMES_{1000}$ (1000 µs pulse duration). Error bars represent 1 standard error.

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CHAPTER 6: GENERAL DISCUSSION

The experiments described in this thesis were designed to determine how the delivery of NMES can be optimised to enhance motor unit recruitment via reflex pathways through the spinal cord (*central* pathways) and to test whether activity through *central* pathways improves the fatigue-resistance of NMES-evoked contractions in people with chronic motor-complete spinal cord injury (SCI). Summarised below are the major findings from each thesis chapter, followed by a discussion of the scientific and/or clinical implications. Limitations of generating contractions through *central* pathways are discussed. To conclude the thesis, we discuss a limitation of our technique, with regards to estimating *peripheral* contributions to evoked contractions, and a promising future direction from this work.

6.1 Effect of NMES site on motor unit recruitment through *peripheral* and *central* pathways

In the first and second research chapters of this thesis, we investigated the effect of NMES site, either over the muscle belly (mNMES) or over the nerve trunk (nNMES), on the contributions of *peripheral* and *central* pathways to evoked contractions of the plantar flexors (Chapter 2; Ref 5) and knee extensors (Chapter 3; Ref 6), in people who were neurologically-intact. In Chapter 2, we generated plantar flexor contractions of 10-30% MVIC, which is sufficient for stepping (20-30% MVIC; Ref 2), while in Chapter 3, we generated knee extension contractions of 6-20 Nm, which is sufficient for NMES-assisted leg cycling (2 to

10 Nm; Ref 15). For the plantar flexors, both NMES sites recruited motor units through *peripheral* and *central* pathways, but the contributions made by these pathways differed markedly. As hypothesised, plantar flexor contractions evoked by nNMES (over the tibial nerve trunk) were generated primarily through *central* pathways (H-reflexes), while mNMES (over the triceps surae muscle belly) generated contractions primarily through *peripheral* pathways (M-waves). Following a brief period of high frequency NMES, both NMES sites generated equivalent increases in torque (extra torque; Ref 11). Extra torque has been attributed to *central* mechanisms (5, 22), such as an increased probability of neurotransmitter release from pre-synaptic terminals associated with post-activation (-tetanic) potentiation (19), and/or increased motor neuron excitability, due to the activation of persistent inward currents in spinal neurons (4, 10, 11). However, the term *extra torque* has also been used to describe small nonlinearity's in torque production due to an intrinsic muscle property (Ref 16; please see our response to this article, in Appendix A, regarding what the authors of Ref 16 describe as *extra torque* compared to what we have used the term to describe). In Chapter 2, the extra torque generated in the plantar flexors by nNMES was accompanied by enhanced H-reflexes, whereas equal levels of extra torque generated by mNMES were generated by enhanced M-waves and asynchronous activity. Thus, a portion of the extra torque during mNMES of the plantar flexors originated from a *peripheral* mechanism.

For the knee extensors, as for the plantar flexors, nNMES generated contractions with a greater contribution through *central* pathways compared with

mNMES.However, unlike the plantar flexors, asynchronous activity was completely absent in the knee extensors during NMES at either site. Further, neither torque nor H-reflexes were augmented following 100 Hz NMES. The lack of such increases indicates that there may be differences in frequency dependant *central* mechanisms (post-activation potentiation or motor neuron excitability) that control the knee extensors, compared to the plantar flexors.

Together, these two studies provide novel insight into how contractions are generated through *peripheral* and *central* pathways based on the site that NMES is delivered on the surface of the skin in leg muscles of people who are neurologically-intact. The importance of making a distinction between contractions that are generated through *peripheral* versus *central* pathways lies in the fact that motor unit recruitment through each pathway differs; recruitment through *peripheral* pathways is random with respect to motor unit type (14, 27) while recruitment through *central* pathways is orderly according to Henneman's size principle (9, 36). Thus, contractions generated through *peripheral* pathways should recruit relatively fewer fatigue-resistant motor units compared with equally sized contractions evoked through *central* pathways. In Chapters 2 and 3, we speculated that nNMES, with its greater *central* contribution, should produce more fatigue-resistant contractions compared with mNMES. In Chapters 4 and 5, we go on to test this idea that contractions with the largest *central* contribution should be the most fatigue-resistant in people with chronic motor-complete SCI.

6.2 Effect of motor unit recruitment through *central* pathways (H-reflexes) on the fatigue-resistance of NMES-evoked contractions

In Chapters 4 and 5, we investigated the potential of recruiting motor units through *central* pathways for generating fatigue-resistant contractions of the plantar flexors in people with chronic motor-complete SCI. In Chapter 4 we took what we learned from Chapters 2 and 3 and tested whether contractions generated by nNMES, which can generate contractions according to Henneman's size principle, are more fatigue-resistant than those evoked by mNMES, in which motor unit recruitment is mainly random with respect to motor unit type. As predicted, we found that nNMES generated contractions that were more fatigue-resistant than mNMES. mNMES generated contractions primarily through *peripheral* pathways (M-waves) while during nNMES contractions were generated through both *peripheral* and *central* (H-reflexes) pathways. Thus, the NMES site with the greatest *central* contribution produced contractions that fatigued the least. Surprisingly, H-reflexes were evoked in only half of the participants tested. However, this provided an opportunity to test more specifically the effect of H-reflexes on the fatigue-resistance of evoked contractions. During nNMES, there was a significantly larger fatigue index (~61%) for the participants in whom H-reflexes contributed to contractions, compared with the participants in whom H-reflexes did not contribute (~29%). Importantly, there was no difference in fatigue index between groups during mNMES when contractions were generated primarily by M-waves in both groups. Thus, since nNMES generated contractions that were more fatigue-resistant than

mNMES, but only when H-reflexes contributed to the evoked contractions, we concluded that recruiting motor units in a more natural *order* (through *central* pathways) improves the fatigue-resistance of NMES-evoked contractions in people with chronic SCI.

In Chapter 5, we tested further the idea that augmenting activity through *central* pathways (H-reflexes) can improve fatigue-resistance of NMES-evoked contractions. In people who are neurologically-intact, the extent to which contractions are generated through *peripheral* and *central* pathways depends on the duration of the NMES pulses (25). Specifically, recruitment curve data showed that longer pulse durations (500 to 1000 μs) generated a larger H-reflex relative to the M-wave than the shorter pulse duration (50 μs; Refs (24, 32, 33). Thus, since H-reflexes improve the fatigue-resistance of NMES-evoked contractions in people with chronic SCI (Chapter 4), then long pulse durations may be more effective than short pulse durations for generating fatigue-resistant contractions. We compared the fatigue-resistance of plantar flexor contractions evoked using a short pulse duration (50 μs; $NMES_{50}$), in which H-reflexes were predicted to be smallest, and a long pulse duration (1000 μs; $NMES_{1000}$), in which H-reflexes were predicted to be largest, in people with chronic SCI. Data collection for this project continues. To date, we have collected complete data sets from 4 participants; statistical analyses of these data have not been conducted as we are presently underpowered. However, qualitative interpretation of the data indicates that $NMES_{1000}$ generated contractions that had a greater contribution

through *central* pathways (H-reflexes) and which were more fatigue-resistant, compared with $NMES_{50}$.

Despite many researchers acknowledging that an altered motor unit recruitment *order* limits the fatigue-resistance of NMES-evoked contractions (1, 18, 21, 26), Chapters 4 and 5 are the first studies to test the effect of a more natural motor unit recruitment order, albeit indirectly (i.e. motor unit recruitment through *central* pathways is assumed to be orderly (9, 36), on the fatigue-resistance of NMES-evoked contractions in people with chronic SCI. Together, Chapters 4 and 5 provide evidence that maximising motor unit recruitment through *central* pathways can improve the fatigue-resistance of NMES-evoked contractions in people with chronic SCI. Thus, motor unit recruitment *order* is indeed important in the maintenance of torque during NMES.

6.3 Limitations of generating contractions through *central* pathways

Despite the observed improvements in fatigue-resistance with contractions generated through *central* pathways, there are a number of practical limitations to consider. Firstly, the position of the electrodes during nNMES tends to be susceptible to movement, making it difficult to deliver consistent current; although this is mainly an issue when nNMES is delivered over the femoral nerve trunk compared to the tibial nerve trunk. Secondly, contractions generated through *central* pathways are less consistent both within (3) and between (3, 6) successive contractions compared with contractions generated only through *peripheral* pathways; however this may only be the case at lower NMES

intensities (3, 6). Thus, it may prove to be difficult to adequately control fine motor tasks through *central* pathways. Thirdly, when NMES is used to restore movement, contributions through *central* pathways should diminish, as it is well known that H-reflexes reduce in size during passive and voluntary movement (7, 8, 12, 20); however, such H-reflex modulation is reduced or absent in people with SCI (23). Fourthly, it is unclear whether contractions with a significant contribution through *central* pathways will be of sufficient amplitude for restoring movement. This is especially true for contractions that require high NMES intensities, such as when NMES intensity is increased in response to fatigue. In this case, the increased NMES intensity will increase levels of antidromic transmission in motor axons (39), thereby reducing any contribution through *central* pathways. However, in *some* participants, we have shown that contractions with a demonstrated *central* contribution (H-reflexes) can reach up to ~30% of a maximum voluntary isometric contraction in people who are neurologically-intact (Chapters 2 and 3), and up to ~27% of the torque that can be generated during maximal tetanic (40 Hz) NMES in people with chronic SCI (Chapters 4 and 5). Lastly, despite optimising NMES to generate contractions through *central* pathways, we found contributions through *central* pathways could not be evoked in every participant. Thus, not everyone will benefit from delivering NMES over the nerve trunk (nNMES) or using long pulse durations ($NMES_{1000}$).

6.4 Limitation of estimating *peripheral* contributions using surface EMG and a promising future direction

During my PhD, I was involved in 3 projects that resulted in publications that are not part of my thesis (28, 31, 37). One project of particular relevance to my thesis tested the effect of NMES site on the spatial distribution of motor units recruited by mNMES (over the tibialis anterior muscle belly) and nNMES (over the common peroneal nerve trunk) of the tibialis anterior (31). We used fine wire recording electrodes inserted into tibialis anterior at separate depths (superficial versus deep recording sites) to compare the spatial distribution of motor units recruited when single pulses were delivered using mNMES versus nNMES. We found that mNMES recruited motor units from superficial to deep with increasing NMES intensity whereas nNMES recruited superficial and deep motor units equally, regardless of NMES intensity. During this study, we also recorded from the surface and confirmed that the surface recording reflects mainly activity in superficial regions of the muscle (17, 29). Figure 1-6 of Chapter 1 provides a schematic of the proposed spatial motor unit recruitment through *peripheral* pathways during mNMES and nNMES. Together, these findings provide two particularly important pieces of information.

First, these findings uncovered a limitation of estimating *peripheral* contributions to evoked contractions using M-waves recorded from the surface. In each research chapter of the present thesis, we used M-wave amplitude as an estimate of the *peripheral* contribution to NMES-evoked contractions, as M-wave amplitude is thought to represent a relative measure of motor axon activation (39).

Since the spatial distribution of recruited motor units through *peripheral* pathways differs between mNMES and nNMES (31), such that motor units in superficial regions of the muscle are recruited preferentially with mNMES, while motor units in both superficial and deep regions of the muscle are recruited with nNMES, and the contribution of superficial and deep motor units to the surface signal are not equal (17, 29, 31), it is inappropriate to compare the amplitude of M-waves evoked between mNMES and nNMES, as done in Chapters 2, 3 and 4. Thus, the M-wave data in these Chapters should be interpreted with caution. However, we have no reason to believe that it is inappropriate to compare the amplitude of H-reflexes evoked between mNMES and nNMES, as done in Chapters 2, 3 and 4, since synaptic recruitment likely recruits a similar population of motor units according to the size principle regardless of NMES site and, thus, is likely to generate a similar signal at the surface regardless of NMES site.

Secondly, based on differences in the spatial distribution of recruited motor units between NMES sites (31), combined with work from the present thesis (mainly Chapters 2, 3 and 4), we have developed the theoretical foundations for an important future study. As discussed in Chapter 1 of the present thesis (Section 1.3.3.1), one way to increase the spatial distribution of recruited motor units, while reducing individual motor unit firing frequencies, during NMES is to rotate the NMES pulses between multiple mNMES sites (Sequential NMES; sNMES; Refs (13, 30, 34, 35). sNMES reduces fatigue of the human triceps surae (30) and quadriceps (13, 34, 35). However, similar to mNMES alone, sNMES has limitations. Firstly, contractions are generated

predominantly by successive M-waves (*peripheral* pathway), in which motor units are recruited in random order with respect to motor unit type (14, 27). Secondly, the depolarisation of motor axons during sNMES recruits superficial motor units preferentially (38), with deep motor units recruited mainly only at relatively high NMES intensities (1), if at all (31). In contrast, during nNMES, the depolarisation of motor axons recruits motor units that are evenly distributed throughout a muscle, regardless of contraction amplitude (31). With this in mind, we developed a form of sNMES whereby the pulses are alternated or *interleaved* between the mNMES *and* nNMES sites (interleaved NMES; iNMES). Like the rationale underlying sNMES (over the muscle belly), the idea is that iNMES will activate largely distinct populations of motor units with every other pulse, allowing for a reduction in the NMES frequency at each site. However, iNMES has the added benefit of recruiting not only superficial, but also deep motor units, regardless of the NMES intensity (31), thereby increasing the number of motor units that can be recruited from the surface and allowing for a greater distribution of the metabolic load. Further, iNMES will also recruit a greater proportion of motor units through *central* pathways compared with mNMES alone since half of the pulses would be generated by nNMES which can generate contractions with large *central* contributions (5, 6). To date, we have conducted experiments using iNMES with the same 8 participants as described in Chapter 4 (see Appendix B). Our original thought was to include these data in Chapter 4 (i.e. compare the fatigue-resistance of contractions evoked by mNMES, nNMES *and* iNMES), however we felt that including iNMES in that Chapter made it difficult to clearly,

and concisely, compare the nNMES and mNMES protocols. We decided, however, that instead of including the iNMES data in Chapter 4, we will collect additional data using *traditional* sNMES (stimulating through multiple mNMES electrodes) to compare those data with the iNMES data collected so far.

6.5 Summary

The work in this thesis contributes to the growing body of knowledge related to the application of NMES for rehabilitation by providing insight into how contractions are generated depending on the NMES site (Chapters 2, 3 and 4). Further, this work also provides the first evidence supporting the conclusion that the fatigue-resistance of NMES-evoked contractions can be improved upon by augmenting activity through *central* pathways (H-reflexes) in people with chronic SCI (Chapters 4 and 5), ideas which have been developing in our lab over the past decade. Future work aimed at determining ways of improving the fatigue-resistance of NMES-evoked contractions should focus not only minimising the firing frequency of individual motor units, but also on the order in which motor units are recruited.

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APPENDIX A: RESPONSE TO FRIGON ET AL.[6]

Frigon A, Thompson CK, Johnson MD, Manuel M, Hornby TG and Heckman CJ. Extra forces evoked during electrical stimulation of the muscle or its nerve are generated and modulated by a length-dependent intrinsic property of muscle in humans and cats. *The Journal of Neuroscience* 31(15):5579-5588, 2011.

A.1 Article

Frigon et al. (2011) applied electrical stimulation over peripheral nerves in decerebrate cats and over the muscles that plantarflex and dorsiflex the ankle in humans in part to *determine whether extra forces/torques are generated by an intrinsic neuronal or muscle property*. They used frequency modulated triangular and *top-hat* stimulation patterns, which we have used previously, to generate contractions in humans via two pathways: 1) *peripheral* pathways, from the activation of motor axons beneath the stimulating electrodes, and 2) *central* pathways, whereby spinal motor neurons are recruited by the electrically evoked sensory volley and generate what we have termed *extra torque* (1, 4-6, 8-10).

Frigon et al. conclude that *extra forces/torques evoked during electrical stimulation of the muscle or nerve are muscle length-dependent and primarily mediated by an intrinsic muscle property*. We are writing this letter to clarify that what Frigon et al. describe as *extra torque* is different from what we have used the

[6] A version of this appendix has been published.
Collins DF and Bergquist AJ. Extra torque during electrically evoked contractions in humans, *Journal of Neuroscience*. Re: Extra forces evoked during electrical stimulation of the muscle or its nerve are generated and modulated by a length-dependent intrinsic property of muscle in humans and cats. Frigon, et al., 31(15):5579-5588

term to describe. We also address the issue of why we routinely find that transmission along central nervous system pathways contributes to electrically evoked contractions, whereas Frigon et al. found no evidence for it.

In the work of Frigon et al., as in several of our previous studies, peripheral nerve block was used to establish the extent to which the central nervous system contributes to electrically evoked contractions in humans. Under *nerve block* conditions, Frigon et al. showed in one participant that more torque was generated by the dorsiflexors at the end of a top-hat stimulation pattern than at the beginning (Figure A-1A), and aptly concluded that this *extra torque* resulted from muscle properties, although a shifting of the motor points beneath the stimulating electrodes may have contributed. In our experiments under similar conditions, less dorsiflexion torque was generated at the end of the top-hat stimulation (Figure A-1B).

However, perhaps the most significant differences between the results of Frigon et al. and our own are those data obtained in *intact* participants, when signals traversing the central nervous system could contribute to the evoked contractions. Frigon et al. saw no differences in the torque generated between the *nerve block* and *intact* conditions in two participants. In contrast, we often measure much more torque in the *intact* condition than during *nerve block* (Figure A-1B) and, as we noted previously (6), the *presence of these additional forces dramatically shifts the force-frequency relation of the stimulated muscle and introduces nonlinearities far greater than known for isolated muscle* (2, 12). We have shown *extra torque* of up to 40% of that generated during a maximum

voluntary contraction and have demonstrated that it originates from transmission through the central nervous system as it does not develop under *nerve block* conditions (3, 5, 6, 9) and it is accompanied by H-reflexes (1, 8, 10) and electromyographic (EMG) activity that is not time-locked to each stimulus pulse (i.e. asynchronous activity; Refs 1 and 6).

Frigon et al. proposed that the *extra torque* that we have reported previously may be attributed to a lack of control in our studies over joint angle, thus suggesting it results from a muscle property effect that they have shown is largest at short muscle lengths. But all of our previous studies have been conducted with the ankle at 90°, an angle which shows no *extra torque* due to muscle properties in our previous *nerve block* data and in the group data of Frigon et al. (Figure 2 in Ref 7).

Frigon et al. also proposed that what we have described as *extra torque* may be due to a tensing-up phenomenon whereby a *central* contribution is provided by involuntary descending drive associated with discomfort. However, we have recorded contractions consistent with a *central* contribution in participants who were asleep (5), those with complete spinal cord injury (6, 11), and at stimulation amplitudes below motor threshold in able-bodied participants (5). Thus, we do not believe that *extra torque* is the result of *tensing up* due to discomfort, although involuntary long-loop reflex pathways through the cortex may contribute (4).

On the other hand, we do see a wide range in the extent to which transmission along *central* pathways contributes to electrically-evoked contractions, with perhaps 20% of our participants showing little to no evidence

of *extra torque*. It is possible that all the participants in the study of Frigon et al., certainly the two selected for the nerve blocks, fell into this group.

As it stands, we are unsure why Frigon et al. did not generate contractions that had a demonstrable *central* contribution in the form of *extra torque*. What they did show is that the previously described non-linearity in torque generating capacity of muscle (2) depends on muscle length and the nervous system must account for this when controlling human movement. Their data also highlight the importance of recording EMG in conjunction with torque measurements when assessing the *central* contribution to electrically-evoked contractions in humans.

Ultimately, it is our hope that experiments designed to identify the reasons for the discrepancies between our work and that of Frigon et al. will provide insight into the mechanisms responsible for the *centrally*-driven contractions that we regularly observe during electrical stimulation of a muscle or its nerve. Presently, it is our thinking that the *central* contribution to electrically-evoked contractions, what we have described as *extra torque*, arises from mechanisms that transform sensory input into motor output during human movement and may include a combination of enhanced pre-synaptic release of neurotransmitter (e.g. post-tetanic potentiation), increased motor neuron excitability (e.g. persistent inward currents), and/or transmission along long-loop reflex pathways through the cortex.

A.2 Figure

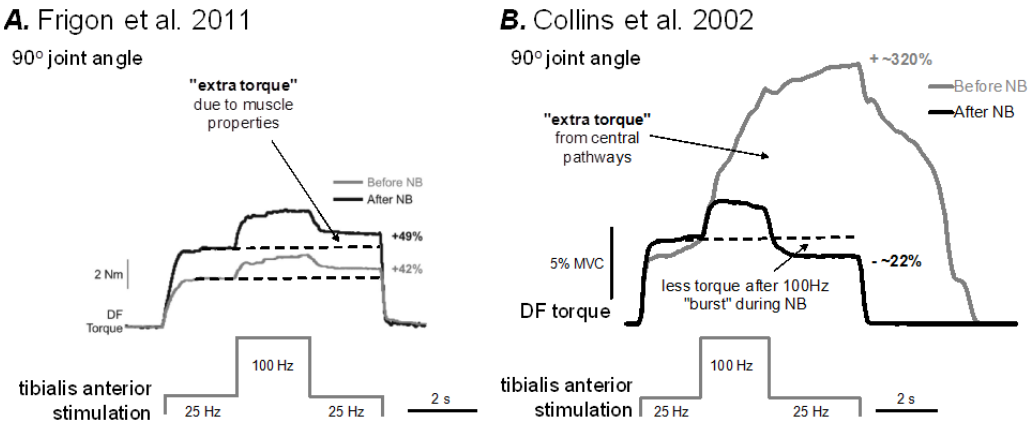

Figure A-1 Dorsiflexion (DF) torque recorded during stimulation over the tibialis anterior muscle before and during a nerve block (NB). Panel B is adapted from Figure 7*C* in Ref 5.

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APPENDIX B: INTERLEAVED NEUROMUSCULAR ELECTRICAL STIMULATION TO IMPROVE THE FATIGUE-RESISTANCE OF EVOKED CONTRACTIONS IN PEOPLE WITH SPINAL CORD INJURY[7]

B.1 Background

Neuromuscular electrical stimulation (NMES) is typically delivered through a single pair of electrodes placed over the muscle belly (mNMES) or over the nerve trunk (nNMES). Unfortunately, rapid contraction fatigue develops when using both of these approaches. Interestingly, rotating the NMES pulses between multiple pairs of electrodes over the muscle belly (sequential NMES; sNMES) can improve the fatigue-resistance of evoked triceps surae (7) and quadriceps (6, 9, 10) contractions. We have developed a form of sNMES, whereby we alternate or *interleave* pulses between the mNMES *and* nNMES sites (interleaved NMES; iNMES). The theoretical advantages of iNMES over *traditional* sNMES are twofold: 1) iNMES will recruit largely different populations of motor units with every other stimulus pulse (i.e. from the nNMES and mNMES sites; Ref 8), thus reducing motor unit firing frequencies and allowing for a greater distribution of the metabolic load and 2) iNMES will recruit a greater proportion of motor units through *central* pathways since half of the pulses are generated by nNMES, which can generate contractions with large *central* contributions (3, 4).

[7] The authors contributing to the work presented in this chapter were:
Bergquist AJ, Okuma Y, Wiest MJ and Collins DF.

Herein we report findings from experiments that demonstrate the *proof of principle* of delivering iNMES to generate contraction of the plantar flexors in people who have had a spinal cord injury (SCI). Thus far, we have conducted experiments using iNMES with the same 8 participants as described in Chapter 4. Our original thought was to include these data in Chapter 4 (i.e. compare the fatigue-resistance of contractions evoked by mNMES, nNMES *and* iNMES), however we felt that including iNMES in that Chapter made it difficult to clearly, and concisely, compare the nNMES and mNMES protocols. Specifically, by excluding the iNMES data, the focus of Chapter 4 remained solely on differences in fatigue-resistance based on motor unit recruitment *order* (i.e. differences in *central* contributions). In addition to the effect of motor unit recruitment order, including iNMES in Chapter 4 would have introduced the effect of reducing motor unit firing frequency on contraction fatigue. We acknowledge that by not including the iNMES data in Chapter 4 we lose information about comparisons between all 3 NMES sites (mNMES, nNMES *and* iNMES). We decided, however, that instead of including the iNMES data in Chapter 4, we will collect additional data using *traditional* sNMES (stimulating through multiple mNMES electrodes) to compare those data with the iNMES data collected so far. Thus, each participant tested will be asked to return for 1 additional experimental session using *traditional* sNMES.

B.2 Methods

Methods for these experiments were equivalent to those reported in Chapter 4. Deviations from those methods are reported below.

B.2.1 Neuromuscular electrical stimulation (NMES)

NMES was delivered using 2 constant-current stimulators (200 μs pulse duration; DS7A and DS7AH Digitimer, Welwyn Garden City, UK). iNMES consisted of alternating every other NMES pulse between 2 pairs of electrodes; 1 pair was placed over the triceps surae muscles, as done previously (mNMES; Chapters 2, 3 and 4), and the other was placed over the tibial nerve trunk, also done previously (nNMES; Chapters 2, 3, 4 and 5). Each electrode pair was stimulated at 10 Hz with a phase shift of 180° with respect to the other, giving a net NMES frequency of 20 Hz.

B.2.2 Setting NMES intensity

To set the NMES intensity for the fatigue protocol, 2 s trains of 10 Hz NMES were delivered 20 s apart while the NMES intensity was adjusted until peak torque was equivalent to 50% of a participants PTT. This was done independently for both the mNMES and nNMES sites. Approximately 10 NMES trains (~5 at each site) were required to set the NMES intensity for the iNMES session. Interleaving NMES between the mNMES and nNMES sites at these intensities generated ~100% of PTT in each participant.

B.3 Results

The present results are divided into two sections. The first section describes results for group (n = 8) data based on *where* NMES was delivered, independent of *how* (M-waves and H-reflexes) contractions were generated. In other words, the first section describes fatigue during iNMES had we not recorded EMG. However, as in a previous study (Chapter 4), only half of the participants generated activity through *central* pathways during iNMES. As such, the second section describes data after participants were divided into groups based on whether contractions were generated with (Group 1; n = 4) or without (Group 2; n = 4) H-reflexes during iNMES.

B.3.1 Fatigue based on *where* NMES was delivered (iNMES)

The peak twitch torque (PTT) generated at the beginning of the iNMES session was 8.17 ± 1.95 Nm. Figure B-1 shows group (n = 8) torque, M-wave and H-reflex data during the iNMES fatigue protocol. Each bin represents data averaged over 5 successive contractions. Torque at the onset of the fatigue protocol (bin 1) was near 100% PTT, but by the end of the fatigue protocol (bin 15), torque had dropped by ~55% (fatigue index [final $torque_{bin15}$ / initial $torque_{bin\ 1}$ x 100] during iNMES was ~45%).

B.3.2 Fatigue based on *how* contractions were generated (M-waves and H-reflexes)

Averaged across the entire fatigue protocol M-waves were 27.7 ± 0.4% M_{max} when NMES was delivered at the mNMES site ($iNMES_{(muscle)}$) and 4.4 ± 2.2% M_{max} when NMES was delivered at the nNMES site ($iNMES_{(nerve)}$).

Averaged across the entire fatigue protocol, H-reflexes were absent when iNMES was delivered at the mNMES site and 11.0 ± 1.1% M_{max} when iNMES was delivered at the nNMES site.

As in a previous study (Chapter 4), H-reflexes contributed to evoked contractions in only half of our participants. Thus, we divided our participants into groups; Group 1 was made up of participants in whom H-reflexes contributed to evoked contractions (n = 4), while Group 2 was made up of participants in whom H-reflexes did *not* contribute to evoked contractions (n = 4). For the participants in Group 1, M-waves were 23.7 ± 14.3% M_{max} and H-reflexes were absent at the mNMES site while M-waves were 2.16 ± 1.1% M_{max} and H-reflexes were 21.2 ± 13.9 % M_{max} at the nNMES site, when averaged across the entire iNMES fatigue protocol. For the participants in Group 2, M-waves were 31.7 ± 17.8% M_{max} and 6.3 ± 2.1% M_{max} at the mNMES and nNMES sites, respectively, when averaged across the entire iNMES fatigue protocol.

Figure B-2 shows torque and EMG data recorded from 2 participants, 1 in whom H-reflexes contributed to evoked contractions (panel A; participant in Group 1) and 1 in whom contractions were evoked by successive M-waves with no measurable H-reflex or asynchronous activity (panel B; participant in Group 2). In panel A, torque during the initial 5 contractions (bin 1) was ~18 Nm, however torque dropped abruptly after the 9th contraction, possibly due to movement of the nNMES electrodes since EMG elicited by this site ($iNMES_{(nerve)}$) changed from predominately M-waves to predominately H-reflexes after the 9th contraction. This was the only participant to show such an abrupt

change in torque and EMG during the fatigue protocol. The decline in torque from the 10^{th} contraction onward was negligible, as after the decline between the 9^{th} and 10^{th} contraction, torque declined by only ~2 Nm by the end of the fatigue protocol, generating ~10 Nm of torque at bin 15. In panel B, torque at bin 1 was ~8 Nm. In this participant, torque declined rapidly during the first 2 min and by the end of the fatigue protocol this participant was generating less than 1 Nm of torque. The fatigue indices (final $torque_{bin15}$ / initial $torque_{bin\ 1}$ x 100) between Groups 1 and 2 are shown in Figure B-3.

B.4 Discussion

These findings provide *proof of principle* regarding the technical feasibility of delivering iNMES in the paralysed plantar flexors. Although a more technically challenging control strategy is required for iNMES than mNMES or nNMES alone, we were able to interleave pulses between each NMES site and generate contractions with relative ease. Conveniently, when the NMES intensity was adjusted at each site to produce ~50% PTT independently using 10 Hz NMES, it produced our desired torque of ~100% PTT in each participant when combined. Whether this relationship exists at other NMES intensities is not known.

When compared with the fatigue indices produced in the same participants during mNMES (~29%) and nNMES (~45%) from a previous study (Chapter 4), iNMES (~45%) was comparable to nNMES. When we compare between studies after participants have been divided into groups based on whether contractions

were generated with (Group 1) and without (Group 2) H-reflexes, we can differentiate between an effect of motor unit recruitment *frequency* and *order* on the fatigue-resistance of the evoked contractions.

In Chapter 4, the fatigue index during mNMES for participants in Group 2 was ~29% and the fatigue index in the same participants during iNMES (presently) was ~37%. This improvement in the fatigue index with iNMES is likely the result of a reduced firing frequency of recruited motor units, since contractions were generated mainly by M-waves during both mNMES and iNMES. We would suggest that alternating the NMES between sites likely recruited, at least in part, distinct subpopulations of motor units with ever other pulse allowing for more time between subsequent activation of each motor units (7). Thus, many of the motor units recruited during iNMES were likely firing at half the frequency of those motor units recruited during mNMES or nNMES alone, closer to physiologically appropriate firing rates (~10 Hz; Refs 1, 2 and 5) thus allowing for greater recovery between successive pulses and lowering metabolic demand.

Data from the present study indicate that there is also a motor unit recruitment *order* effect on the fatigue-resistance of evoked contractions, since the fatigue index was largest for participants who generated contractions with H-reflexes (Group1; ~49%) compared with participants in whom contractions were generated mainly be successive M-waves (Group 2; ~37%). During iNMES in these participants, half of each contraction is generated with activity through *central* pathways (every second pulse), and thus we would suggest that a good

portion of the motor units are recruited according to Henneman's *size principle*, thereby contributing to improved fatigue- resistance observed in Group 1.

From the present data, we suggest that future work related to improving the fatigue-resistance of NMES-evoked contractions should focus not only on reducing the firing frequency of individual motor units, but also on the order in which motor units are recruited. When compared with a data from Chapter 4, the present data show both a motor unit recruitment *frequency* and *order* effect. In the future, it may be advantageous to *combine* traditional sNMES and iNMES (i.e. rotating pulses between multiple pairs of electrodes over the muscle belly *and* 1 pair over the nerve trunk), thus maximising the *frequency* effect of rotating pulses between electrodes, while still providing a *central* contribution (*order* effect) by incorporating nNMES.

There are a number of practical limitations to consider before using iNMES in the clinic. First, iNMES is technically more complicated and would require a much more sophisticated stimulator, which is presently not available, compared with mNMES or nNES alone. Second, setting the NMES intensity is more challenging during iNMES, compared with mNMES or nNMES alone, as iNMES requires that the user attend to more than 1 stimulator. Third, iNMES, like nNMES, is suitable only for muscles in which the innervating nerve trunk is accessible from the surface. Fourth, as the NMES intensity is increased, the frequency reducing effect of iNMES will be reduced due to greater overlap between motor units recruited by the mNMES and nNMES sites.

Although much remains to be learned about optimal delivery of iNMES (e.g. Is it best to rotate between NMES sites pulse-by-pulse or train-by-train?) these initial experiments provide evidence for the potential of iNMES strategies.

B.5 Figures

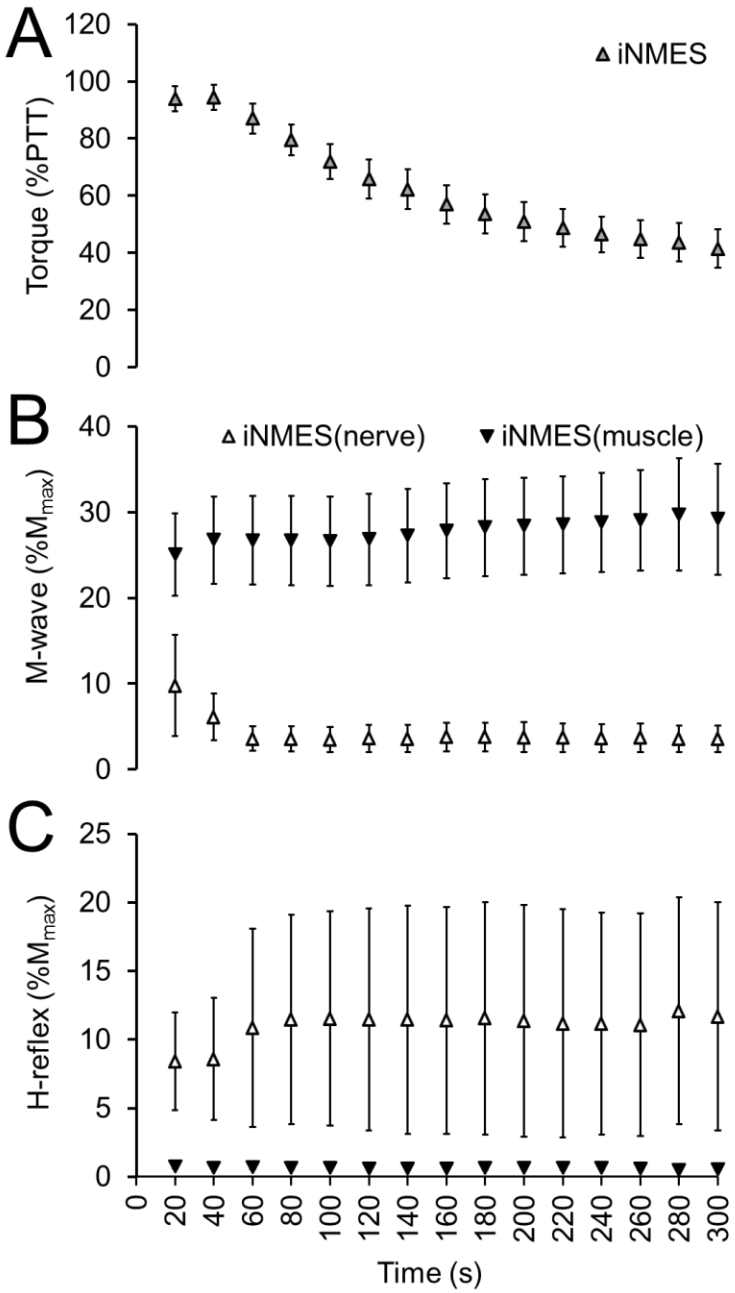

Figure B-1 Torque (A), M-waves (B) and H-reflexes (C) during the iNMES fatigue protocol (n = 8). Each symbol represents data averaged over 20 s (5 consecutive contractions; 1 bin). Error bars represent 1 standard error. $iNMES_{(muscle)}$: recordings from mNMES pulses; $iNMES_{(nerve)}$: recordings from nNMES pulses.

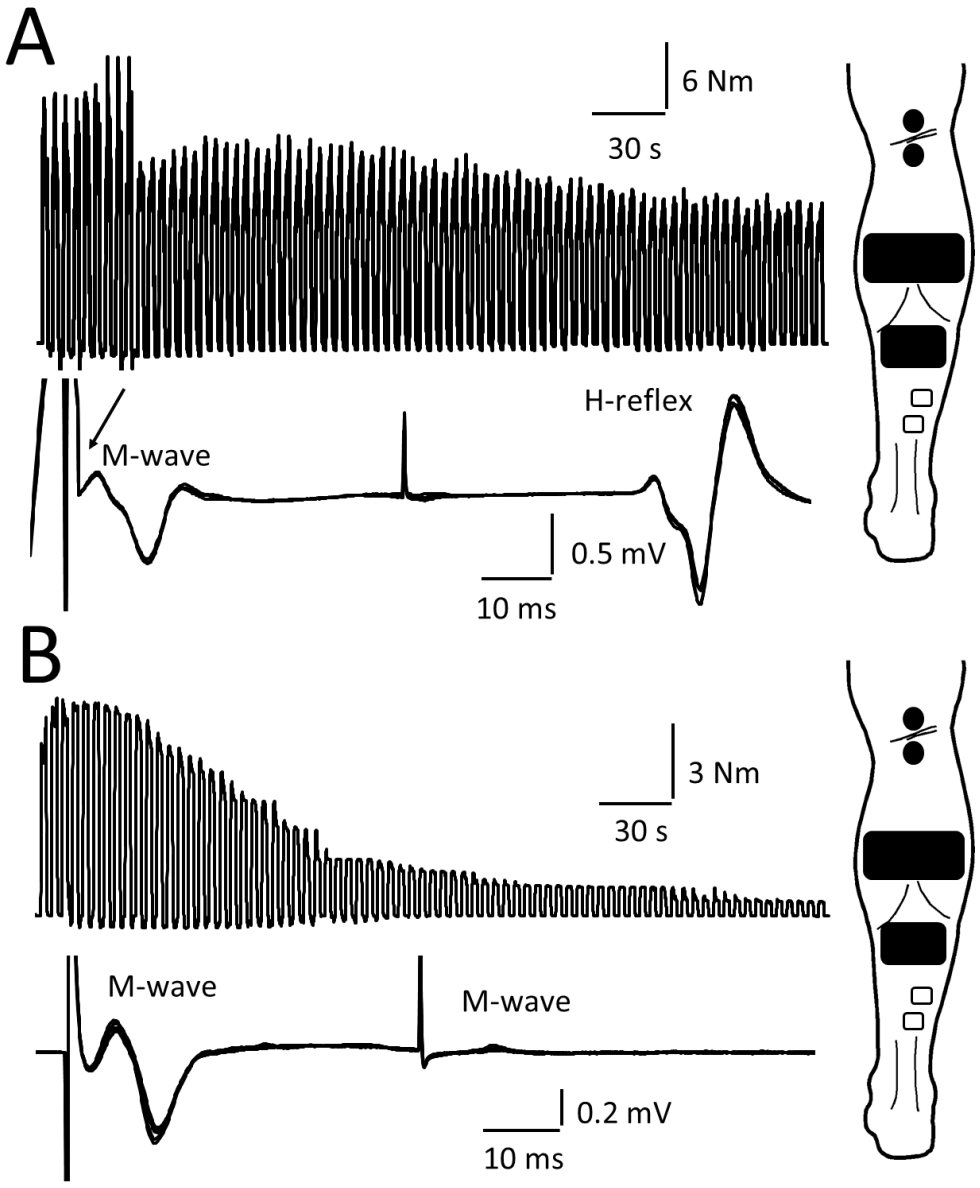

Figure B-2 Torque and EMG evoked by iNMES in a participant who generated contractions with H-reflexes (Group 1; panel A) and a participant who generated contractions without H-reflexes (Group 2; panel B). In the top of each panel, the solid line represents torque in response to the 5 min, 2-s-on-2-s-off, fatigue protocol (75 contractions). The bottom of each panel shows EMG in response to the last 2 NMES pulses (left: $NMES_{(muscle)}$; right: $iNMES_{(nerve)}$) for each of the last 5 contractions. The arrow points to where the tails of the preceding H-reflexes were removed.

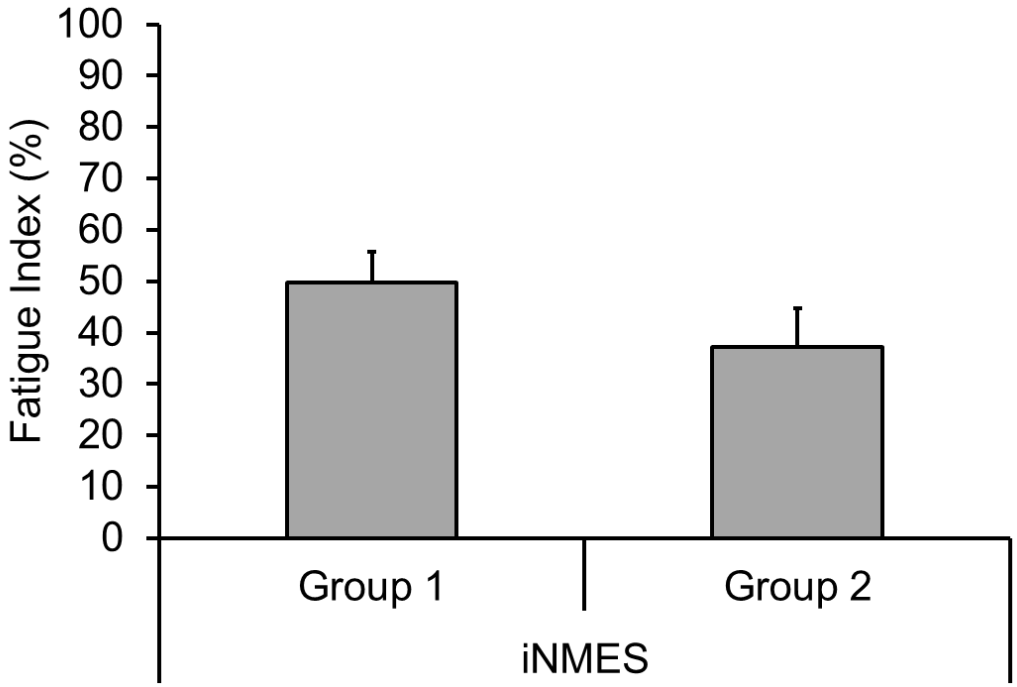

Figure B-3 Fatigue indices (mean $torque_{bin15}$ / mean $torque_{bin\ 1}$ x 100) for the iNMES fatigue protocol for participants who generated contractions with (Group 1; n = 4) and without (Group 2; n = 4) H-reflexes. Error bars represent 1 standard error.

B.6 References

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