Interleaved Neuromuscular Electrical Stimulation

by

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ABSTRACT

Neuromuscular electrical stimulation (NMES) is used to produce contractions to restore movement and reduce secondary complications for individuals experiencing paralysis. NMES is traditionally delivered through electrodes over a muscle belly (mNMES) or superficial nerve trunk (nNMES). Unfortunately, both methods are limited by rapid fatigability of the evoked contractions due in part to the non-physiologically high frequencies at which motor units (MUs) discharge. In order to reduce this fatigability, interleaved NMES (iNMES) was developed, which involves alternating stimulus pulses between the mNMES and nNMES sites. iNMES takes advantage of the fact that different MUs can be recruited by each NMES site. Therefore, alternating stimulus pulses reduces the discharge rate of recruited MUs, resulting in less fatigability than during traditional NMES. The experiments described in this thesis were designed to address gaps in knowledge about iNMES to develop a better understanding about how best to use iNMES for rehabilitation.

The first project, described in Chapter 2[1], was designed to estimate the effect of *stimulation amplitude* on the overlap between MUs recruited by mNMES, delivered over the tibialis anterior (TA) muscle belly, and nNMES, delivered over the common peroneal nerve. We showed that overlap increased progressively with increases in stimulation amplitude until overlap reached 72%. Further, trains of iNMES delivered at the stimulation amplitude that produced the least overlap (5%) generated contractions of 25% of a maximal voluntary isometric contraction (MVIC).

[1] This Chapter is under review in the Muscle & Nerve journal (submission date: November 10th 2015).

The second project, described in Chapter 3, characterized the effect of *stimulation frequency* and *pathway* on torque generated by mNMES, nNMES and iNMES delivered to TA and the triceps surae (TS). In general, iNMES generated more torque at the same "net" frequency as mNMES and nNMES in both muscles. For TA, contractions were generated predominantly by the activation of motor axons, thus through peripheral pathways, independent of NMES type. For the TS, mNMES produced contractions predominantly through peripheral pathways; however, central pathways predominated during nNMES and, to a lesser extent during iNMES. Plantarflexion torque during nNMES reached a steady state at a lower frequency (20 Hz) than mNMES (60 Hz) or iNMES (80 Hz) which was most likely due to frequency-dependent depression of transmission along central pathways.

The final project, described in Chapter 4, was designed to compare *discomfort* when the three types of NMES were delivered to generate submaximal contractions and identify the *maximal torque* that could be generated by each type of NMES before discomfort became a limiting factor. Results indicate there were no differences in discomfort between NMES types when torque was low (5-20% MVIC). However, during the maximal torque trials, nNMES produced significantly more torque (65% MVIC) than mNMES (33% MVIC) with significantly less discomfort. iNMES generated contractions of 49% MVIC with discomfort that was not different than mNMES or nNMES.

Collectively, this series of experiments address gaps in our knowledge about iNMES. Interestingly, iNMES can produce five times the torque required to dorsiflex the ankle during the swing phase of walking when stimulation amplitude and, consequently, overlap were low. iNMES also produced more torque at a given net frequency than traditional NMES. Both of these results bode well for producing contractions of sufficient amplitude to be used for rehabilitation while minimizing fatigability by reducing MU discharge rates. iNMES provided

these major advantages with a similar level of discomfort as traditional NMES, offering an overall superior method of NMES delivery than traditional NMES. In conclusion, iNMES reduces MU discharge rates without compromising the capacity to generate functionally relevant torque, making it a potentially valuable new rehabilitative tool after paralysis. Further research is necessary to test if these advantages translate into improved outcomes of NMES-based rehabilitation programs.

PREFACE

This thesis is an original work by Matheus Joner Wiest. The research projects described here received approval from the University of Alberta Research Ethics Board, project names: “Electrical stimulation of human muscle”, No. Pro00032035, 07/03/2012; “Comparing three ways to use electrical stimulation to generate muscle contractions”, No. Pro00049084, 06/23/2014; and “Comfort during neuromuscular electrical stimulation”, No. Pro00054856, 03/30/2015.

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LIST OF ABBREVIATIONS

ANOVA, analysis of variance

H-reflex, Hoffman reflex

$iNMES_{(m)}$, mNMES delivered during interleaved NMES

$iNMES_{(n)}$, nNMES delivered during interleaved NMES

iNMES, interleaved neuromuscular electrical stimulation

LS_{torque}, linear summation of the torque produced by mNMES and nNMES

m+nNMES, mNMES and nNMES delivered together

$MAX_{amplitude}$, nNMES delivered at maximal tolerable stimulation amplitude

MAX_{torque}, maximal torque produced at the maximal tolerable stimulation amplitude during

nNMES, neuromuscular electrical stimulation over a nerve trunk

mNMES, neuromuscular electrical stimulation delivered over a muscle belly

MU, motor unit

MVIC, maximal voluntary isometric contraction

M-wave, motor wave

NMES, neuromuscular electrical stimulation

PTT, peak twitch torque

R^2, coefficient of determination

rmANOVA, repeated measures ANOVA

TA, tibialis anterior muscle

TFR, torque-frequency relationship

$T_{m+nNMES,}$ torque evoked when mNMES and nNMES were delivered together

$T_{mean\ mNMES\&nNMES,}$ average torque of mNMES and nNMES

T_{mNMES}, torque evoked by mNMES alone

$T_{nNMES,}$ torque evoked by mNMES alone

TS, triceps surae muscle

VAS, visual analogue scale

CHAPTER 1. GENERAL INTRODUCTION

1.1 Overview

Neuromuscular electrical stimulation (NMES) is often used for rehabilitation for individuals experiencing paralysis due to central nervous system disorders such as a spinal cord injury or stroke (Peckham et al., 2005). The disuse resulting from paralysis causes secondary complications including reduced muscle mass and strength, loss of bone mineral density and reduced cardiovascular health (Shields, 2002). NMES has been shown to minimize these complications (Deley et al., 2015); however, the benefits of using NMES for rehabilitation are limited by rapid fatigability and discomfort.

A main focus of this thesis was to compare a new type of NMES, called interleaved NMES (iNMES), with two traditional types of NMES. Traditionally, NMES is delivered through a single pair of electrodes placed over a muscle belly (mNMES) or superficial nerve trunk (nNMES) using relatively low frequencies of 30 to 50 Hz. These traditional types of NMES recruit one group of motor units (MUs) synchronously at frequencies higher than occurs physiologically, resulting in rapid fatigability (Gorgey et al., 2009). One way to reduce this rapid fatigability is to deliver NMES through multiple pairs of electrodes to recruit different groups of MUs from each stimulation site and reduce MU discharge rates (Nguyen et al., 2011; Petrofsky, 1979). iNMES was developed with this goal in mind and involves alternating or "interleaving" stimulation pulses between the mNMES and nNMES sites. This approach relies on the fact that mNMES and nNMES recruit MUs in different portions of the muscle (Okuma et al., 2013). It has been shown that iNMES reduces fatigability compared to mNMES and nNMES (Lou et al, under review). However, the effect of the *amplitude of stimulation* on the overlap of MUs recruited by the mNMES and nNMES sites, the effects of *frequency of stimulation* and *pathway* on torque and how does *discomfort* and *maximal torque* during iNMES compare to that during mNMES and nNMES are unknown. The experiments described in this thesis were designed to address these knowledge gaps and contribute to a better understanding about how best to use iNMES to reduce the secondary complications resulting from paralysis.

In this introductory chapter, I describe how NMES is used for rehabilitation (Section 1.2), how NMES generates contractions (Sections 1.3.1 and 1.3.2) and provide an overview of different NMES parameters (Section 1.3.3). Limitations of using NMES for rehabilitation are discussed in Section 1.4. In Section 1.5 I describe some of the different ways that have been used to deliver NMES, including the two traditional methods (mNMES and nNMES) and one new method (iNMES), the latter being the main focus of the research described in this thesis. The subsequent section (Section 1.6), describes topics specific to each of the three experimental chapters. The examples provided in this first chapter will focus on NMES delivered to lower limb muscles, specifically the tibialis anterior (TA) and triceps surae (TS), as these are the muscles on which the experiments described in this thesis were performed. Both these muscles are important when using NMES to generate functional movements such as walking (Everaert et al., 2010; Higuchi et al., 2006). Moreover, NMES delivered over the TA and TS muscles can generate contractions through different pathways (see Section 1.3.2), a factor that was important for the experiments described in Chapter 3. The final section of this General Introduction (Section 1.7) introduces the objectives of the three experimental projects that comprise my PhD thesis research.

The three main research projects of this thesis are described in Chapters 2, 3 and 4. The experiments described in Chapter 2 were designed to test the effect of *stimulation amplitude* on the overlap between MUs recruited by mNMES and nNMES delivered to the TA muscle. The experiments described in Chapter 3 were designed to compare the influence of *stimulation frequency* and *pathway* on the torque produced during mNMES, nNMES and iNMES. Chapter 4 describes experiments designed to assess *discomfort* and *maximal torque* during mNMES, nNMES and iNMES. Finally, Chapter 5 comprises the General Discussion in which the main results of the three experimental chapters are summarized and integrated, and clinical implications and suggestions for how to overcome the main limitations of NMES are presented.

The work described in Appendix A represents the first project that I developed when starting my PhD program which is unrelated to iNMES. This project was designed to compare the effect of muscle length and stimulation type on torque generated by mNMES and nNMES.

1.2 NMES applications and benefits

NMES can be used to produce therapeutic effects (sometimes called therapeutic electrical stimulation) or to generate functional movements (sometimes called functional electrical stimulation). Therapeutic electrical stimulation is used to promote recovery from secondary complications of paralysis by increasing muscle mass, strength or bone mineral density [for review see (de Kroon et al., 2005)]. This type of stimulation is also used as part of a conditioning protocol before someone enters a functional electrical stimulation program. Functional electrical stimulation consists of activating muscles to produce coordinated movements that are functionally useful. Some examples include cycling with the legs (Faghri et al., 1992; Hopman et al., 2002) or arms (Davis et al., 1990; Raymond et al., 1997), rowing (Taylor et al., 2011; Wheeler et al., 2002), standing (Gillette et al., 2008), walking (Everaert et al., 2010; Higuchi et al., 2006) and grasping (Gan et al., 2012). In addition to producing useful movements, functional electrical stimulation can improve cardiovascular function (Hooker et al., 1992), strength (Wheeler et al., 2002), resistance to fatigability (Crameri et al., 2002), muscle mass (Crameri et al., 2002) and bone mineral density (Belanger et al., 2000). Therefore, both therapeutic and functional electrical stimulation are effective in reducing the secondary complications that accompany paralysis (Sabatier et al., 2006; Thomas et al., 1997). Throughout this thesis the term NMES will be used as a general term to describe therapeutic and functional electrical stimulation.

1.3 How are contractions generated during NMES?

1.3.1 Contractions are generated by depolarizing axons

During NMES, multiple pulses of stimulation are delivered repetitively as a "train". These pulses generate contractions by recruiting axons beneath the stimulating electrodes and not by the direct activation of muscle fibres. Axons are recruited at lower stimulation amplitudes than muscle fibres because the current required to initiate action potentials in muscle fibres is at least six times higher than for axons (Mortimer, 2011). Although it is possible to use NMES to generate contractions of denervated muscle, by depolarizing muscle fibres directly, NMES is much more commonly used to activate muscles with intact innervation thus by stimulating axons. Within this thesis, the experimental focus was on

NMES used to generate contractions of muscles with intact innervation and thus also the focus of the General Introduction. NMES depolarizes axons within peripheral mixed nerves, which can contain motor, sensory and autonomic axons (Hultman et al., 1983; Rattay et al., 2003). Table 1-1 provides an overview of how these axons can be differentiated based on their diameter, conduction velocity, rheobase and threshold for activation.

Table 1-1. Peripheral nerve fibre classification.

Myelinated						
Somatic		**Autonomic**				
Afferent	**Efferent**	**Afferent**	**Efferent**	**d[1] (µm)**	**CV[1] (m/s)**	**Stim Threshold***
Ia and Ib **(1.26 mA)[2]**	α-MN **(2.46 mA)[2]**			13-20	70-120	Very low
Group II and cut.	γ-MN	Group II		5-12	30-70	Low
Group III and cut.		Group III	B fibers	1-5	4.5-30	Medium
Unmyelinated[1]						
Group IV		Group IV	C fibers	0.2-1.5	0.4-3	High

Cut., cutaneous; *d*, axonal diameter; *CV*, axonal conduction velocity; *MN*, motor neuron; * Stimulation amplitude necessary to activate the axons (threshold).

[1] Erlanger and Gasser, 1937; Cats data.

[2] Mogyoros, 1996; rheobase of motor and sensory axons in humans.

NMES depolarizes axons by producing an electrical potential that influences the movement of ions across the axonal membrane. At rest, there is a difference in membrane potential between the inside and outside of an axon (i.e. resting membrane potential), where the inside is negatively charged relative to the outside. During NMES, the negatively charge cathode repels negative ions towards the outside of the axonal membrane. This makes the outside of the membrane relatively more negative, reducing the voltage difference between the inside and outside of the membrane, effectively depolarizing the axonal membrane. An opposite change occurs under the anode, where positive ions are repelled from the positively charged anode, increasing the polarization of the membrane, causing hyperpolarization. If the amplitude of stimulation is large enough, voltage-gated sodium channels under the cathode open, causing an inflow of sodium ions and initiating an “all or none” action potential which travels in both directions away from the stimulation site. Thus, these action potentials travel antidromically along motor axons to the cell body or along sensory axons towards the receptor

and orthodromically along motor axons to the muscle fibres or along sensory axons to the spinal cord. The orthodromic signals in motor and sensory axons are the ones that can generate contractions during NMES as described in the next section.

1.3.2 NMES can generate contractions through peripheral and central pathways

Traditionally it has been assumed that NMES produces contractions by the direct activation of motor axons, and thus via signals that are restricted to "peripheral" pathways (Figure 1-1A; motor volley). When contractions are generated in this way, the discharge of MUs is time-locked to each stimulation pulse, resulting in the synchronous discharge of MUs at the frequency of stimulation. Such MU discharge is recorded as successive "motor-" or M-waves in the electromyographic (EMG) signal (Figure 1-1B). The latency between each stimulus pulse and the discharge of the MUs recruited as M-waves depends on the distance between the stimulation site and the muscle. When the tibial nerve in the popliteal fossa is stimulated, for example, the M-wave is recorded in the soleus muscle 3 to 5 ms after the stimulation.

Action potentials initiated in sensory axons can also produce contractions, in this case via signals that traverse via "central" pathways. The sensory volley (Figure 1-1A) travels to the spinal cord along different types of axons including Ia and II afferents from muscle spindles, Ib afferents from Golgi tendon organs, and types III and IV from cutaneous and noxious receptors (see Table 1-1). A contraction is generated when the sensory volley is sufficient to initiate action potentials in motor neurons. This "reflexive" recruitment of MUs can be recorded as a "Hoffman" or H-reflex in the EMG signal (Figure 1-1B). When the tibial nerve is stimulated in the popliteal fossa, the latency of the H-reflex in the soleus muscle is approximately 30 ms. The difference in latency between M-waves and H-reflexes is due to the differences in the length of pathways along which action potentials travel. A second way that MUs discharge when recruited by the electrically-evoked sensory volley is represented as EMG activity that is not time-locked to each stimulus pulse and has therefore been described as "asynchronous activity" (Bergquist et al., 2011a; Bergquist et al., 2012b). The mechanism that underlies this asynchronous activity has been suggested to be related to the activation of persistent inward currents in motor neurons in the spinal cord (Collins et al., 2001; Dean et al., 2014).

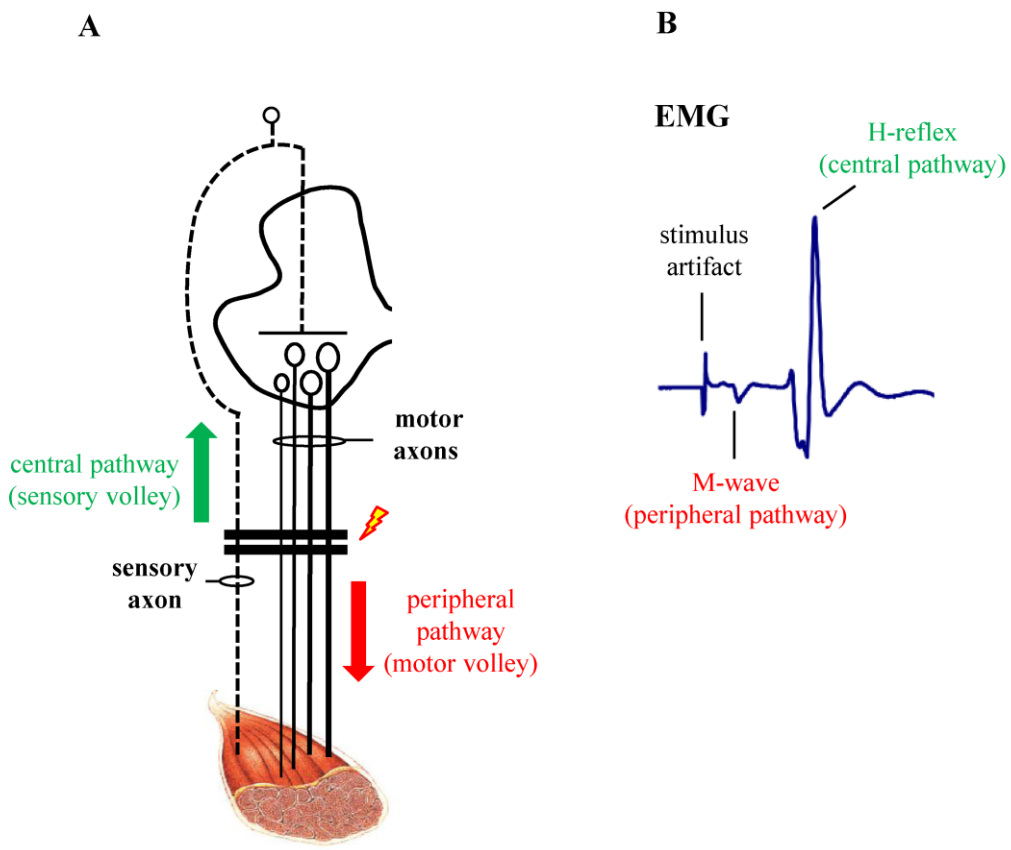

Figure 1-1. Central and peripheral pathways can contribute to contractions evoked by NMES in humans. **(A)** Diagram of pathways involved in generating the contractions during NMES. **(B)** Electromyographic (EMG) recording during one pulse of NMES. See Section 1.3.2 for further information.

1.3.3 NMES parameters influence how torque is produced

During voluntary movements, torque can be modulated by recruiting and de-recruiting MUs (Henneman et al., 1965b) and by increasing or decreasing MU discharge rates (i.e. "rate coding") (Bigland et al., 1954). This section will describe how NMES parameters, which include pulse amplitude, frequency and duration, can be manipulated to modulate torque during NMES. Moreover, I will discuss how some of these parameters influence the recruitment of MUs through peripheral and central pathways. The following discussion is based on monophasic rectangular pulses, since this is the waveform that is most commonly used when NMES is used for rehabilitation and is used in all the experiments described in this thesis.

1.3.3.1 Stimulation amplitude

Stimulation amplitude during NMES refers to the amplitude of current delivered by each pulse of stimulation. Common stimulators used for research and designed to deliver stimulation using surface electrodes typically have peak output currents between 100 to 1000 mA. When stimulation amplitude is high enough, axons are depolarized above the threshold for action potential initiation and torque starts to be produced. A progressive increase in stimulation amplitude results in a progressive recruitment of more axons and consequently more torque (Adams et al., 1993; Gorgey et al., 2006). Eventually, when stimulation amplitude is sufficiently high to recruit all of the axons under the electrodes, further increases in stimulation amplitude do not result in further increases in torque. Therefore, stimulation amplitude is one of the parameters that can be manipulated to modulate the number of MUs recruited and thus the torque produced by NMES.

1.3.3.2 Pulse duration

Pulse duration refers to the time over which current is delivered during one phase of a single stimulus pulse. Common pulse durations used during traditional NMES are 0.3 to 0.6 ms (Eser et al., 2003; Janssen et al., 2004). For similar reasons as increasing stimulation amplitude, increasing pulse duration during NMES results in more torque due to the recruitment of more axons (Gorgey et al., 2006).

One important feature of manipulating pulse duration during NMES that is relevant for the research described in this thesis is that the relative recruitment of motor and sensory axons, and thus the peripheral and central contribution to the evoked contractions, can be altered by changing pulse duration. Grill and Mortimer (1996) showed that motor axons are preferentially activated by relatively short pulse durations, between 0.05 and 0.4 ms. Sensory axons, on the other hand, are more effectively recruited by pulse durations between 0.5 and 1 ms (Kiernan et al., 1996; Mogyoros et al., 1996). The reasons behind these differences in the recruitment of sensory and motor axons is that sensory axons have a lower rheobase and longer strength-duration time constant than motor axons (Panizza et al., 1998; Veale et al., 1973) resulting in a more effective recruitment of sensory axons when using long pulse durations (Mogyoros et al., 1996). This fact must be considered when one aims to manipulate the relative transmission along central (i.e. contractions produced by a sensory volley that recruits motor neurons in the spinal cord) or peripheral pathways (i.e. contractions generated

by the direct activation of motor axons, bypassing the spinal cord) during NMES as was the case for the experiments described in Chapters 2 and 3. In Chapter 2, we wanted to reduce the probability of generating H-reflexes in the TA muscle and maximize the contribution made by MUs recruited as M-waves by using a short pulse duration. In Chapter 3, we chose a short pulse duration for the TA, for the same reason as in Chapter 2, and a long pulse duration for TS to increase the probability of evoking H-reflexes in the soleus muscle to maximize the extent to which contractions were produced via central pathways. A relatively long pulse duration was also used in the experiments described in Appendix A, where we wanted to maximize the central contribution to the torque generated by NMES.

1.3.3.3 Stimulation frequency

Stimulation frequency refers to the number of pulses delivered per unit of time during a train of NMES and is another NMES parameter that can be manipulated to modulate torque during NMES. In general, NMES is delivered at frequencies ranging from 30 to 50 Hz to generate strong fused contractions (Deley et al., 2015; Gregory et al., 2007; Kesar et al., 2008). The increase in torque with increases in frequency is not due to the recruitment of more MUs, but rather is due to an increase in MU discharge rate (Black et al., 2008; Gorgey et al., 2006). The effect of manipulating stimulation frequency on torque during NMES is discussed further in Section 1.6.2 and is the focus of the experiments described in Chapter 3.

1.4 Limitations of using NMES for rehabilitation

The benefits of using NMES for rehabilitation are limited by fatigability and discomfort. Fatigability during NMES manifests as a rapid decrease in torque over time (Enoka et al., 2008) and is due in part to the non-physiological way in which NMES generates contractions which include the random recruitment order and high discharge rates of MUs. Fatigability limits the duration that a given task can be produced by NMES. Discomfort during NMES manifests as pain and originates from the recruitment of nociceptor afferents. Discomfort reduces the number of individuals who can participate in NMES programs and limits the amount of torque that NMES can generate in those who do participate. Both fatigability and discomfort limit the extent to which NMES can produce positive neuromuscular and cardiovascular adaptations since NMES-based exercises can only be performed at relatively low intensities for short periods. The next two sections will discuss fatigability and discomfort

during NMES in more detail and will describe how the experiments in this thesis were designed to address both these limitations.

1.4.1 Fatigability during traditional NMES

In general, fatigability limits the time and intensity that a given task can be performed using NMES. It has been recommended that individuals affected by paraplegia should perform voluntary exercises five times a week, for 20 to 60 minutes in each session at an intensity of 50 to 80% maximal heart rate or 50 to 80% of a single maximal repetition (Jacobs et al., 2004; Vincent, 2013). To our knowledge, there is no such guideline for electrically-induced exercises. However, it is improbable that NMES can produce exercises of such volume and intensity given that torque decreased by 44% during a 3 min NMES-fatiguing protocol in individuals with no impairment (Bickel et al., 2004). Therefore, rapid fatigability is an important limitation of the benefits of NMES used for rehabilitation. It should be acknowledged that fatigability during NMES is even greater in people experiencing paralysis than those with no neurological impairment; torque decreased by 67% over the course of the same protocol that resulted in a 44% decrease in individuals with no impairment (Bickel et al., 2004). Thus, at least part of the rapid fatigability in individuals experiencing paralysis is due to compromised muscle quality. It is well known that individuals experiencing paralysis have a conversion in muscle fibre type from slow to fast-fatigable phenotypes (Castro et al., 1999) accompanied by deficits in muscle mass (Crameri et al., 2002) and strength (Wheeler et al., 2002). However, since fatigability developed rapidly in both individuals with and without paralysis, resistance to fatigability during NMES is not only due to disuse-related adaptations of the muscle, but is also due to the way NMES generates contractions where during NMES MUs are recruited in a random order according to type and at frequencies higher than during voluntary contractions (Bickel et al., 2011; Gregory et al., 2005; Jubeau et al., 2007), as described in more detail in the next two paragraphs.

Rapid fatigability is due in part to the order in which MUs are recruited during NMES. Generally, the literature supports the hypothesis that MU recruitment order during traditional NMES is random (Jubeau et al., 2007; Slade et al., 2003) with respect to Henneman's size principle (Henneman et al., 1965a, 1965b). Henneman's size principle states that during voluntary contractions, small motor neurons which innervate muscle fibres with high resistance to fatigability, are recruited first, followed by progressively larger motor neurons

which innervate muscle fibres with progressively lower resistance to fatigability. This "orderly" recruitment is due to differences in input resistance of the cell bodies of motor neurons of different sizes. For a given input, a larger excitatory postsynaptic potential will be generated in a small motor neuron because it has a higher input resistance than a larger motor neuron. The random recruitment order of motor axons during traditional NMES results from the fact that contractions are generated by the recruitment of motor axons and not by synaptic input to motor neuron cell bodies. When axons are recruited by stimulation delivered through superficial electrodes, both axon diameter and distance between the axon and the stimulating electrode play important roles in determining which axon, and thus MU, will be recruited first. Theoretically, large diameter axons are activated at lower stimulation amplitudes than small axons due to their lower axial resistance (Enoka, 2002). If this was the only factor involved it would result in a reversed order of recruitment of motor neurons compared to voluntary contractions. However, when NMES is applied through the skin, the distance from the stimulating electrode is also an important factor as action potentials may be initiated in small diameter axons located closer to the electrode at a lower stimulation amplitude than larger axons located farther away (Grill et al., 1995). Thus, axon diameter and distance from the stimulating electrode are both important when using surface electrodes and recruitment order during NMES is thought to be random with respect to MU type (Gregory et al., 2005).

Rapid fatigability during NMES is also a result of the non-physiologically high discharge rates of recruited MUs (Gorgey et al., 2006; Jubeau et al., 2007; Kim et al., 1995; Maffiuletti, 2010). During voluntary contractions, MUs are recruited asynchronously from one another, resulting in fused torque at relatively low discharge rates (Bellemare et al., 1983). During voluntary contractions, the average discharge rate of MUs of the TA muscle, for example, is between 10 to 20 Hz during submaximal smooth contractions (De Luca et al., 2010) and ~27 Hz during MVICs (De Luca et al., 2010), although they can discharge from 60 to 120 Hz for very short periods of time during a brisk ballistic contractions (Desmedt et al., 1977). On the other hand, when MUs are recruited by the depolarization of motor axons, as occurs during traditional NMES, the discharge of MUs is time-locked to each stimulation pulse and thus all MUs discharge synchronously. This synchronous recruitment of MUs during NMES increases the minimal frequency of stimulation required to produce strong fused contractions. Accordingly, NMES frequencies between 30 to 50 Hz have been recommended

to produce fused torque of sufficient amplitude to be useful for rehabilitation (Deley et al., 2015; Gregory et al., 2007; Kesar et al., 2008). Therefore, rapid fatigability during NMES is, in part, a consequence of the synchronous recruitment of MUs at frequencies that exceed the high end of the physiological firing rate of submaximal contractions which increases the metabolic cost of the evoked contractions (Bridges et al., 1991; Vanderthommen et al., 2003).

Although none of the experiments described in this thesis were designed to reduce fatigability directly, the results contribute to the development of iNMES which reduces fatigability (Lou et al., under review) by reducing the discharge rate of recruited MUs recruited by each NMES site.

1.4.2 Discomfort during NMES

Discomfort during NMES represents an individual's perception that results from the excitation of nociceptor afferents in the skin and musculotendinous structures (Delitto et al., 1992; Gracanin et al., 1975; Matthews et al., 1997; Vodovnik et al., 1965). These nociceptive afferents include group III fibres that convey information about mechanical stimuli and heat and produce a rapid pain response (Adriaensen et al., 1983; Georgopoulos, 1977) and group IV fibres that respond to mechanical, thermal and chemical stimulation (Hallin et al., 1982; Vanhees et al., 1972) and produce a more diffuse sensation of pain. It has been suggested that when NMES is delivered to produce small contractions, discomfort is associated with the activation nociceptor afferents in the skin (characterized as a "sharp" sensation). When large contractions are generated an additional source of discomfort is associated with the activation of nociceptor afferents in the muscle since the description of discomfort was as "muscle tearing" or "diffused or aching discomfort" (Delitto et al., 1992; Laufer et al., 2011; Matthews et al., 1997; Yu et al., 2001). It is important to emphasize that discomfort is a limitation only when an individual has intact or at least partial sensation. Discomfort does not limit participation in NMES programs for individuals who have no sensation, such as those with a complete spinal cord injury; however, the depolarization of nociceptor afferents can be problematic for individuals prone to autonomic dysreflexia (Ashley et al., 1993).

The success of NMES for producing positive neuromuscular adaptations is known to depend on the initial tolerance of stimulation amplitudes required to produce functionally relevant torque (Giavedoni et al., 2012; Vivodtzev et al., 2012; Vivodtzev et al., 2014). As previously discussed (Section 1.3.3), torque during NMES can be manipulated by changing

NMES parameters such as amplitude, pulse duration and frequency, with amplitude being the parameter that is most commonly manipulated. However, an increase in amplitude or pulse duration will recruit not only more MUs and produce more torque, but will also recruit more nociceptor afferents and produce more discomfort. Contractions of at least 10% MVIC are necessary to improve muscle function during an NMES training protocol (Melo et al., 2013; Talbot et al., 2003). Therefore, participants who cannot withstand the stimulation amplitude or pulse duration required to generate such low contraction amplitudes will likely not benefit from NMES as a rehabilitation tool. In fact, such individuals may not complete or even start NMES-based programs. The success of NMES training also depends on an individual's capacity to withstand progressive increases in overload which depend on increasing stimulation amplitude (Giavedoni et al., 2012; Vivodtzev et al., 2012; Vivodtzev et al., 2014). Therefore, discomfort can also limit the benefits of NMES in individuals who may be able to tolerate the initial stimulation amplitude but cannot tolerate further increases. Importantly, discomfort during NMES can be minimized by manipulating electrode size, NMES type and/or stimulation parameters (Lyons et al., 2002) as discussed in Section 1.6.3.

Therefore, it is important to identify ways to reduce the discomfort produced by NMES without compromising torque. The experiments described in Chapter 4 were designed to address the possible benefits of using iNMES to reduce discomfort when compared to traditional types of NMES.

1.5 NMES types

This section provides an overview of some of the different ways that NMES has been delivered to produce contractions of human muscles. Traditionally, NMES has been delivered through a single pair of surface electrodes positioned over a muscle belly (mNMES) or superficial nerve trunk (nNMES) using relatively narrow pulse durations and low frequencies. This traditional form of NMES is thought to produce contractions by the direct activation of motor axons, thus via peripheral pathways. Recently, novel NMES parameters such as wider pulse durations and higher frequencies (Bergquist et al., 2011a; Klakowicz et al., 2006) have been used to amplify the central contribution to the torque generated by NMES and produce contractions more similar to the ones produced voluntarily. Other novel ways of delivering NMES have also been developed, such as sequential (Nguyen et al., 2011) and interleaved

NMES (Lou et al., 2015, under review), to reduce fatigability and improve the effectiveness of NMES for rehabilitation. It should be noted that NMES can also be delivered through implanted electrodes, but this method is more expensive, invasive and potentially less safe than surface NMES. NMES delivered using implanted electrode systems is beyond the scope of the research described in this thesis and will not be discussed further in this section, although such approaches are discussed briefly in Sections 1.5.2.1 and 1.6.1.

1.5.1 NMES over the muscle belly or the nerve trunk

Traditional NMES was developed with the goal of depolarizing motor axons under the stimulating electrodes to produce contractions through peripheral pathways. Throughout this thesis, traditional NMES is defined as when mNMES or nNMES are delivered through one pair of electrodes using pulse durations between 0.3 to 0.6 ms and frequencies between 30 to 50 Hz. The next Sections (1.5.1.1 and 1.5.1.2) will discuss the advantages and limitations when using different types of NMES.

1.5.1.1 mNMES

mNMES is the most common way that NMES has been delivered for rehabilitation. This is likely because it is the most user friendly method since the muscle belly of superficial muscles are readily identifiable, making electrode placement relatively easy. mNMES consists of positioning stimulating electrodes over the muscle belly with the goal of recruiting the axons innervating a given muscle. Further, by placing the electrodes directly over the muscle belly (Figure 1-2A; black filled squares), large muscles can be activated relatively independently with little or no activation of adjacent muscles that produce torques that may be counterproductive. In most cases during mNMES, the cathode is positioned over the motor point to minimize the stimulation amplitude required to produce contractions (Gobbo et al., 2014).

Peripheral pathways (Figure 1-1A; motor volley) are the primary way that contractions are generated by traditional mNMES (Bergquist et al., 2011a; Bergquist et al., 2012b; Mortimer, 2011). This is due in part to the fact that the narrow pulse durations (i.e. 0.3 to 0.6 ms) recruit primarily motor axons (Mogyoros et al., 1996). One important advantage of generating contractions through peripheral pathways is that torque is more consistent (~10% coefficient of variation) than when central pathways (18% coefficient of variation) are involved since motor axons are activated directly without modulation of spinal mechanisms

(Baldwin et al., 2006; Bergquist et al., 2012b). A disadvantage of recruiting motor axons directly is that recruitment order is random in relation to MU type, resulting in rapid fatigability (Bickel et al., 2011; Binder-Macleod et al., 1995; Gregory et al., 2005; Jubeau et al., 2007). In general, the central contribution during mNMES is low when mNMES is delivered using traditional NMES parameters (Baldwin et al., 2006; Okuma et al., 2013) but it can be increased by using non-traditional wide pulse high frequency stimulation (Collins et al., 2001, 2002; Nickolls et al., 2004). Regardless of the stimulation parameters used, the central contribution during mNMES is smaller than during nNMES, even in muscles with a high probability of generating H-reflexes, such as the TS (Baldwin et al., 2006).

At low stimulation amplitudes, mNMES generates contractions by recruiting MUs close to the stimulating electrodes, thus in superficial portions of the muscle (Adams et al., 1993; Okuma et al., 2013), as depicted in Figure 1-2B. Increases in stimulation amplitude result in the activation of deeper MUs and the production of larger torque (Adams et al., 1993; Okuma et al., 2013). It can be difficult to activate the whole muscle during mNMES since high stimulation amplitudes are necessary to recruit motor axons in deep portions of the muscle belly. It has also been suggested that the most torque that can be produced by mNMES delivered to the TA was 40% MVIC before discomfort was a limitation (Milner et al., 1969). On the other hand, mNMES delivered to the quadriceps generated contractions between 50% MVIC (Scott et al., 2014) to 88% MVIC (Kramer, 1987), suggesting that discomfort is less of a limitation when mNMES is delivered to this muscle. The differences between these muscles regarding to the torque that can be produced before limited by discomfort could be associated with the density of nociceptor afferents in different areas of the leg, where the density would be larger in the TA skin area resulting in more discomfort and lower maximal torque. This hypothesis has yet to be tested.

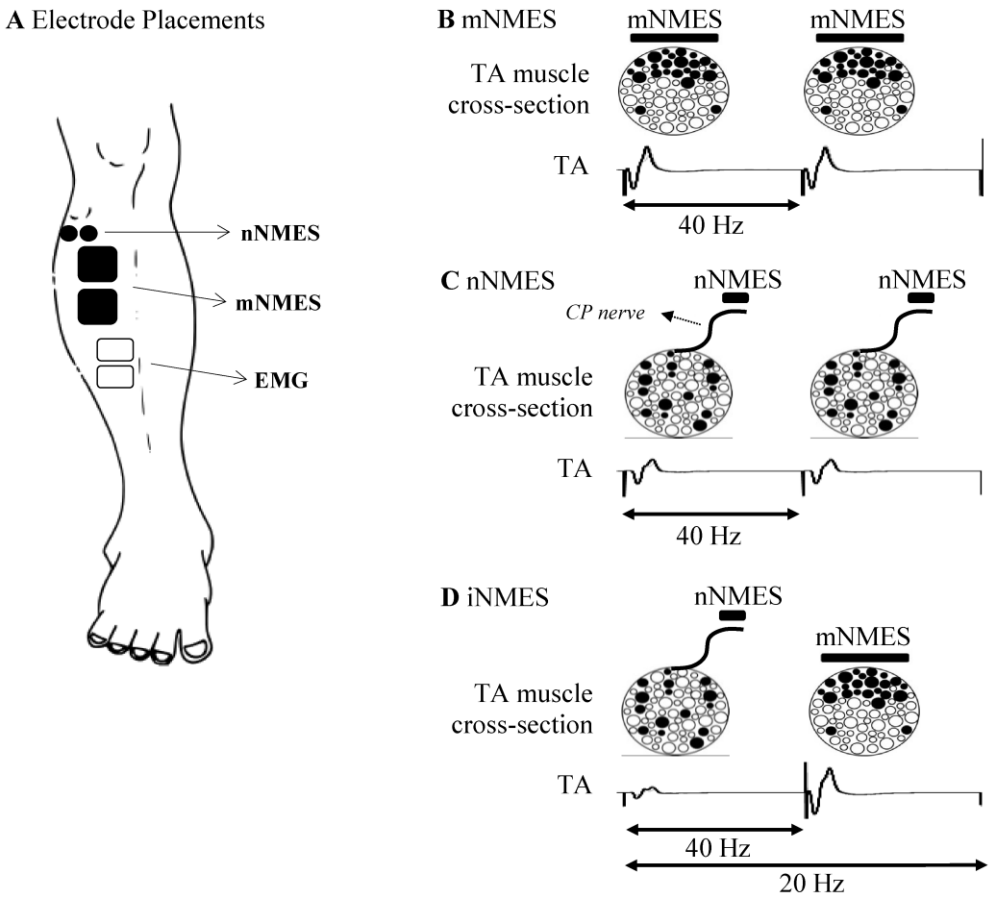

Figure 1-2. Spatial recruitment of MUs during mNMES, nNMES and iNMES delivered to the TA. **(A)** Electrode placement for mNMES over the muscle belly of the TA muscle (black squares), nNMES over the common peroneal nerve (black circles), and when both stimulation sites were stimulated during iNMES. White squares represent the location where EMG was recorded from the TA and the grey square represents the location of the reference electrode over the tibia bone. **(B)** Representation of the superficial recruitment of MUs (filled circles in the cross-section of the TA muscle) during mNMES. The traces under the muscle cross-sections represent the EMG (M-waves) recorded in response to each stimulation pulse of the train. **(C)** Representation of the more wide-spread recruitment of MUs in the TA muscle during nNMES delivered to the common peroneal nerve with the associated EMG recordings. **(D)** Representation of the recruitment of different groups of MUs with every other stimulus pulse during an iNMES train. Note that the net frequency delivered during iNMES is the same as during mNMES or nNMES (40 Hz) but the frequency delivered to each stimulation site is half the net frequency (20 Hz). EMG is shown for each stimulation site.

In summary, the main advantages of mNMES are its' ease of application and consistent torque production. Despite these advantages, the torque produced by mNMES can be limited by discomfort and rapid fatigability.

1.5.1.2 nNMES

nNMES is delivered by positioning stimulating electrodes on the skin over a nerve trunk where it runs close to the skin surface, as represented by the black circles in Figure 1-2A. Thus, instead of targeting axons dispersed throughout the muscle belly, such as during mNMES, nNMES activates axons bundled in a mixed nerve trunk innervating a given muscle.

nNMES can generate contractions through peripheral pathways by the direct recruitment of motor axons, similar to mNMES. The contractions generated by mNMES and nNMES through peripheral pathways are prone to rapid fatigability since MUs are recruited randomly according to MU type and synchronously at high frequencies (Bickel et al., 2011; Maffiuletti, 2010).

An important characteristic of nNMES is that it can generate contractions with a strong central contribution, due to the recruitment sensory axons. This central contribution to contractions produced by nNMES has been demonstrated for the TA (Klakowicz et al., 2006), quadriceps (Bergquist et al., 2012b) and TS (Bergquist et al., 2011a; Klakowicz et al., 2006; Lagerquist et al., 2010) muscles. Collins and colleagues (Bergquist et al., 2011a; Klakowicz et al., 2006), found that the central contribution to contractions generated by nNMES can be augmented when a train of stimulation at 20 Hz is interspersed with a brief "burst" of wide-pulse (1 ms) high-frequency stimulation (100 Hz). This pattern of stimulation resulted in torque larger than expected or "extra torque", after the burst of high frequency stimulation. Torque increased up to 40% of a MVIC after the burst of high frequency stimulation (Collins et al., 2002). The central origin of extra torque has been confirmed because it coincides with enhanced H-reflexes (Bergquist et al., 2011a; Klakowicz et al., 2006), asynchronous activity (Bergquist et al., 2011a) and the finding that it was abolished during an anaesthetic block of the nerve between the stimulation site and the central nervous system (Collins et al., 2001, 2002; Lagerquist et al., 2009a). Extra torque has been suggested to be due to the activation of persistent inward currents in motor neurons and/or post-tetanic potentiation of neurotransmitter release from afferent terminals (Collins et al., 2001). Regardless of the

mechanism, motor neurons activated synaptically by the electrically-evoked sensory volley are recruited according to Henneman's size principle (Buchthal et al., 1970; Henneman, 1957; Trimble et al., 1991) and generate contractions that are more resistant to fatigability than contractions generated exclusively by peripheral pathways (Bergquist et al., 2014).

Instead of preferentially recruiting MUs in the superficial portions of the muscle such as during mNMES, at least in the TA muscle, nNMES recruits MUs that are distributed evenly throughout the muscle, regardless of stimulation amplitude (Okuma et al., 2013), as represented in Figure 1-2C. Moreover, the clustering of axons in the nerve trunk close to the stimulating electrodes reduces the stimulation amplitude necessary to produce a given torque which typically results in less discomfort than during mNMES (Lyons et al., 2004; Naaman et al., 2000).

nNMES can be limited by the specificity and consistency of torque production. The torque produced by nNMES can be compromised because axons innervating more than one muscle may be bundled inside the stimulated nerve trunk. Therefore, the selective activation of a single muscle or synergistic muscles can be more challenging than during mNMES. The stimulation of the common peroneal nerve, for example, can result in the activation of not only ankle dorsiflexors but also evertors, which produce plantarflexion torque. Another limitation of nNMES is that the torque produced through central pathways is less consistent than the torque produced through peripheral pathways (Baldwin et al., 2006; Bergquist et al., 2012b). The reduced consistency is due to in part because motor neurons recruited through central pathways are exposed to complex inhibition and excitation modulatory mechanisms, mainly in the spinal cord.

In summary, nNMES has the potential to produce large torque with low discomfort while reducing fatigability if central pathways are involved. However, the torque produced during nNMES can be less consistent than mNMES, and localizing the nerve is not as simple as the muscle belly. Moreover, fatigability develops rapidly when nNMES generates contractions mainly through peripheral pathways since MU recruitment order is random; on the other hand, contractions driven mainly through central pathways result in less fatigability since MU recruitment order occurs according to Henneman's size principle, providing a possible mechanism to reduce fatigability during NMES.

1.5.2 New ways to deliver NMES

1.5.2.1 Sequential NMES

Sequential NMES was developed to minimize fatigability by recruiting different MUs from different stimulation sites, thereby reducing discharge rates of recruited MUs. To achieve this goal, sequential NMES is delivered through one large electrode subdivided into four small cathodes paired with one large anode (Nguyen et al., 2011) or through four to six cathodes placed over different portions of the muscle sharing a common anode (Downey et al., 2014; Malesevic et al., 2010). Stimulation is then rotated between pairs of electrodes, reducing the frequency delivered to each pair of electrodes without affecting the overall frequency delivered to the muscle (Nguyen et al., 2011; Popovic et al., 2009). In this way, for example, 40 Hz delivered to the whole muscle can be achieved by rotating stimulation at 10 Hz between four stimulation sites. Although this method is relatively new for surface NMES, similar ideas were initially tested in animal models using implanted electrodes.

The idea of stimulating different groups of MUs using different channels of stimulation (different pairs of electrodes) was initially tested in an animal model by Rack and Westbury (1969). These authors demonstrated that small fused contractions could be produced by rotating stimulation pulses between five electrodes implanted into the ventral roots of cats. Petrofsky and colleagues (Lind et al., 1978; Petrofsky, 1979) showed that maximal tetanic tension could be produced at frequencies within the physiological range when stimulation was rotated between electrodes in different ventral roots of cats. Later, Yoshida et al. (1993a) delivered stimulation to two pairs of electrodes embedded to a implanted cuff electrode to produce 25% of the maximal force and showed that fatigability was reduced compared to when stimulation was delivered to a single pair of electrodes. Similarly, Mushahwar and Horch (1997) showed that stimulation delivered to the lumbo-sacral spinal cord of cats through one pair of electrodes caused rapid and high fatigability; however, stimulation alternated between two electrodes eliminated fatigability.

In humans, sequential NMES applied through four pairs of electrodes over the quadriceps produced fused contractions, even when the stimulation frequency delivered to each of the four stimulation channels located over the quadriceps muscle was as low as 4 Hz (Downey et al., 2014). This approach produced less fatigability compared to traditional mNMES, in individuals with no neurophysiological impairments (Downey et al., 2014) and in

individuals with spinal cord injury (Downey et al., 2015; Nguyen et al., 2011; Popovic et al., 2009; Sayenko et al., 2013). Downey et al. (2015) showed that, in individuals with spinal cord injury, traditional mNMES delivered over the quadriceps muscle at 32 Hz caused a decrease in torque of 55% of the initial value while sequential NMES delivered at a "net" frequency of 32 Hz (i.e. 8 Hz to each pair of electrodes) resulted in only 39% decrease in torque. These authors found similar results when mNMES and sequential NMES were compared in individuals with no impairment: torque decreased by 39% during mNMES but only by 11% during sequential NMES. Sequential NMES also reduced fatigability when compared to mNMES in the biceps femoris, TA and TS of individuals with and without neurophysiological impairments (Sayenko et al., 2014a). These results show that sequential NMES can be used to produce fused contractions even when low stimulation frequencies delivered to each pair of electrodes and that it can significantly reduce fatigability. Potential disadvantages of sequential NMES are that high stimulation amplitudes are necessary to recruit MUs located in deep portions of the muscle and generate large torque, resulting in discomfort. Moreover, the overlap in MU recruitment between stimulation sites can be large if the electrodes are positioned close together; however, both these hypotheses have yet to be tested.

1.5.2.2 iNMES

Similar to sequential NMES, iNMES was developed to reduce fatigability by reducing MU discharge rates. Specifically, iNMES was developed in our lab to reduce fatigability of TA by alternating stimulus pulses between pairs of electrodes over the mNMES and nNMES sites. This approach relies on the fact that at low to moderate stimulation amplitudes MUs in different portions of the TA muscle are recruited from the mNMES and nNMES sites [Figure 1-2D; (Okuma et al., 2013)]. Therefore, similar to sequential NMES, without changing the overall frequency delivered to the muscle, MU discharge rates are reduced using iNMES compared to when mNMES or nNMES are delivered alone. However, the extent to which sequential NMES and iNMES reduces fatigability depends on the amount of overlap between MUs recruited by each stimulation site. Indeed, if both sites recruit the same MUs, iNMES would have no advantage compared to traditional mNMES or nNMES. Identifying the effect of stimulation amplitude in the overlap of MU recruitment by mNMES and nNMES was the main goal of the experiments described in Chapter 2.

Lou et al. (under review) showed that iNMES delivered to activate the TA and generate an initial torque of ~15% MVIC resulted in less fatigability than mNMES or nNMES. Torque decreased 39% over the course of 240 contractions (12 minutes) during iNMES while it decreased 67% during mNMES and 58% during nNMES. The contractions generated in these experiments were mainly due to the direct activation of motor axons (i.e. peripheral pathways). iNMES may reduce fatigability even further in muscles with high probability of generating contractions through central pathways. In muscles with strong H-reflexes such as the TS and quadriceps (Zehr, 2002), MU recruitment could occur according to Henneman's size principle if muscle contractions were generated, at least partially, through central pathways. Theoretically, this would further reduce fatigability intrinsic to NMES. Therefore, it is important to identify the extent to which contractions can be generated through central pathways during iNMES, which was one of the goals of the experiments described in Chapter 3.

In addition to the finding that iNMES reduces fatigability compared to mNMES and nNMES, Lou et al.'s study (under review) also provided two other interesting results that are relevant for the experiments described in this thesis: 1) a trend for lower current at each stimulation site during iNMES compared to mNMES and nNMES; and 2) a trend for less discomfort during iNMES compared to mNMES and nNMES. However, Lou et al.'s experiments were not specifically design to test discomfort and limitations in their protocols prompted us to explore discomfort during iNMES more rigorously in the work described in Chapter 4. For example, in the Lou et al. study, only one stimulation amplitude was tested and discomfort was assessed mid-way through a protocol designed to test fatigability. Further, the different NMES types were tested on different days, which may have compromised the discomfort and current measurements due to changes in skin impedances and electrode placements between days. The experiments described in Chapter 4 were specifically designed to compare discomfort during mNMES, nNMES and iNMES applied on the same day over a range of torque amplitudes to more adequately address the topic.

In summary, iNMES has potential to minimize the main limitations of using NMES for rehabilitation: iNMES can reduce fatigability (Lou et al., under review) and we expected that it would reduce discomfort without compromising torque.

1.6 Addressing knowledge gaps about iNMES

The overall goal of this thesis was to contribute to the background knowledge about iNMES to develop a better understanding of how best to use iNMES for rehabilitation. To achieve this goal some important gaps in our knowledge about iNMES have to be addressed. These gaps include: 1) the effects of the *amplitude of stimulation* on the overlap of MUs recruited by the mNMES and nNMES sites; 2) the effect of *frequency of stimulation* and *pathway* on torque; and 3) how does *discomfort* and *maximal torque* during iNMES compare to that during mNMES and nNMES. The following sections summarize what is known about techniques used to estimate MU recruitment overlap (Section 1.6.1), the effect of frequency on the torque produced during NMES (Section 1.6.2) and discomfort during NMES (Section 1.6.3).

1.6.1 Estimating MU recruitment overlap during NMES

As previously mentioned, iNMES will only effectively reduce fatigability when different MUs are recruited from the mNMES and nNMES sites. However, how the overlap in MUs recruited by these two sites influences the torque produced by iNMES is unknown. The first experimental chapter of this thesis was designed to address this initial knowledge gap by using an overlap estimation technique previously used to quantify overlap of MUs recruited between different pairs of electrodes in implanted multi-electrode arrays (McDonnall et al., 2004b). This technique initially involves constructing separate twitch torque recruitment curves for two different pairs of electrodes of the array (Branner et al., 2001; Rutten et al., 1991; Yoshida et al., 1993a). A twitch torque recruitment curve is constructed by delivering single pulses of stimulation over a wide range of stimulation amplitudes, to generate twitches ranging from the smallest to the largest possible and then plotting twitch amplitude versus stimulation amplitude. A third recruitment curve is then constructed when both pairs of electrodes are stimulated together, where the second electrode is stimulated in the absolute refractory period (1 to 3 ms after the stimulation of the first electrode) induced by the stimulation of the first electrode. At a given stimulation amplitude, the sum of the torque produced by each pair alone is compared to the torque produced when both are stimulated together. If the torque produced by the stimulation of both pairs of electrodes together is equal to the sum of the torque produced by each pair alone, overlap is calculated to be 0%. If the torque produced by the

stimulation of both electrode pairs together is the same as the torque produced by the two pairs stimulated separately, this corresponds to 100% overlap.

This technique used to estimate overlap in MU recruitment for multi-electrode arrays was initially used animal models. Yoshida et al. (Yoshida et al., 1993b) found that the lowest overlap they could obtain was 27% between two pairs of intrafascicular wire electrodes implanted into the tibial nerve of cats. Several studies have tested the overlap in MU recruitment between different pairs of electrodes of large electrode arrays implanted into nerves of cats (Branner et al., 2001; McDonnall et al., 2004a, 2004b). It was found little to no overlap at stimulation amplitudes that produced less than 20% of the maximal twitch and that overlap increased progressively with increases in stimulation amplitude. This overlap estimation technique was recently used to quantify MU recruitment overlap in implanted electrodes in humans. In two studies, Fisher and colleagues (Fisher et al., 2011; Fisher et al., 2013) implanted cuff electrodes around the femoral nerve of humans. Overlap in MU recruitment between the two pairs of electrodes in each cuff increased with increases in stimulation amplitude. More importantly, functionally relevant torque, sufficient to lock the knee during standing, could be generated at a stimulation amplitude that produced little (10%) to no overlap.

For rehabilitation, however, NMES is mainly delivered through surface electrodes, due to the simplicity, reduced cost and safety compared to implanted systems. No studies have been designed to estimate MU recruitment overlap between surface NMES electrodes in humans. The first estimation of overlap in MU recruitment during surface stimulation is described in Chapter 2. The goal of these experiments was to estimate the overlap in MUs recruited by mNMES and nNMES delivered to the human ankle dorsiflexors over a range of torque amplitudes. The results of these experiments address the first knowledge gap and provide information about how best to use iNMES for rehabilitation, since this technique strongly depend on the recruitment of different groups of MUs from different stimulation sites to reduce fatigability. Moreover, the results of these experiments contribute to the understanding of how contractions are generated during iNMES.

1.6.2 Relationship between torque and NMES frequency

The second knowledge gap that the experiments described in Chapter 3 were designed to address the effect of frequency of stimulation and pathway on the torque generated by iNMES

compared to mNMES and nNMES. It is important to address this effect since it is not currently known if the fact that iNMES can recruit different MUs at each NMES site contributes to an increased capacity of generating torque at different frequencies of stimulation when compared to traditional NMES. This "torque-frequency relationship" (TFR) is typically described by a sigmoidal curve when torque is plotted against stimulation frequency (Binder-Macleod et al., 1992). The TFR has been mainly used to identify the mechanisms that contribute to the development of fatigability (Binder-Macleod et al., 1992; Edwards et al., 1977; Millet et al., 2011b). A reduction in torque generating capacity after a fatigability protocol specifically at low frequencies of the TFR has been attributed to impairments in excitation-contraction coupling (Edwards et al., 1977; Millet et al., 2011a). A reduction in torque produced at high frequencies of the TFR has been attributed to reduced neurotransmitter release at the neuromuscular junction and impaired action potential propagation across the sarcolemma (Edwards et al., 1977; Millet et al., 2011a). Although most of the current TFR data derives from studies designed to investigate fatigability, the TFR can be used to simply describe changes in torque due to changes in frequency when, for example, different muscle lengths (Gerrits et al., 2005) or NMES types are compared.

Most of the experiments designed to characterize the TFR during NMES have been performed on the human quadriceps muscle using mNMES (Binder-Macleod et al., 1992; Edwards et al., 1977; Gerrits et al., 2005; Periard et al., 2014). In this muscle (Binder-Macleod et al., 1992), low frequencies of stimulation (<5 Hz) produce individual twitches with full recovery. At frequencies from 5 to 10 Hz, individual twitches can still be seen but there is no complete relaxation between twitches (i.e. torque is unfused) and torque starts to sum. Torque is fused at frequencies of around 20 Hz. With further increases in frequency, torque increases and reaches a steady state at approximately 50 Hz, where further increases in frequency do not result in further increases in torque. Only a few studies have investigated the TFR when mNMES was delivered to the TA (Harridge et al., 2002; Mela et al., 2001; Orizio et al., 2004) and TS (Rafolt et al., 1999; Sale et al., 1982; Stackhouse et al., 2005) muscles. These studies did not use a wide range of frequencies, often limited to a maximum of 50 Hz. The torque steady state frequency for the TA was not defined by any of the previous studies. It was previously suggested that the torque steady state frequency for the TS was 50 Hz during mNMES (Sale et al., 1982; Stackhouse et al., 2005). Together, these results show that the TFR

is well described when mNMES was delivered to the quadriceps; however, the TFR is poorly studied in the TA and TS muscles during mNMES even though both these muscles are stimulated when NMES is used for walking or standing (Bajd et al., 1999).

To our knowledge, only two studies have documented the TFR during nNMES. Shields and Chang (1997) reported a steady state in plantarflexion torque at 30 Hz when nNMES was delivered to the tibial nerve. Meyers et al. (2001) showed a steady state frequency of 40 Hz when the femoral nerve was stimulated and knee extension torque was recorded. Therefore, there is a major lack of data regarding the TFR during nNMES. An important topic that previous studies did not address was the pathway involved in generating contractions during the TFR protocols, independent of whether mNMES or nNMES were used.

The pathway by which contractions are generated during NMES can potentially affect the TFR. Most of the previously mentioned studies presumably generated contractions through peripheral pathways by using mNMES and testing muscles such as the TA, which have a low probability of generating H-reflexes (Zehr, 2002), or by using high amplitudes of stimulation which results in a preferential recruitment through peripheral pathways due to antidromic block (Gottlieb et al., 1976; Pierrot-Deseilligny et al., 2000). It could be expected that contractions generated through central pathways would alter the TFR since transmission along central pathways can depend on stimulation frequency. The amplitude of the H-reflex, for example, is depressed with increases in frequency due to homosynaptic depression of neurotransmitter release from Ia afferent terminals (Hultborn et al., 1996). If the recruitment of motor neurons and, consequently, torque is mainly a consequence of the transmission along central pathways, torque may decline with increases in frequency as H-reflexes are progressively depressed; however, this has yet to be tested. On the other hand, high frequencies of NMES can increase asynchronous MU activity, which would increase torque (Bergquist et al., 2011a; Bergquist et al., 2012b). Therefore, a torque steady state may be reached at lower frequencies of stimulation for centrally-driven contractions compared to those generated through peripheral pathways.

Together, these results show that there is still a considerable gap in our knowledge about the TFR during mNMES delivered to the TA and TS and during nNMES independent of muscle. Moreover, there are no studies describing the TFR during iNMES, comparing the TFR between the three NMES types or testing the effects of central contribution on the TFR. The

main goal of the experiments described in Chapter 3 was to compare the TFR between the three NMES types delivered to muscles with different levels of central contribution. These results will help to increase the knowledge base about the effect of frequency on torque when these types of NMES are delivered to these muscles.

1.6.3 Minimizing discomfort during NMES

The third and final knowledge gap that the experiments described in this thesis addressed was how does the discomfort and maximal torque produced by iNMES compare to the discomfort and maximal torque produced by mNMES and nNMES. As mentioned in Section 1.4.2, discomfort limits the benefits of NMES by reducing enrollment, treatment completion and the capacity to generate large amounts of torque. One of the most common methods used to assess discomfort during NMES is the visual analogue scale (VAS) (Broderick et al., 2011; Broderick et al., 2010; Naaman et al., 2000). The VAS consists of a 100 mm line, where the left end refers to "no discomfort" and the right end refers to "worst imaginable discomfort". Participants are instructed to make a mark on the VAS, the distance of the mark from the beginning of the line is then measured, denoting the amount of discomfort (Dexter et al., 1995). The VAS has been shown to have high reliability and reproducibility when used to access discomfort (Rosier et al., 2002; Wewers et al., 1990). The VAS has been used to evaluate discomfort during NMES when manipulating electrode size or NMES parameters such as frequency and pulse duration. Most of the research about discomfort during NMES was performed using mNMES and to a smaller extent nNMES. A comparison of the results between studies is difficult since the NMES protocols used were significantly different.

Multiple studies have shown that larger electrodes (ranging from 36 to 81 cm^2) produced less discomfort than small electrodes (ranging from 2.25 to 20 cm^2) when mNMES was delivered to the quadriceps (Alon, 1985; McNeal et al., 1988; Patterson et al., 1991), hamstrings (McNeal et al., 1988) and TS (Alon et al., 1994). However, opposite results were shown for the TA muscle, where small electrodes produced the least discomfort (Forrester et al., 2004; Milner et al., 1969). These results suggest that the effect of electrode size on discomfort may be different for different muscles; however, none of these studies tested same stimulation parameters and same electrode sizes in different muscles.

Conflicting results have also been reported when pulse duration was manipulated to minimize discomfort during mNMES. In several studies, medium pulse durations (0.1 to 0.3 ms) produced less discomfort than short (from 0.05 to 0.1 ms) and long pulse durations (from 0.3 to 0.5 ms) when mNMES was delivered to the quadriceps muscle (Bowman et al., 1985a; Bowman et al., 1985b; Gracanin et al., 1975; Scott et al., 2009). In contrast, one study showed that long pulses (0.5 ms) produced less discomfort than short pulses (0.2 ms) (Scott et al., 2014) and another found that different pulse durations had no effect on discomfort during mNMES delivered to the quadriceps muscle (Liebano et al., 2013). The disagreement in the results of these studies could again be linked to the different stimulation protocols used.

The manipulation of frequency to minimize discomfort during mNMES has also yielded contradictory results. Some studies showed that low frequencies (~20 Hz) produced the least discomfort (Laufer et al., 2008; Vaz et al., 2012; Ward et al., 2006); others showed that high frequencies (80 to 100 Hz) produce the least discomfort (Fukuda et al., 2013; Maffiuletti et al., 2008; Szecsi et al., 2007); and some studies showed no difference in discomfort during different frequencies of stimulation (Bircan et al., 2002; Dantas et al., 2015). Drawing conclusions from the above studies about how discomfort can be minimized by manipulating electrode size, pulse duration or frequency during mNMES is problematic since different muscles, electrode positioning, pulse waveform and pulse amplitudes were used.

Only a few studies have tested ways to minimize discomfort during nNMES. Kramer et al. (1987) showed that high frequency nNMES of the femoral nerve (50 and 100 Hz) applied while the individual performed MVICs produced less discomfort than low frequency stimulation (20 Hz). Verhoeven et al. (2006) found that smaller electrodes produced more discomfort than larger electrodes when nNMES was delivered to the tibial nerve while Naaman et al. (2000) found no difference in discomfort when different sizes of electrode were used to stimulate the common peroneal nerve. The low number of studies, different NMES protocols and different muscles tested may account for the lack of agreement in the results of the aforementioned studies.

Only two studies have compared discomfort between mNMES and nNMES. Naaman et al. (2000) found that nNMES delivered over the common peroneal nerve to activate the TA muscle produced less discomfort than mNMES when both were delivered to generate a full dorsiflexion. The authors attributed this result to the lower current used during nNMES than

mNMES. The second study did not intentionally compare mNMES and nNMES but one of the four electrode positions tested involved electrodes placed over the pathway of the tibial nerve (Lyons et al., 2004). These authors showed that the electrode placement equivalent to nNMES produced less discomfort than the electrode placement equivalent to mNMES when producing a given torque amplitude.

As discussed throughout this section, multiple studies have tested diverse stimulation parameters to minimize discomfort during NMES. However, the variation in the protocols makes comparisons between studies difficult. Moreover, a factor that none of the aforementioned studies addressed was the comparison between NMES types over a range of torque amplitudes or described discomfort during iNMES. The experiments described in Chapter 4 of this thesis compared discomfort produced by mNMES, nNMES and iNMES delivered to the ankle dorsiflexors at same torque amplitudes and identified the maximal torque that could be generated before limited by discomfort.

1.7 Thesis objectives

NMES is one of the best ways for individuals with paralysis to maintain regular exercise and reduce secondary complications, yet the way in which NMES generates contractions limits the benefits of NMES-based rehabilitation programs. Therefore, it is important to develop and study new ways to deliver NMES, such as iNMES. Gaps in our knowledge about how best to use iNMES for rehabilitation are addressed in this thesis. The effects of the *amplitude of stimulation* on the overlap of MUs recruited by the mNMES and nNMES sites, the effect of *frequency of stimulation* and *pathway* on torque and the *discomfort* and *maximal torque* during iNMES were explored.

The experiments described in Chapter 2 were designed to quantify the overlap between MUs recruited by mNMES over the TA muscle belly and nNMES over the common peroneal nerve trunk, across a full range of stimulation amplitudes. The hypothesis was that the amount of overlap between MUs recruited by mNMES and nNMES would increase with increases in stimulation amplitude. In these experiments, we also quantified the torque produced when iNMES was delivered at the stimulation amplitude that produced the lowest overlap.

The experiments described in Chapter 3 were designed to: 1) identify the effect of different stimulation frequencies on torque produced during mNMES, nNMES and iNMES

delivered to the TA and TS muscles; and 2) to identify how the pathway by which NMES generates contractions (central vs. peripheral) influences the TFR. The hypotheses of this study were that: 1) torque produced by iNMES (i.e. at 40 Hz) would be the linear summation of torque produced by mNMES (i.e. at 20 Hz) and nNMES (i.e. at 20 Hz) alone, for both TA and TS; and 2) torque generated by central pathways would reach a steady state at lower frequencies than torque produced by peripheral pathways.

The goals of the experiments described in Chapter 4 were to compare discomfort between mNMES, nNMES and iNMES during contractions that generated 5-30% MVIC and quantify the maximal torque that could be produced by each NMES type. The hypotheses of this study were that at a same torque amplitude, iNMES would require less current and produce less discomfort than mNMES and nNMES and that in the maximal trials discomfort would be similar between NMES types; however, torque would be larger during iNMES than during mNMES or nNMES.

CHAPTER 2. INTERLEAVED NEUROMUSCULAR ELECTRICAL STIMULATION: MOTOR UNIT RECRUITMENT OVERLAP[2]

2.1 Introduction

Neuromuscular electrical stimulation (NMES) can be delivered over a muscle belly (mNMES) or over a peripheral nerve trunk (nNMES) to restore movement and reduce secondary complications after injury or disease of the central nervous system [for review see Sheffler and Chae (2007) and Bergquist et al. (2011b)]. At low stimulation intensities, these 2 types of NMES activate different portions of the tibialis anterior muscle (TA), as was shown using fine-wire electromyography (EMG) electrodes inserted into superficial and deep portions of the muscle (Okuma et al., 2013). Consistent with previous data (Farina et al., 2004; Mesin et al., 2010), low intensity mNMES recruited motor units (MUs) in superficial portions of TA, with increases in stimulation intensity recruiting MUs in progressively deeper portions (Okuma et al., 2013). In contrast, nNMES over the common peroneal nerve recruited MUs that were distributed throughout the TA, regardless of stimulation intensity (Okuma et al., 2013). These data suggest that mNMES and nNMES recruits different populations of MUs, at least at low to moderate stimulation intensities.

Independent of where NMES is applied, one of the main problems with electrically-evoked contractions is their rapid fatigability, which manifests as a decrease in torque produced over time. Fatigability during NMES has been attributed, in part, to the synchronous activation of MUs at discharge rates that are higher than those that occur during voluntary contractions [for review see Bergquist et al. (2011b) and Bickel et al. (2011)]. Indeed, fatigability is lower when NMES is delivered at lower frequencies compared to higher frequencies (Binder-Macleod et al., 1997; Gregory et al., 2007). To address the fatigability problem, a novel approach was developed for delivering NMES to activate the ankle dorsiflexor muscles, termed "interleaved" NMES (iNMES; Lou et al. 2015, under review).

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Interleaved NMES was developed to reduce fatigability by reducing the unnaturally high discharge rates associated with mNMES and nNMES. The iNMES approach involves alternating every other stimulus pulse between mNMES delivered over the TA muscle belly and nNMES delivered over the common peroneal nerve trunk, thereby taking advantage of the fact that NMES applied at these 2 sites recruits different MUs (Okuma et al., 2013). Theoretically, the extent to which iNMES reduces fatigability will depend on the amount of "overlap" between MUs recruited by mNMES and nNMES. It is expected that iNMES will most effectively reduce fatigability when overlap is low since different MUs would be recruited by each stimulation site. In contrast, iNMES will be less effective at reducing fatigability if overlap is high, since most or all MUs will be activated by both sites. Thus, the main goal of the present study was to characterize the overlap of MUs recruited by mNMES and nNMES delivered to dorsiflex the ankle.

The present experiments were conducted in 2 parts. In part 1, experiments were conducted to estimate the amount of overlap between the MUs recruited by mNMES over the TA muscle belly and nNMES over the common peroneal nerve trunk, across a full range of stimulation intensities. To assess MU recruitment overlap, a technique was adapted from one used to assess overlap between the MUs recruited by pairs of electrodes in implanted multi-electrode arrays (Branner et al., 2001; Dowden et al., 2012; Fisher et al., 2013; McDonnall et al., 2004b; Schiefer et al., 2013). This technique involves comparing the torque generated when single pulses were delivered at each stimulation site separately (i.e. mNMES *or* nNMES) with that generated when the both sites were activated together (m+nNMES). The hypothesis of this study was that the amount of overlap between MUs recruited by mNMES and nNMES would increase with increases in stimulation intensity. Thus, it was expected that 1) at low stimulation intensities, the twitch torque generated by m+nNMES would be the linear summation of the twitch torques produced by mNMES and nNMES alone (Figure 2-1A), consistent with 0% overlap and, 2) when twitch torques were maximal, m+nNMES would generate the same amount of torque as mNMES or nNMES alone (Figure 2-1B), consistent with 100% overlap. In Part 2, experiments were conducted to quantify the torque produced when iNMES was delivered at a frequency of 40 Hz and at a stimulation intensity that had the lowest MU overlap, as identified in Part 1. The term m+nNMES refers exclusively to the stimuli used to generate single twitches, thus when single stimulus pulses

were delivered at both the mNMES and nNMES sites in Part 1. The term iNMES refers to when the stimulus pulses were "interleaved" repetitively between the mNMES and nNMES sites during the trains of stimulation in Part 2. Together, these experiments help us understand the range over which iNMES may reduce fatigability of electrically-evoked contractions of TA and demonstrates that functionally relevant contractions can be generated by iNMES with low MU recruitment overlap.

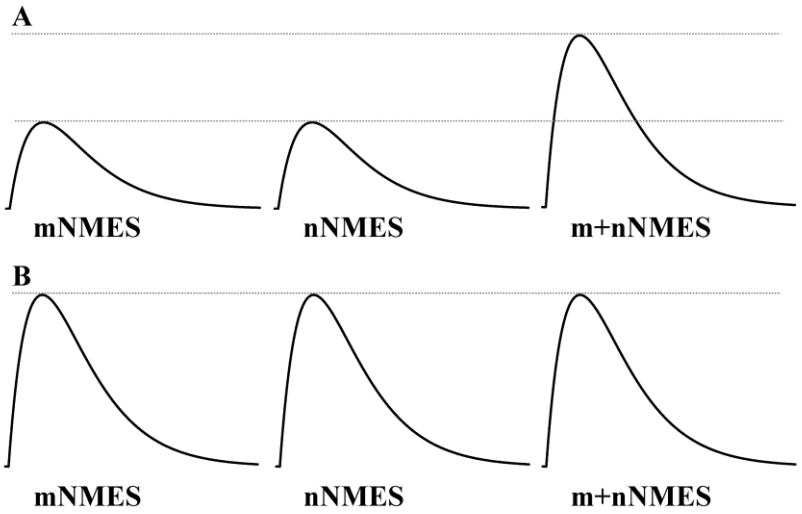

Figure 2-1. Stylised torque profiles for twitches used to estimate motor unit recruitment overlap. In both panels, torque amplitude was matched between mNMES and nNMES. In panel **(A)**, torque produced by m+nNMES is the linear summation of mNMES and nNMES, representing 0% overlap between the MUs recruited by mNMES and nNMES. In panel **(B)**, the torque produced by m+nNMES has the same amplitude as mNMES and nNMES, representing 100% overlap.

2.2 Methods

2.2.1 Participants

Eleven individuals with no known neuromusculoskeletal impairment volunteered for the present study. This study was conducted in 2 parts. In part 1, single pulses of NMES were delivered to estimate the overlap between MUs recruited by mNMES and nNMES. Nine participants (3 females and 6 males; 30 ± 9 years; mean ± standard deviation) volunteered for 1 experimental session lasting ~2 hours. In part 2, the torque produced when iNMES was

quantified when delivered at the intensity that resulted in the lowest overlap, as determined in Part 1. Five individuals (5 males; 35 ± 11 years) volunteered for these experiments which lasted ~30 min and were performed on a separate day from Part 1. Three individuals participated in both Parts 1 and 2. The experimental protocols were approved by the Human Research Ethics Board at the University of Alberta.

2.2.2 Protocol

2.2.2.1 Torque

A Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York) was used to measure isometric dorsiflexion torque with the hip and knee positioned at ~90° and ankle at ~100°, where 90º is the neutral position of the ankle. The centre of rotation of the ankle joint was aligned with the axis of the dynamometer. All procedures were performed on the right leg.

2.2.2.2 Electromyography (EMG)

Surface EMG was recorded from the distal portion of the TA muscle using recording and analyses techniques described elsewhere (Okuma et al., 2013). These analyses were conducted to assess the prevalence and magnitude of H-reflexes. M-waves were not quantified because we were unable to accurately quantify M-waves recorded during mNMES and m+nNMES in most participants, and nNMES in some participants, due contamination of the EMG by the stimulus artifacts. Further, due to a technical issue during data collection, the EMG data were not saved during 4 of 8 trials for one participant. H-reflexes were evoked occasionally in 4 participants. In one of those participants H-reflexes were evident during both nNMES and m+nNMES, in one other participant H-reflexes were evident during nNMES only and in two participants H-reflexes were evident during m+nNMES only. H-reflexes were not evoked by mNMES in any participant. Overall, H-reflexes were evoked in 14 of the 558 twitches that were generated across the 9 participants and were always less than 4% the maximal M-wave. Given that H-reflexes were evoked rarely and when they were present they were small, these data are not discussed further.

2.2.2.3 Neuromuscular Electrical Stimulation

NMES was delivered over the TA muscle belly (mNMES), common peroneal nerve trunk (nNMES), or both together (m+nNMES in Part 1; iNMES in part 2) to generate

dorsiflexion torque. The TA muscle was chosen because: 1) it is a target for NMES to reduce "foot-drop" after central nervous system injury or disease (Everaert et al., 2010); 2) mNMES and nNMES recruit separate populations of MUs in TA, at least at low to moderate stimulation intensities (Okuma et al., 2013), and; 3) H-reflexes in TA are typically small or non-existent (Pierrot-Deseilligny et al., 1981; Zehr, 2002); using the present approach, Hoffman reflexes (H-reflex) would confound the estimation of overlap between the MUs recruited by each stimulation site. A short 100 μs pulse duration was used to preferentially activate motor axons (Brooke et al., 1997; Lagerquist et al., 2009b; Veale et al., 1973) and thus further minimise the possibility of generating H-reflexes.

Constant-current stimulators (Digitimer, Welwyn Garden City, UK) were used to deliver rectangular pulses to the mNMES (model DS7AH), nNMES (model DS7A) sites. At the mNMES site, a pair of adhesive gel electrodes (5.08 x 5.08 cm; model 400-899, Richmar, Chattanooga, TN, USA) were placed over the largest portion of TA, consistent with the location of the main motor point of the TA (Botter et al., 2011), with the anode ~1 cm distal to the cathode. At the nNMES site, a pair of adhesive gel electrodes (3.2 cm round, model 400-864, Richmar, Chattanooga, TN, USA) were placed over the common peroneal nerve at a location where dorsiflexion was produced with minimal or no eversion. The cathode was placed distal to the fibular head while the anode was positioned ~1 cm anterior to the cathode along the anticipated path of the nerve trunk.

2.2.2.4 Peak twitch torque

All twitch torque data were normalised to peak twitch torque (PTT). To identify PTT, single pulses of mNMES and nNMES were delivered (separately) at increasing stimulation intensities until the torque produced by the evoked twitches no longer increased. Three of these maximal twitches were recorded for each type of stimulation and the torque recorded during the largest twitch was defined as PTT, regardless of whether it was produced by mNMES or nNMES. During the experiments that comprised Part 1, PTT was generated by nNMES for 6 participants and by mNMES for 3 participants. PTT was generated by nNMES for all 5 participants in Part 2.

2.2.2.5 Part 1: MU recruitment overlap between mNMES and nNMES

Recruitment curves were constructed for each type of stimulation by plotting the torque produced during twitches evoked across a range of stimulation intensities. To generate these

recruitment curves, stimulation intensity was adjusted to evoke twitches of similar amplitude by mNMES and nNMES (i.e. twitch amplitudes were "matched"), and then without changing the stimulation intensity, both sites were stimulated together (m+nNMES). Our criteria for matching twitch amplitudes between mNMES and nNMES required that the torque evoked by both sites be within 4% PTT. When this matching was achieved successfully, 3 twitches were recorded for each of the 3 types of stimulation. This process was repeated for as many twitch amplitudes as could be matched between the mNMES and nNMES sites for a given participant. The order of the twitch amplitudes during the recruitment curves was randomized between participants. It was often difficult to produce twitches that met the matching criteria (within 4% PTT) between mNMES and nNMES, and it was not possible to produce consistent increments in twitch amplitude (e.g. increases of 5% PTT). It was especially difficult to produce small and consistent increments in torque during nNMES since small increases in stimulation intensity resulted in larger increases in torque. Therefore, there were different numbers of increments in torque for each participant, resulting in 6 to 12 twitch amplitudes (7.0 ± 2.1) that met the matching criteria and were included in the data analyses across participants.

To ensure that MUs were recruited simultaneously from the 2 sites during m+nNMES, mNMES was delivered 1 to 4 ms after nNMES to account for the longer pathway from the nNMES site to the muscle. The difference in time between delivering mNMES and nNMES was identified by finding for each participant the delay at which m+nNMES delivered with mNMES and nNMES at maximal twitch amplitude produced a twitch that did not exceed PTT. Across all participants this delay was 2.8 ± 0.8 ms.

2.2.2.6 Part 2: Torque generated by iNMES

Part 2 was conducted to quantify the torque generated when iNMES trains were delivered at the intensity that produced the lowest overlap. The experiments in Part 1 showed that the least overlap occurred when twitches evoked by mNMES and nNMES generated 10% PTT, the lowest torque amplitude tested. Part 2 was conducted to quantify the torque generated when iNMES trains were delivered at this low intensity. The same electrode configurations were used as described for Part 1. The intensity for PTTs was identified prior to the maximal voluntary isometric contractions (MVICs). Participants initially performed 2 dorsiflexion MVICs, 60 s apart, while receiving verbal encouragement to maximize

performance. If two consecutive MVICs had a large variation, a third was recorded. Three maximal twitches were recorded, 5 s apart, beginning immediately after the last MVIC according to a protocol used previously by Verges et al. (2009) and these twitches were used to calculate PTT. Immediately after the MVICs, 3 PTTs were recorded to reduce the variability of the PTTs by inducing post activation potentiation (Tillin et al., 2009). Stimulation amplitude was then adjusted to generate twitches with an amplitude of ~10% PTT from both the mNMES and nNMES sites (separately). Three twitches at this amplitude were recorded for both stimulation sites. Without changing the stimulation intensity, 3 trains of mNMES at 20 Hz, nNMES at 20 Hz and iNMES at 40 Hz were recorded separately. Interleaved NMES was delivered at 40 Hz by alternating every other stimulus pulse between the mNMES and nNMES sites and, thus, the frequency at each site was half (20 Hz) the frequency delivered by the stimulator. Each type of NMES was delivered for 1 s with 5 s between trains.

2.2.3 Data Acquisition and Analyses

Data were acquired at 25 kHz for the experiments in Part 1 and at 5 kHz for the experiments in Part 2 using custom-written Labview software (National Instruments, Austin, Texas). All data were then stored on a computer for analyses that was performed *post-hoc* using custom-written Matlab software (The Mathworks, Natick, Massachusetts). Torque was filtered using a low-pass filter with cut-off frequency of 80 Hz prior to data analyses. Twitch amplitude was measured from baseline to peak. Time to peak was measured from the initial deflection from the baseline to the peak of the twitch.

2.2.3.1 Part 1: MU overlap between nNMES and mNMES

Data from recruitment curves were normalized by PTT and averaged across the 3 twitches for each twitch amplitude. A curve fitting method was used to enable averaging and statistical analysis of the data from the group. Two best-fit curves were generated: one for mNMES vs. nNMES, and the other for mNMES or nNMES (whichever produced PTT) vs. m+nNMES (Figure 2-2A). Linear curve fitting was used for mNMES vs. nNMES because the twitch amplitudes of mNMES and nNMES were always matched. The x coordinate in the linear equation represents the stimulation site that generated PTT (nNMES for most participants) and the y coordinate represents the other stimulation site (mNMES for most participants). Multiple fitting equations were tested and a 2^{nd} order polynomial fit was found

to be most appropriate for mNMES or nNMES vs. m+nNMES. The choice of the linear and 2nd order polynomial fittings was based on low residual variability and a high coefficient of determination (R^2). Residuals represent the difference between original data and the data predicted by the curve fitting method; the larger the difference, the lower the accuracy of the curve fitting method. The *y* coordinate in the polynomial equation represents the twitch amplitude of m+nNMES, while the *x* coordinate represents the twitch amplitude of the stimulation site that generated PTT. After curve fitting, the predicted values (y-axis) were calculated for input values (x-axis) of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100, representing 10 different twitch torque levels (% PTT). Using this method, twitch amplitude was estimated for mNMES, nNMES and m+nNMES in these 10 torque levels allowing comparisons between participants, independent of the number of increments in twitch amplitude that were recorded for each participant (Figure 2-2B).

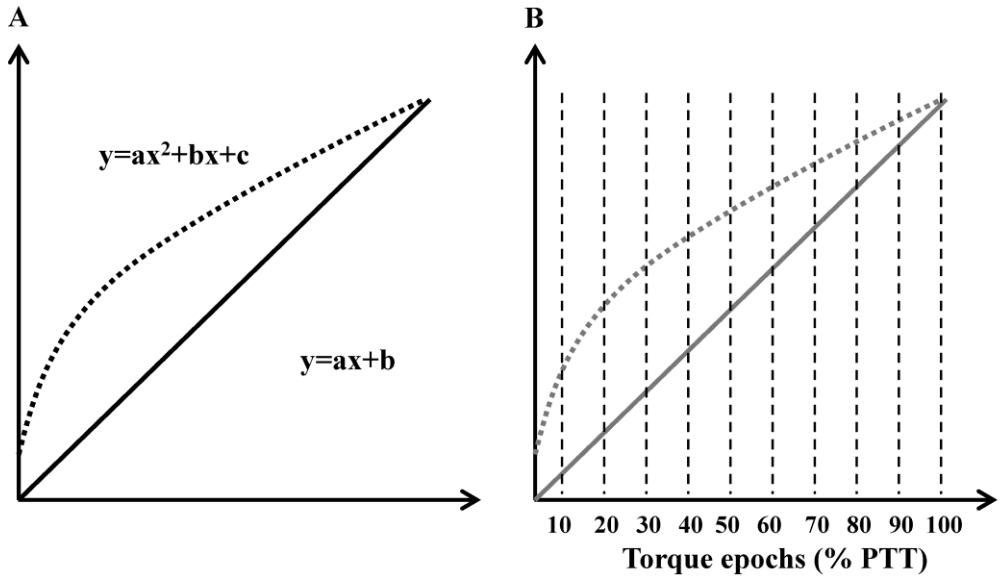

Figure 2-2. (A) A linear curve fit was used to find the equation for mNMES vs. nNMES (solid black line) while a 2nd order polynomial fit was used to find the equation for mNMES or nNMES vs. m+nNMES (dotted black lines). Raw data was used to identify the equations. **(B)** Twitch amplitude of mNMES, nNMES and m+nNMES was estimated for ten torque levels (from 10 to 100% PTT; vertical dotted lines) using the best curve fit equations.

Equation 1 was used to estimate the percent overlap between mNMES and nNMES using the data resulting from the linear and 2nd order polynomial fittings:

$$\% \text{ overlap} = \frac{100 \times (T_{mNMES} + T_{nNMES} - T_{m+nNMES})}{T_{mean\ mNMES\&nNMES}}$$

Equation 2-1. Overlap equation.

where T_{mNMES} is the twitch amplitude of mNMES alone, T_{nNMES} is the twitch amplitude of nNMES alone and $T_{m+nNMES}$ is the twitch amplitude when mNMES and nNMES were delivered together (m+nNMES); $T_{mean\ mNMES\&nNMES}$ is the average twitch amplitude of mNMES and nNMES ([mNMES + nNMES]/2). If mNMES and nNMES produced the same twitch amplitude and m+nNMES produced a twitch torque that was the sum of these 2 torques (i.e. mNMES + nNMES), this would indicate 0% overlap (Figure 2-1A), suggesting that different groups of MUs were recruited by mNMES and nNMES. In contrast, if mNMES, nNMES and m+nNMES produced the same twitch amplitude, this would indicate 100% overlap (Figure 2-1B), suggesting that mNMES and nNMES recruited the same MUs (Branner et al., 2001; McDonnall et al., 2004b).

All statistical analyses were performed on group data using Statistica 12 software (StatSoft, Tulsa, Oklahoma). The distribution normality was tested by visual inspection of quantile-quantile plots and using the Shapiro-Wilk test. A 2-way repeated measures ANOVA (rmANOVA; 3 x 10) was used to compare the twitch amplitude generated by each stimulation site (mNMES vs. nNMES vs. m+nNMES) at each torque level (10, 20, 30, 40, 50, 60, 70, 80, 90, 100% PTT). A 1-way rmANOVA (1 x 10) was used to compare percent overlap across the 10 torque levels. A 2-way rmANOVA (2 x 3) was used to compare the time to peak generated by each stimulation site (mNMES and nNMES) at three twitch amplitudes (small, medium and large). Significant main effects and interactions were tested post-hoc using Tukey's honestly significant difference test. The level of significance was set at 0.05. All data are reported as mean ± standard deviation.

2.2.3.2 Part 2: Torque generated by trains of iNMES

Torque data from the 3 single twitches of mNMES and nNMES were averaged and normalized by the PTT recorded after the MVICs. Torque generated by the 3 trains of mNMES, nNMES and iNMES was averaged and reported as % MVIC and in Nm. The goal of

the experiments in Part 2 was to demonstrate the magnitude of the torque produced during trains of iNMES. Therefore, only descriptive statistics were used to report the data from Part 2. Data are reported as mean ± standard deviation.

2.3 Results

2.3.1 Part 1: MU overlap between mNMES and nNMES.

The mean torque recorded during twitches evoked by mNMES, nNMES and m+nNMES are shown for a single participant in Figure 2-3A. For this participant, we were able to generate twitches of 12 different amplitudes that could be matched between mNMES and nNMES. These twitch amplitudes ranged from torque threshold to 85% PTT (0 to 3.8 Nm). Although larger twitches could be evoked by nNMES for this participant, it was not possible to produce twitches larger than 85% PTT with mNMES. Figure 2-3B shows the mean twitch amplitudes for mNMES vs. nNMES for this participant and the linear curve fit calculated for those data. These data show that the amplitudes of twitches evoked by mNMES and nNMES were closely "matched" and that the curve fit adequately captures the linear relationship between these 2 variables (R^2=0.99). Similarly, Figure 2-3C shows the mean twitch amplitudes for m+nNMES vs. nNMES and that the polynomial fit describes adequately those data (R^2=0.95). For this participant, PTT was generated during nNMES and thus the curve fitting was done using the nNMES twitch amplitude data on the *x* axis. In general for this participant, m+nNMES produced twitches that were approximately twice as large as nNMES when twitch amplitudes were small (left side of panel 2-3C), consistent with little or no overlap of MUs recruited by mNMES and nNMES. In contrast, when twitch amplitudes became progressively larger (moving right along the x-axis), torque produced by either muscle or nerve NMES compared to combined m+nNMES became progressively more similar. This is consistent with an increasing amount of overlap between mNMES and nNMES. The 2 curves shown in panels 2-3B and 2-3C were used to estimate twitch amplitudes for each of 10 torque levels for mNMES, nNMES and m+nNMES.

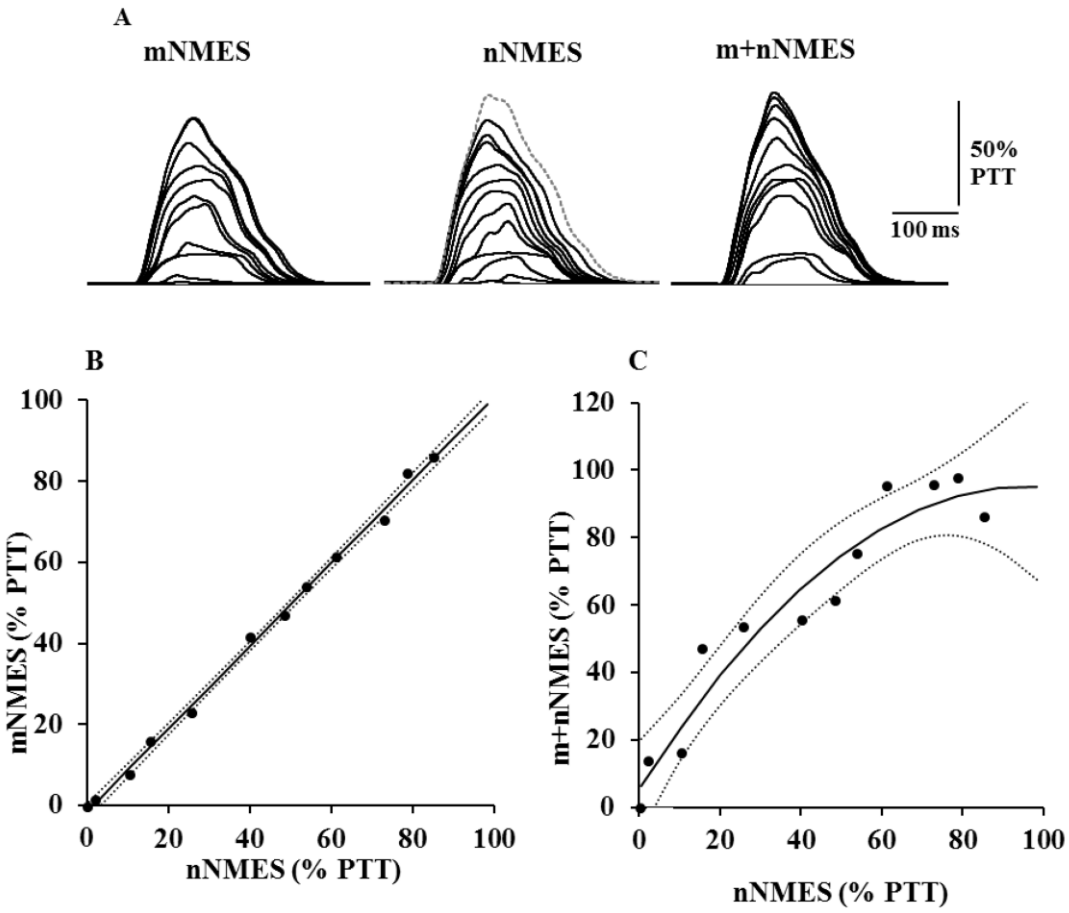

Figure 2-3. (A) Torque traces (black lines) recorded from a single participant during twitches evoked by mNMES, nNMES and m+nNMES. Twitch amplitudes were matched between mNMES and nNMES. Each trace represents the average of the torque recorded during three twitches. The grey dotted line on the nNMES graph (middle) represents the peak twitch torque for this participant. **(B)** Linear fitting used to estimate twitch amplitude for nNMES vs. mNMES. Grey dotted lines represent the upper and lower prediction boundaries (95% confidence interval) calculated based on the distribution of the raw data represented by the black filled circles. The black solid line represents the linear curve fit estimated based on raw data. **(C)** Second order polynomial fitting estimated based on the twitch amplitude recorded from nNMES vs. m+nNMES. Data are shown in the same format as in B. Torque is reported as % of PTT.

The residuals of the linear and 2nd order polynomial fittings for the group data (n=9) are shown in Figure 2-4. Figures 2-4A and 2-4B show that most of the residuals resulting from the linear and polynomial fittings are within the 95% confidence intervals. The visual inspection of the quantile-quantile plots, which compares the probability of the distribution of the quantiles of 2 data sets, suggested that the data was normality distributed. The normality was confirmed by the Shapiro-Wilk test (p=0.97 for the linear fitting and p=0.27 for the polynomial fitting). Across participants, the linear and polynomial fits had an average R^2 value of 0.99 ± 0.001 and 0.95 ± 0.03, respectively.

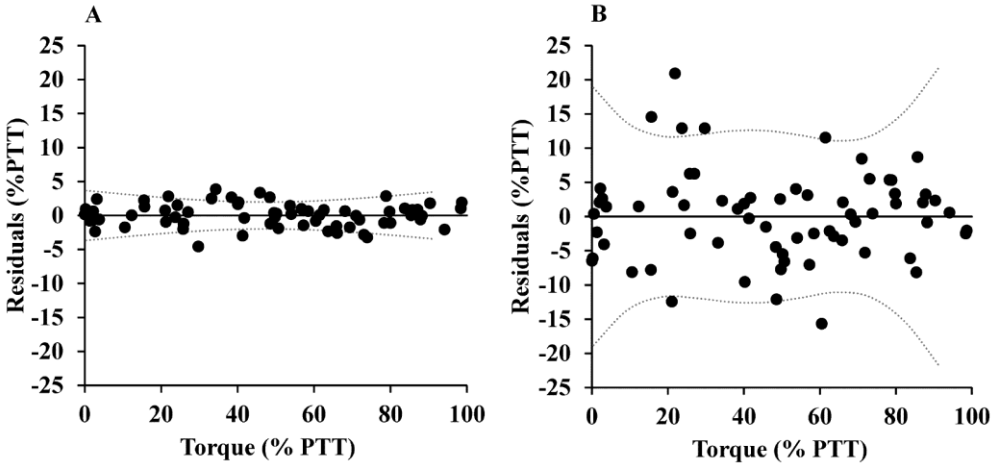

Figure 2-4. Residual distribution for the overlap experiments for all 9 participants. **(A)** Residual data from the linear fit. Grey dotted lines represent the upper and lower prediction boundaries, black solid line the mean and black filled circles the residuals. **(B)** Residual data from the second order polynomial fit. Colours and symbols represent the same as in A.

The average of the twitch amplitude data from the 9 participants was calculated for each of the 10 torque levels, as shown in Figure 2-5. There were no significant differences between twitch torque produced by mNMES and nNMES at any torque level [p=1.00]. Twitches generated by m+nNMES were significantly larger [$F_{(18,144)}$=5.0855, p<0.001, observed power=1.00] than those produced by mNMES at torque levels up to 80% PTT [p<0.01] and were not significantly different at 90 and 100% PTT [p=0.302 and p=1.00, respectively]. Twitches generated by m+nNMES were significantly larger than nNMES at torque levels up

to 90% PTT [p<0.001] and were not significantly different at the 100% PTT torque level [p=1.00]. The average PTT evoked by mNMES and nNMES was 3.0 ± 0.9 Nm (n=3) and 3.3 ± 1.2 Nm (n=6), respectively. We compared the time to peak of twitches evoked by mNMES versus nNMES for small (~5% PTT), medium (~50% PTT) and large (the largest twitch recorded) twitches for each participant. The ANOVA analyses of these data showed no significant main effects [p>0.52] or interactions [p=0.58]. The times to peak of small (3.5 ± 3.7% PTT), medium (50.2 ± 6.2% PTT) and large (87.9 ± 9.7% PTT) twitches during mNMES were 87.1 ± 33.6 ms, 94.6 ± 16.5 ms and 99.2 ± 16.9 ms, respectively. The times to peak of small (3.1 ± 3.8% PTT), medium (50.9 ± 6.1% PTT) and large (88.3 ± 10.1% PTT) twitches during nNMES were 86.2 ± 41.6 ms, 95.7 ± 11.3 ms and 92.4 ± 27.2 ms, respectively.

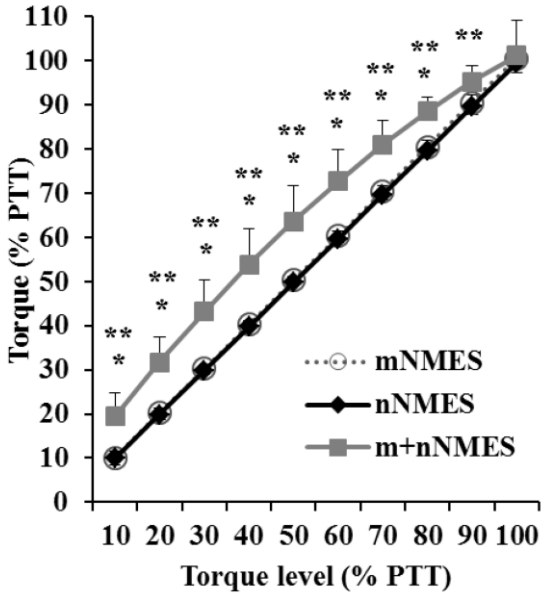

Figure 2-5. Twitch torque data across a full range of stimulation amplitudes for all participants (n=9). Single asterisks represent a significant difference between mNMES vs. m+nNMES within the same torque level. Double asterisks represent a significant difference between nNMES vs. m+nNMES at the same torque level. Torque is reported as % PTT. * and **(p<0.05).

The percent overlap between MUs recruited by mNMES and nNMES was estimated using Equation 1 and is shown in Figure 2-6. The 1-way rmANOVA indicated that the percent

overlap for 10% PTT torque level, when twitches produced by mNMES and nNMES were smallest (5.01%), was significantly [$F_{(9,72)}$=20.614, p<0.001, observed power=1.00] lower than all other torque levels [p<0.01], indicating that this is where the lowest overlap occurred. Percent overlap was lower at the 20% PTT torque level compared to 50-100% PTT torque levels [p<0.02]; the 30% PTT torque level compared to 80-100% PTT [p<0.01]; and the 40% PTT compared to 100% PTT [p=0.01]. Overlap was not significantly different [p>0.05] between torque levels from 50 to 100% PTT.

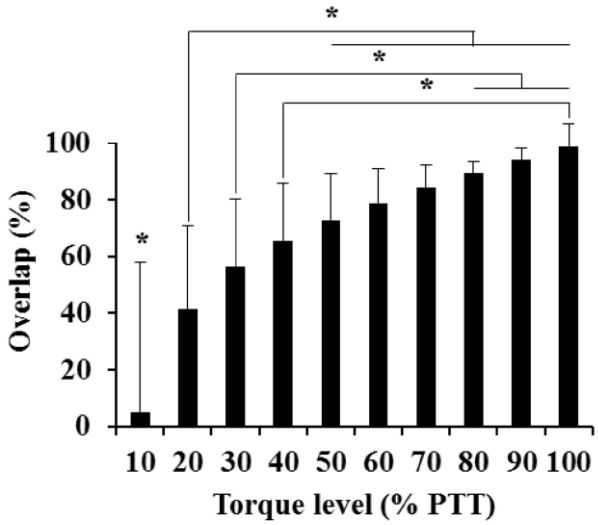

Figure 2-6. Motor unit recruitment overlap across a full range of stimulation amplitudes averaged across all participants (n=9). Each bar represents the percent of overlap estimated using Equation 1. The cross over the 10% PTT bar indicates significant difference from all other torque levels. *(p<0.05).

2.3.2 Part 2: Torque generated by trains of iNMES.

Figure 2-7 shows the torque produced when trains of iNMES were delivered at 40 Hz at the intensity that resulted in the least overlap in Part 1. Data from a single participant are shown in Figure 2-7A and mean data for the group (n=5) are shown in Figures 2-7B and 2-7C. The left side of panel 2-7A shows the torque produced by twitches evoked when single pulses were delivered to produce ~10% PTT. In this participant the twitches produced on average 8.8 and 6.3% PTT and 0.3 and 0.2 Nm during mNMES and nNMES, respectively. When mNMES and nNMES were delivered separately at 20 Hz at these intensities they produced an average of 16.7% MVIC. When iNMES was delivered at 40 Hz it produced 38% MVIC. Figure 7B

shows the average torque generated by twitches delivered at the mNMES and nNMES sites for the group. On average these twitches produced 8.3 ± 1.0% PTT. For the group, PTT was 9.0 ± 3.8% MVIC. Torque produced by trains of mNMES, nNMES and iNMES are shown in Figure 2-7C. For the group, the trains of mNMES, nNMES and iNMES generated ~12.6, 14.4 and 25.8% MVIC or 5.7, 6.3 and 11.6 Nm, respectively.

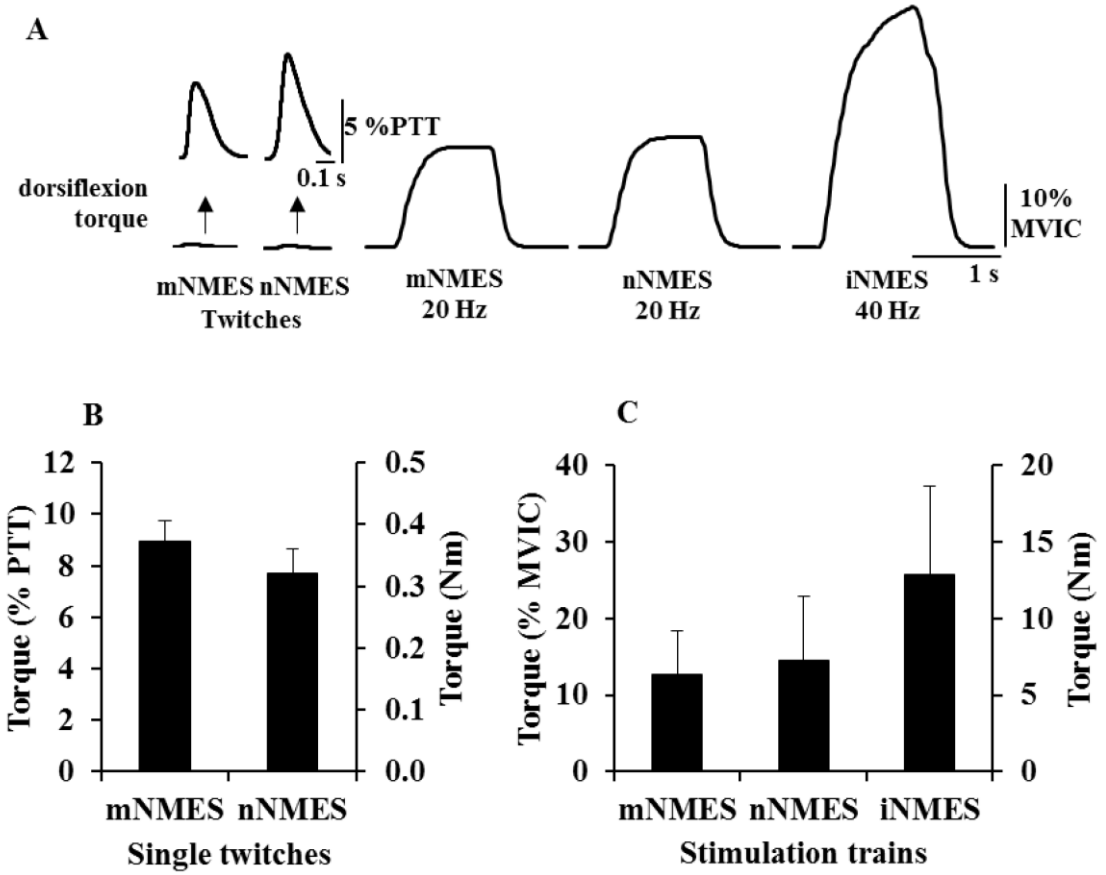

Figure 2-7. Single participant and group data of the experiments performed in Part 2. **(A)** Single twitches for mNMES and nNMES as well as the torque produced by mNMES and nNMES at 20 Hz and iNMES at 40Hz in a single participant. **(B)** The average amplitude of single twitches (group data) generated by mNMES and nNMES where torque described as % PTT and in Nm. **(C)** Average amplitude of trains of stimulation for group data when stimulation was delivered by mNMES, nNMES and iNMES at the stimulation amplitude described in 7B. Torque is described in % MVIC and Nm**.**

2.4 Discussion

To reduce fatigability of muscle contractions generated by conventional NMES (mNMES or nNMES), we developed iNMES in which stimulus pulses are interleaved between the mNMES and nNMES sites (iNMES) (Lou et al. 2015, under review). This technique is based on the finding that mNMES and nNMES recruit separate portions of the TA muscle (Okuma et al., 2013), at least at low to moderate intensities. Theoretically, iNMES will be most effective for reducing fatigability when the overlap between MUs recruited from the 2 sites is low, as this will reduce the firing rates of most of the recruited MUs by half, compared to mNMES or nNMES delivered alone. However, currently there are no data regarding MU recruitment overlap between mNMES and nNMES. Accordingly, the main goal of the present study was to estimate the amount of overlap between MUs in TA recruited by mNMES and nNMES across a range of stimulus intensities.

As hypothesized, overlap increased with increases in stimulation intensity. However, even at the lowest intensity tested (10% PTT), the torque generated by m+nNMES was not the linear summation of the twitch torques produced by mNMES and nNMES alone, which would have been consistent with 0% overlap. Rather, m+nNMES produced twitches 1.9x larger than mNMES or nNMES at 10% PTT, instead of 2x larger as predicted, resulting in a calculated overlap of ~5%. Thus, even at this low stimulus intensity, there was some overlap between MUs recruited by the 2 sites, consistent with the finding that even at low stimulus intensities, both mNMES and nNMES recruit some MUs in superficial portions of the muscle (Okuma et al., 2013). As stimulation intensity was increased, the overlap between MUs recruited by mNMES and nNMES also increased, such that when twitches evoked by mNMES and nNMES were maximal (100% PTT), overlap was 98%, indicating that mNMES and nNMES recruited virtually the same MUs.

In the present study, overlap was estimated based on the torque produced by single pulses of stimulation because it allowed comparison over a range of stimulation intensities from torque threshold to PTT. Trains of stimulation were not used to test overlap because during pilot experiments, contractions greater than 30% MVIC (intensities that generate twitches of ~57% PTT) were not well tolerated by the participants, mainly during trains of mNMES. However, to generate muscle contractions for rehabilitation, NMES is delivered in trains. Presently, when trains of iNMES where delivered at the intensity identified to have the

least overlap (10% PTT) between mNMES and nNMES, contractions of approximately 25% MVIC or 11 Nm were generated. Dorsiflexion of the ankle during the swing phase of walking requires ~2 Nm (Bogey et al., 2010; Davy et al., 1987). Therefore, at this low intensity, iNMES generated torque 5 times larger than what is necessary to dorsiflex the ankle during the swing phase of walking, with very little overlap. It would be reasonable to expect that at stimulation intensities at which mNMES and nNMES generated twitches smaller than 10% PTT, functionally-relevant contractions could be produced with even less overlap.

Theoretically, the extent to which iNMES reduces fatigability compared with mNMES or nNMES alone will be affected by the amount of MU recruitment overlap between these sites during iNMES. The present study design did not allow for a determination of the stimulation intensity where overlap is so high that iNMES loses its advantage compared to conventional mNMES or nNMES, because fatigability measurements were not within the scope of this study. Based on our results, however, if single twitches delivered to mNMES and nNMES exceed 50% PTT, there may be little to no advantage when using iNMES compared to conventional NMES, since the overlap is not significantly different from 100%. This means that mostly the same MUs are recruited at intensities that generate twitches larger than 50% PTT. Further, the type of MUs recruited by mNMES and nNMES may affect the fatigability of the evoked contractions. Interestingly, in the present data there were no differences in time to peak of twitches evoked by mNMES or nNMES or between twitches of different amplitudes, suggesting that recruitment order of TA MUs was random according to type for both types of NMES.

Another type of NMES that has been developed to reduce MU discharge rates and fatigability is sequential NMES (Nguyen et al., 2011; Popovic et al., 2009; Sayenko et al., 2015; Sayenko et al., 2014b). Sequential NMES is based on a similar principle as iNMES, but instead of alternating pulses between mNMES and nNMES, the stimulation is rotated between multiple mNMES electrodes with the goal of recruiting different MUs from each site. The extent to which different MUs are recruited by the mNMES sites during sequential NMES has not been tested. One could hypothesize that there is greater overlap between stimulation sites during sequential NMES than iNMES due to the greater proximity of the stimulation electrodes to each other over the muscle belly. The approach used in the present study would be appropriate to test overlap between the different mNMES sites of sequential NMES.

The results of this study may not be generalizable to NMES delivered to other muscles. Overlap between mNMES and nNMES for different muscles may be affected by different probabilities of Hoffman-reflexes between different muscles [e.g. ankle plantarflexors (Pierrot-Deseilligny et al., 1981) vs. dorsiflexor (Pierrot-Deseilligny et al., 1981)] as reflex pathways provide a 3rd (if nNMES recruits sensory axons), and maybe 4th (if mNMES also recruits sensory axons), input to the stimulated muscle when compared to stimulation driven mainly by the direct activation of motor axons during mNMES and nNMES (Bergquist et al., 2012a; Bergquist et al., 2013). Inputs arriving at different time at the muscle, due to the length of different pathways, could result in under-estimation of overlap resulting from catch-like effects (Burke et al., 1970, 1976). Moreover, different muscles may have different spatial recruitment patterns during mNMES and nNMES. For example, the spatial recruitment of MUs during NMES differs between different muscles of the quadriceps (Rodriguez-Falces et al., 2013). For vastus lateralis, mNMES recruited MUs from superficial-to-deep with increasing stimulus intensity, however, nNMES recruited MUs evenly throughout the muscle regardless of stimulus intensities. In contrast, there was no difference in spatial recruitment of MUs between mNMES or nNMES of the vastus medialis. Further, for thin "flat-shaped" muscles such as the trapezius, differences in the spatial distribution of motor units recruited by nNMES and mNMES are likely to be small, which would result in a large overlap of MUs recruited by the two sites regardless of stimulation intensity. During these experiments the nNMES electrodes were positioned to activate TA with little or no activation of the peroneal muscles which can counteract the torque produced by TA. However, in a few participants, some activation of the peroneal muscles was unavoidable and this may have led to an underestimation of MU recruitment overlap in those participants.

2.5 Conclusions

The overlap between motor units recruited by the mNMES and nNMES sites increased with increasing stimulation intensity up to the intensity at which single stimuli delivered at each site separately generated 50% PTT (i.e. 72% overlap). The lowest overlap (5%) was identified at the lowest stimulation intensity tested, when single pulses delivered to the mNMES and nNMES sites separately generated twitches of 10% PTT. Torque generated by trains of iNMES delivered at this intensity produced contractions 5 times larger than what is

necessary to dorsiflex the ankle during the swing phase of walking. Thus, torque was not only of a functionally-relevant amplitude, but was generated with little overlap between MUs recruited by every other stimulus pulse. These results bode well for using iNMES for reducing the fatigability of TA contractions for rehabilitation.

CHAPTER 3. TIBIALIS ANTERIOR AND TRICEPS SURAE TORQUE-FREQUENCY RELATIONSHIP WHEN ELECTRICAL STIMULATION IS DELIVERED OVER THE MUSCLE BELLY, NERVE TRUNK OR INTERLEAVED BETWEEN BOTH[3]

3.1 Introduction

Neuromuscular electrical stimulation (NMES) can be used for rehabilitation to produce contractions of paralyzed muscles (Peckham et al., 2005; Sheffler et al., 2007). Torque produced by such contractions, as with voluntary contractions, can be modulated by changing the number of motor units (MU) recruited (Adams et al., 1993) and/or the frequency at which MUs fire (Binder-Macleod, 1995; Peckham et al., 2005). During NMES, manipulating NMES amplitude (i.e. current) or pulse duration changes the number of recruited MUs, whereas manipulating stimulation frequency changes the firing rates of recruited MUs. The goal of this study was to compare the effects of stimulation frequency, and thus MU discharge, on torque between two traditional and one novel type of NMES delivered over the muscles that dorsiflex and plantarflex the ankle.

Traditionally, NMES is delivered through a single pair of electrodes over a muscle belly (mNMES) or superficial nerve trunk (nNMES). Recently, we developed interleaved NMES (iNMES), which involves alternating stimulation pulses between pairs of electrodes over the mNMES ($iNMES_{(m)}$) and nNMES ($iNMES_{(n)}$) sites. In this way, during iNMES the NMES frequency at the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites is reduced by half, compared to traditional mNMES or nNMES delivered at the same net frequency. Interleaved NMES takes advantage of the fact that at low to moderate stimulation amplitudes, mNMES and nNMES recruit different MUs in the tibialis anterior muscle [TA; (Okuma et al., 2013)]. Given the well-known relationship between stimulation frequency and fatigability, with higher frequencies resulting in greater fatigability (Binder-Macleod et al., 1997; Gregory et al., 2007), iNMES effectively reduces fatigability by reducing MU discharge frequencies, compared to traditional

[3] The contributing authors to the work presented in this chapter were: Austin J Bergquist, Leilane Rocha, Kelvin E Jones, and David F Collins.

NMES (Lou et al., under review). What is still not clear is how torque produced by iNMES is influenced by stimulation frequency compared to traditional mNMES and nNMES. The effect of stimulation frequency on torque production during NMES is typically described by a sigmoidal curve in the torque versus frequency relationship (TFR) (Binder-Macleod, 1995; Binder-Macleod et al., 1998; Edwards et al., 1977; Newham et al., 1983).

For the TA (Klakowicz et al., 2006) and triceps surae (TS) (Bajd et al., 1999; Bergquist et al., 2011a) muscles, mNMES generates contractions mainly by the recruitment of motor axons under the stimulating electrodes. This type of recruitment generates contractions through pathways restricted to the peripheral nervous system (i.e. peripheral pathways) and is represented by motor-, or M-waves, in the electromyographic (EMG) signal. On the other hand, nNMES, delivered to activate the TS muscle, generates contractions mainly through pathways that traverse the central nervous system (i.e. central pathways), particularly when a wide pulse duration is used (1 ms) (Lagerquist et al., 2010; Lagerquist et al., 2009a). Motor unit recruitment through central pathways is represented in the EMG signal by Hoffman-reflexes (H-reflexes) (Bergquist et al., 2011a; Bergquist et al., 2012b; Klakowicz et al., 2006) and "asynchronous activity", which is MU discharge that is not time-locked to each stimulation pulse (Bergquist et al., 2011a; Bergquist et al., 2012b). Little is known about how the pathway by which electrically-evoked contractions are produced influences the TFR.

The first objective of this study was to compare the effect of stimulation frequency on torque produced by mNMES, nNMES and iNMES. The second objective was to identify whether the pathway by which NMES generates contractions (central vs. peripheral) affects the TFR for TA and TS muscles. We hypothesized that: 1) torque produced by iNMES (i.e. at 40 Hz) would be the linear summation of torque produced by mNMES (i.e. at 20 Hz) and nNMES (i.e. at 20 Hz) alone, for both TA and TS muscles; and 2) torque generated by central pathways would reach a steady state at lower frequencies than torque produced by peripheral pathways. The results of this study are important for rehabilitation since they allow for a better understanding of the effect of stimulation frequency on torque production, especially during iNMES. Moreover, the TA muscle is commonly electrically stimulated to correct foot-drop resulting from central nervous system impairments (Everaert et al., 2010) and the TS muscle is important for walking (Anderson et al., 2003) and standing (Loram et al., 2002) and can be affected by central nervous system injuries (Bajd et al., 1999; Kesar et al., 2008).

3.2 Methods

3.2.1 Participants

For each participant data were collected during two sessions on different days: one session for TA and one for TS muscle. Each experimental session lasted ~2 hours. Data were collected from 9 participants for both the TA (4 females, 5 males; aged 27 ± 4.7 years) and TS (3 females, 6 males; aged 27 ± 4.7 years) experiments. Four participants took part in both experiments. For these 4 participants, the TA and TS sessions were performed at least 48h apart. Participants had no known neuromusculoskeletal impairments. The experimental protocols were approved by the local Human Research Ethics Board.

3.2.2 Torque

A Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York) was used to measure isometric torque around the ankle joint. The center of rotation of the ankle was aligned to the axis of the dynamometer. All experiments were performed on the right leg. During the TA experiments, dorsiflexion torque was recorded with the hip at ~90°, knee at 90° and ankle at 100°, where 90º is the neutral position of the ankle. During the TS experiments, plantarflexion torque was recorded with the hip at ~90°, knee at 90° and ankle at 90°.

To reduce the variability of torque generated during the TFR for each NMES type, the muscle was potentiated prior to data collection using previously established protocols (Binder-Macleod et al., 1998; Tillin et al., 2009). Initially, three MVICs were recorded, each were 3-5s long and were separated by 2 min (Tillin et al., 2009). Participants received verbal encouragement to perform maximally and visual feedback of torque produced during each MVIC. The MVICs were followed by 5 to 10 trains of mNMES and nNMES separately (6 pulses, 100 Hz), until a consistent amount of torque was produced by successive trains (Binder-Macleod et al., 1998). The stimulation amplitude used for these potentiation trains was adjusted to generate ~20% MVIC with the first train. The recording of the TFR data commenced within 10 min of this potentiating protocol (Binder-Macleod et al., 1998; Tillin et al., 2009).

3.2.3 EMG recordings

The skin was lightly abraded with a fine grain sandpaper and cleaned with alcohol prior to electrode application. Figure 3-1 shows the EMG electrode positioning for TA (left panel)

and TS (right panel) muscles. Two adhesive electrodes (2.25 cm^2; Vermed Medical, Vermont, USA) arranged in a bipolar configuration were used to record EMG from TA and soleus. The electrodes were placed distal to the mNMES electrodes and parallel to the predicted direction of the muscle fibers with 1 cm inter-electrode distance. The ground electrode was placed over the tibia. The EMG signals were amplified from 500 to 1000 times and band-pass filtered at 30-1000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

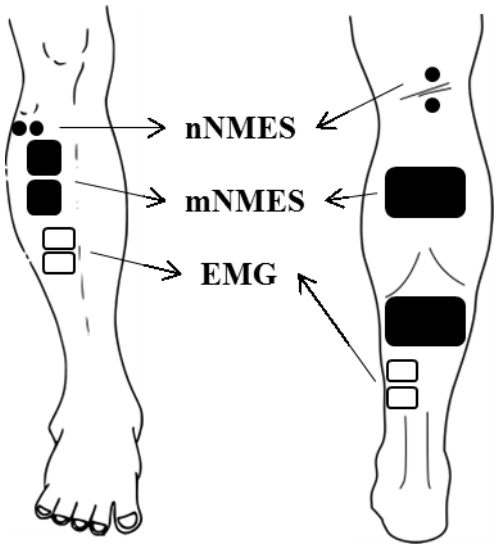

Figure 3-1. Schematic showing the NMES and EMG sites for the TA **(left)** and TS **(right)** muscles.

3.2.4 NMES - TA

For TA, 50 μs rectangular pulses were used during all three types of NMES to reduce the probability evoking H-reflexes (Brooke et al., 1997; Lagerquist et al., 2009a; Pierrot-Deseilligny et al., 1981; Veale et al., 1973; Zehr, 2002). The locations of the stimulating electrodes are shown in the left side of Figure 3-1. For mNMES, the stimulating electrodes (5.08 x 5.08 cm; model 400-899, Richmar, Chattanooga, TN, USA) were placed over the largest portion of the TA muscle belly consistent with the location of the main motor point of TA (Botter et al., 2011), with the cathode proximal. The nNMES was delivered through two adhesive gel electrodes (3.2 cm round, model 400-864, Richmar, Chattanooga, TN, USA) with the cathode distal to the fibular head over the common peroneal nerve and the anode 1 cm distal to the cathode along the anticipated path of the nerve. The location for the nNMES

electrodes was adjusted for each participant to produce dorsiflexion with minimal or no eversion.

3.2.5 NMES - TS

Rectangular pulses with relatively long duration (1 ms) were used when all three types of NMES were delivered to the TS muscle. The longer pulse duration was chosen to optimize the activation of sensory axons and consequently the extent to which contractions are generated through central pathways (Collins et al., 2001, 2002; Lagerquist et al., 2008). Locations of the stimulating electrodes are shown in the right side of Figure 3-1. For mNMES, the anode (5.08 x 8.89 cm; model 400-899, Richmar, Chattanooga, TN, USA) was placed over the largest portion of the gastrocnemius muscle belly and the cathode was just distal to the gastrocnemius over the soleus muscle. The nNMES was delivered through two adhesive gel electrodes (3.2 cm round, model 400-864, Richmar, Chattanooga, TN, USA) placed on the skin over the tibial nerve in the popliteal fossa separated by 1 cm with the cathode proximal.

3.2.6 NMES - torque-frequency relationship protocol

Two constant-current stimulators were used to deliver the TFR protocol (Digitimer, Welwyn Garden City, UK), one for mNMES (DS7AH), one for nNMES (DS7A) and both for iNMES. The two stimulators were controlled by a computer running custom-written Labview scripts. For each type of NMES, 2 s long trains of stimulation were delivered at each frequency (10, 20, 30, 40, 60, 80, and 100 Hz). Each frequency was delivered twice but not consecutively. The order of testing each NMES protocol and each frequency was randomised for each participant. Stimulation amplitude was set such that 2 s of each type of NMES at 20 Hz generated 20% MVIC during the second half of the stimulation train. For iNMES, stimulus pulses were alternated between the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites, with the first pulse always delivered to the $iNMES_{(n)}$ site. To set stimulation amplitude for iNMES, current delivered to the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites was initially set to generate 10% MVIC at 10 Hz. If these stimulation amplitudes did not result in 20% MVIC when iNMES was delivered at 20 Hz, the stimulation amplitudes were re-adjusted until the desired torque was reached. During this process, $iNMES_{(m)}$ and $iNMES_{(n)}$ were always set to generate approximately the same amount of torque at 10 Hz.

3.2.7 Data acquisition and analyses

Data were acquired at 5 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for post-hoc analysis that was conducted using custom-written Matlab software (The Mathworks, Natick, MA).

Torque generated during NMES was normalized by torque produced during the MVICs. The MVIC torque was quantified by averaging a 500 ms window centered on the point of peak torque. Torque generated by NMES was calculated over the second half of each stimulation train. Torque was then averaged over the two stimulation trains delivered at each stimulation frequency by the same NMES type. The amplitudes of M-waves and H-reflexes were quantified only for trials in which NMES was delivered at 10 and 20 Hz, as stimulation artifacts at higher stimulation frequencies result in contamination of the EMG. The objective of this EMG analysis was to provide insight into the pathways that contributed to the evoked-contractions and determine whether response amplitude was influenced by stimulation frequency. Waveforms in the EMG were identified as M-waves and H-reflexes based on their latency and amplitude. Initially, the EMG data were visually inspected for the presence of waveforms with latencies that were "time-locked" to the stimulus pulses at ~5 ms for M-waves and 25-30 ms for H-reflexes. To be included in the analysis as an M-wave or H-reflex the waveforms had to be larger than the average plus 2 standard deviations of the baseline EMG that was calculated over a 5 ms window before the stimulation artifacts. M-waves and H-reflexes were measured peak-to-peak and were then averaged over the responses to each stimulus pulse at a given frequency (10 or 20 Hz) and NMES type. Responses evoked by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites during iNMES were quantified separately.

At low stimulation amplitudes, single pulses of mNMES and nNMES recruit different MUs in TA (Okuma et al., 2013). To test our second hypothesis and provide an estimate of the amount of overlap between MUs recruited from the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites during iNMES, a similar approach adapted from the one used on Chapter 2 to estimate "overlap". The linear sum of the torque (LS_{torque}) generated when mNMES and nNMES were delivered separately at half the iNMES frequency was calculated and compared that to torque generated by iNMES. For example, torque generated when mNMES and nNMES were delivered at 10 Hz was summed (LS_{torque}) and the result was compared to the torque produced when iNMES was delivered at a net frequency of 20 Hz (i.e. 10 Hz at each stimulation site). In this way, if

mNMES and nNMES recruited completely different MUs, the LS_{torque} of mNMES and nNMES at 10 Hz would be equal to torque generated by iNMES at 20 Hz. In contrast, if mNMES and nNMES recruited the same MUs, the LS_{torque} of mNMES and nNMES at 10 Hz would be double torque generated by iNMES at 20 Hz.

All statistical analyses were performed on group data using Statistica 12.0 software (StatSoft, Tulsa, OK). The normal distribution of the data was tested and confirmed using a Shapiro-Wilk test. A two-way repeated measures analysis of variance test (rmANOVA; 3x7) was used to compare torque data with *NMES type* (mNMES, nNMES and iNMES) and *Stimulation Frequency* (10, 20, 30, 40, 60, 80 and 100 Hz) as factors. Tukey's post-hoc tests were used when main effects or interactions were identified. The amplitudes of M-waves or H-reflexes were compared separately using a two-way rmANOVA taking in consideration the factors *NMES type* (i.e. mNMES and $iNMES_{(m)}$) and *Frequency* (10 and 20 Hz). The amplitude of M-waves or H-reflexes, separately, were only compared within the same NMES type since the spatial distribution of the MUs recruited by mNMES and nNMES and, consequently, the contribution to the EMG signal varies according to the NMES type and wave recorded (Okuma et al., 2013; Vanderthommen et al., 2000). Tukey post-hoc tests were used when main effects or interactions were identified. Dependent Student's t-tests were used to compare the LS_{torque} when mNMES and nNMES were delivered at 10, 20, 30 and 40 Hz to the torque produced by iNMES at 20, 40, 60 and 80 Hz, respectively, to assess the overlap in the MUs recruited by mNMES and nNMES. Dependent Student's t-tests were also used to compare the torque generated by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites when delivered at the amplitude that was used during the iNMES TFR protocol. The level of statistical significance was set at 0.05. All data are reported as mean ± standard deviation.

3.3 Results

3.3.1 TA experiment

3.3.1.1 Torque

Figure 3-2 shows torque and EMG recorded from a single participant. Torque generated by mNMES at 10 and 100 Hz ranged from 1.1 to 6.8 Nm, representing a difference of 29% MVIC between the two frequencies.

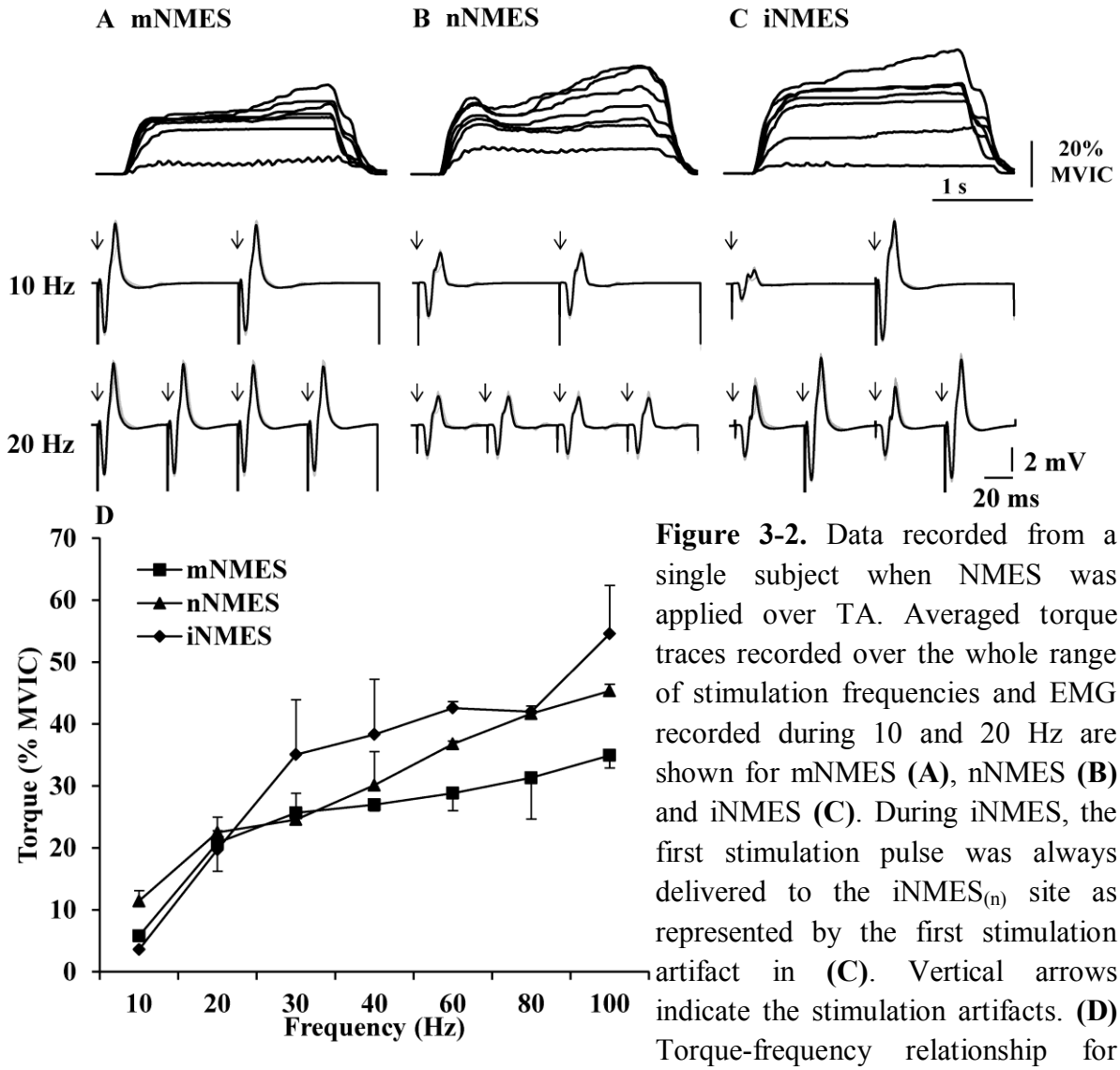

Figure 3-2. Data recorded from a single subject when NMES was applied over TA. Averaged torque traces recorded over the whole range of stimulation frequencies and EMG recorded during 10 and 20 Hz are shown for mNMES **(A)**, nNMES **(B)** and iNMES **(C)**. During iNMES, the first stimulation pulse was always delivered to the $iNMES_{(n)}$ site as represented by the first stimulation artifact in **(C)**. Vertical arrows indicate the stimulation artifacts. **(D)** Torque-frequency relationship for mNMES, nNMES and iNMES.

For this subject, the contractions generated by mNMES were mainly driven by M-waves. Torque generated by nNMES at 10 and 100 Hz ranged from 2.2 to 8.8 Nm, representing a 34% increase between the two frequencies. Contractions where mainly generated by M-waves during nNMES, however, small H-reflexes were identified. At low stimulation frequencies (i.e. 10-30 Hz) torque had a "flat" profile while in some trials at higher frequencies (i.e. 100 Hz) there was a gradual increase in torque over time. This increase in torque during the stimulation was largest and occurred most frequently during nNMES. An increase in torque during the NMES trains was observed in 3 out of 9 participants, although

the increases were smaller and less frequent than for the participant whose data are shown in Figure 3-2. Torque generated by iNMES at 10 and 100 Hz ranged from 0.6 to 10.6 Nm, a 51% MVIC increase in torque between the two frequencies, and the contractions where mainly generated by M-waves independent of frequency. The TFR described in Figure 3-2D was calculated based on the raw traces shown on the first row of Figures 3-2A, 3-2B and 3-2C. Although statistical analyses were not performed on data from individual subjects, torque produced by iNMES was generally larger than mNMES and nNMES at frequencies above 20 Hz (except 80 Hz) and torque generated by nNMES was larger than mNMES at frequencies higher than 40 Hz.

The torque generated by each NMES type at each stimulation frequency averaged across the 9 participants is shown in Figure 3-3A. No significant difference [$p>0.05$] was identified between NMES types at 20 Hz since stimulation amplitude was manipulated to generate 20% MVIC at 20 Hz for all three NMES types. During iNMES, torque generated by $iNMES_{(m)}$ and $iNMES_{(n)}$ was set to generate approximately the same torque amplitude at half the desired net frequency (10 Hz). Torque generated by $iNMES_{(m)}$ was 6.9 ± 1.8% MVIC and $iNMES_{(n)}$ was 8.6 ± 2.7% MVIC and there was no significant difference between the two [$t_{(8)}$=-2.25; $p=0.053$]. For torque, there was a significant interaction between NMES type and frequency [$F_{(12,96)}$=23.68; $p<0.001$; observed power=1.0]. No significant difference [$p>0.9$] between torque generated by mNMES and nNMES was identified at any frequency; iNMES generated significantly more torque than mNMES and nNMES at frequencies equal to or greater than 30 Hz [$p<0.01$]. The average torque increase from 10 Hz to 100 Hz during mNMES, nNMES and iNMES was 21, 22 and 44% MVIC, respectively. Torque reached a steady state when there was no significant difference between a given frequency and torque produced at 100 Hz. Torque generated by mNMES reached steady state at 30 Hz [$p>0.07$], nNMES at 60 Hz [$p>0.85$] and iNMES at 80 Hz [$p=0.11$]. Figure 3-3B shows the comparison between torque generated by iNMES and LS_{torque}, which was used to estimate the overlap in MU recruitment between mNMES and nNMES. The LS_{torque} was not significantly different [$p>0.05$] than iNMES torque at any of the frequencies tested (iNMES torque vs. LS_{torque} at 20 [$t_{(8)}$=1.79; $p=0.11$], 40 [$t_{(8)}$=0.73; $p=0.48$], 60 [$t_{(8)}$=-0.2; $p=0.84$] and 80 [$t_{(8)}$=-1.34; $p=0.21$] Hz), suggesting that different groups of MUs were recruited by mNMES and nNMES at this stimulation amplitude.

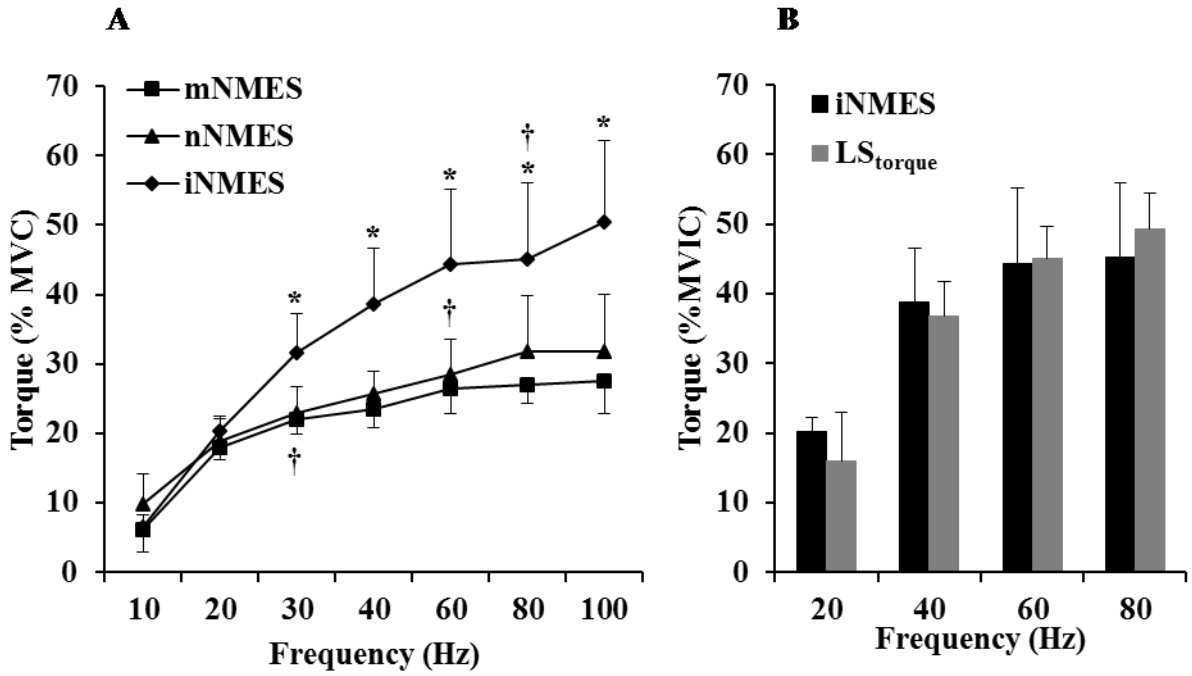

Figure 3-3. Group data from the experiments performed on the TA muscle. TFR recorded from the TA muscle during mNMES, nNMES and iNMES **(A)** († torque steady state: no difference from 100 Hz; * iNMES vs. mNMES and nNMES; $p<0.05$). Comparison between iNMES torque and LS_{torque} **(B)**.

3.3.1.2 EMG

The amplitude of M-waves and H-reflexes were quantified when mNMES, nNMES and iNMES were delivered at 10 and 20 Hz as shown in Figure 3-4. M-waves generated during iNMES were quantified separately for each stimulation site: $iNMES_{(m)}$ and $iNMES_{(n)}$; both were delivered at 5 and 10 Hz (half the net frequency used during iNMES: 10 and 20 Hz). There was no main effect of frequency on M-wave [$F_{(3,24)}=1.03$; $p=0.39$; observed power=0.24] or H-reflex amplitude [$F_{(3,24)}=1.422$; $p=0.26$; observed power=0.32] during mNMES and $iNMES_{(m)}$ when the net frequency was 10 and 20 Hz. Similarly, no main effect frequency on the amplitude of M-waves [$F_{(3,24)}=0.38$; $p=0.76$; observed power=0.11] or H-reflexes [$F_{(3,24)}=0.02$; $p=0.99$; observed power=0.05] was identified when nNMES and $iNMES_{(n)}$ were delivered at 10 and 20 Hz (Figure 3-4C). On average, the mean baseline EMG amplitude was 0.037 ± 0.013 mV during mNMES and 0.016 ± 0.008 mV during nNMES.

In three subjects small H-reflexes were evident in the EMG when mNMES was delivered at 10 Hz (0.21, 0.14 and 0.31 mV) and 20 Hz (0.09, 0.07 and 0.24 mV); and when nNMES was delivered at 10 Hz (0.13, 0.25 and 0.2 mV) and 20 Hz (0.17, 0.2 and 0.3 mV). One of these subjects had relatively large H-reflexes when iNMES was delivered at 10 Hz (only during $iNMES_{(n)}$) which had an average amplitude of 0.72 mV. For this subject, when iNMES was delivered at 20 Hz the H-reflex amplitude decreased to 0.16 mV.

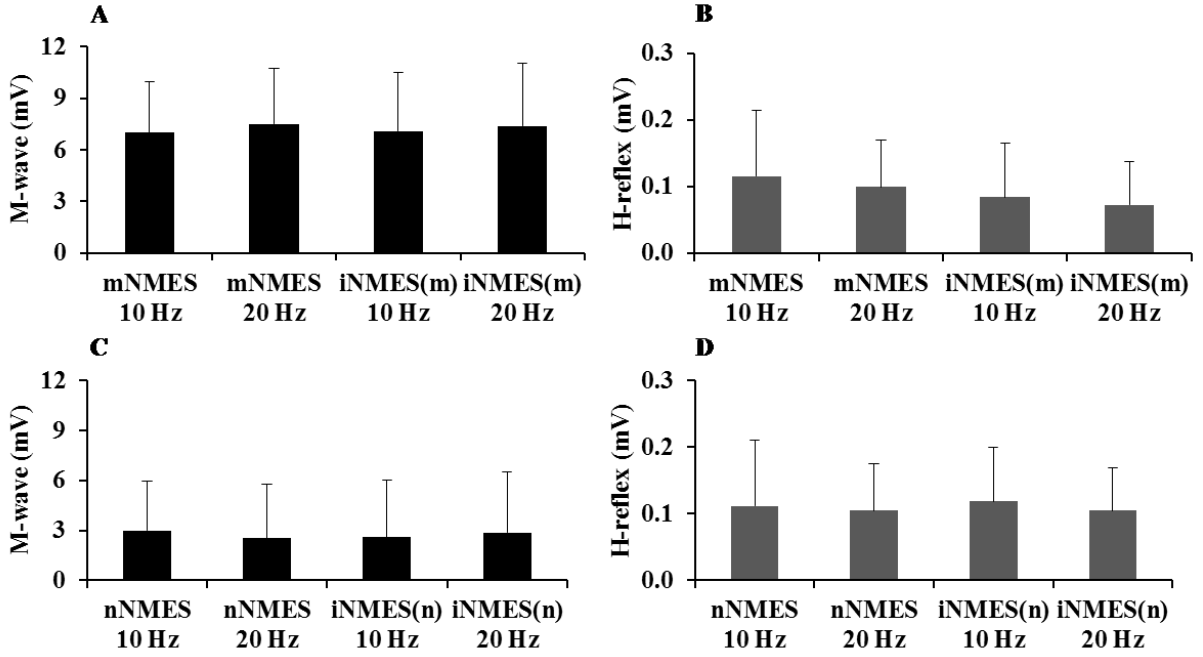

Figure 3-4. Average M-wave (**A** and **C**) and H-reflex (**B** and **D**) amplitudes recorded when NMES was delivered at 10 and 20 Hz to the TA. Panels **(A)** and **(B)** show the M-wave and H-reflex amplitudes when the stimulation was delivered by over the muscle belly (mNMES or $iNMES_{(m)}$). Panels **(C)** and **(D)** show M-wave and H-reflex amplitudes when the stimulation was delivered over the common peroneal nerve trunk (nNMES or $iNMES_{(n)}$).

3.3.2 TS experiment

3.3.2.1 Torque

Figure 3-5 shows, in the same format as Figure 3-2, the averaged torque and EMG recorded from a single participant when NMES was applied over the TS muscle.

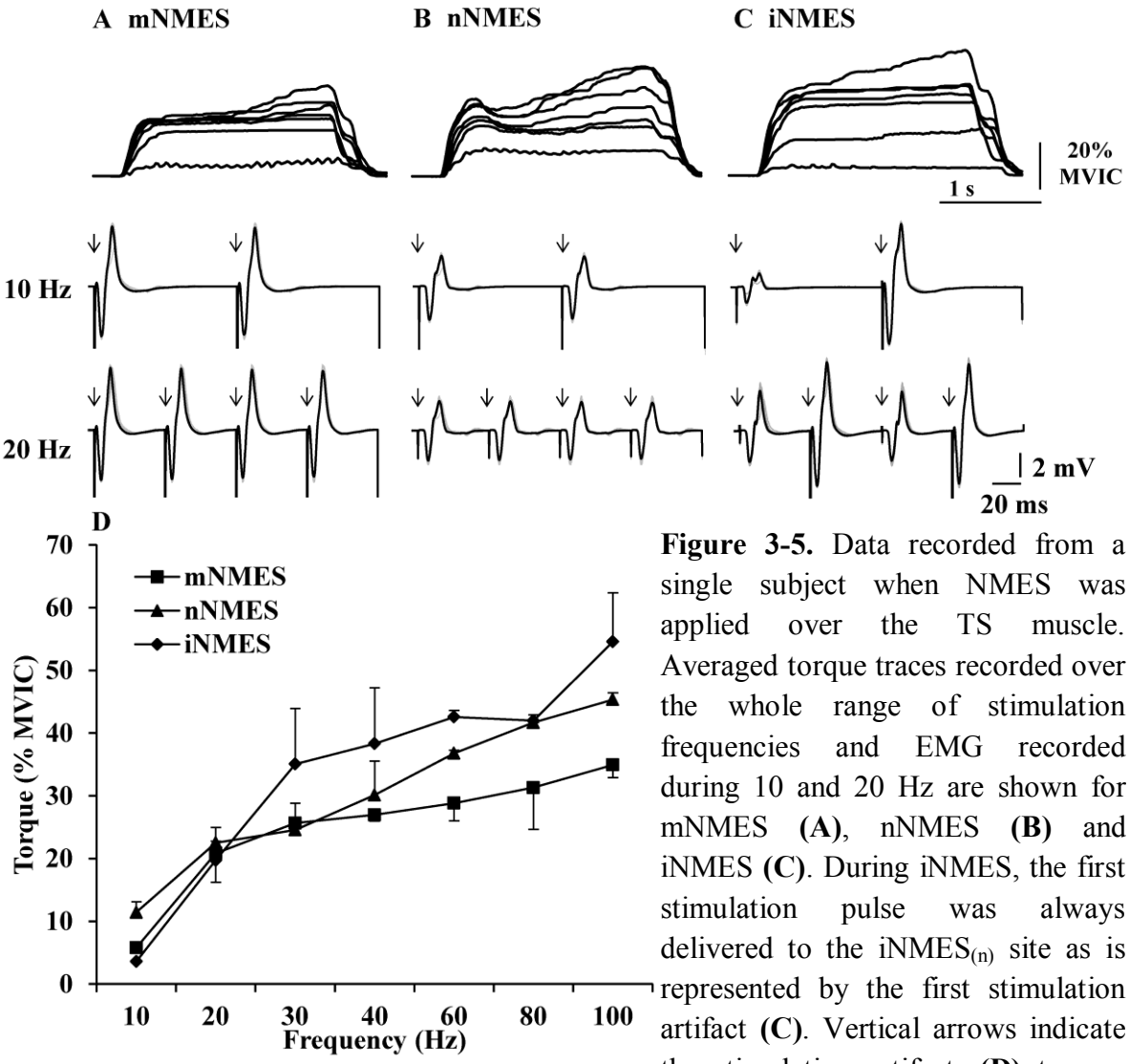

Figure 3-5. Data recorded from a single subject when NMES was applied over the TS muscle. Averaged torque traces recorded over the whole range of stimulation frequencies and EMG recorded during 10 and 20 Hz are shown for mNMES **(A)**, nNMES **(B)** and iNMES **(C)**. During iNMES, the first stimulation pulse was always delivered to the $iNMES_{(n)}$ site as is represented by the first stimulation artifact **(C)**. Vertical arrows indicate the stimulation artifact. **(D)** torque-frequency relationship for mNMES, nNMES and iNMES.

There was a 30% MVIC increase in torque when mNMES was delivered at 100 Hz compared to 10 Hz as it increased from 13.6 Nm at 10 Hz to 46.5 Nm at 100 Hz. For this subject, contractions generated by mNMES were mainly driven by M-waves independent of frequency (i.e. 10 or 20 Hz) although small H-reflexes were identified at both frequencies. Torque generated by nNMES increased only 0.7% MVIC when the stimulation frequency increased from 10 to 100 Hz ranging from 23.6 to 24.4 Nm. During nNMES, contractions where mainly generated by H-reflexes but small M-waves were present. The H-reflexes were

qualitatively larger at 10 Hz compared to 20 Hz while the M-waves were of similar amplitude between the two frequencies. Torque generated by iNMES increased 30% MVIC when frequency increased from 10 to 100 Hz and ranged from 13.5 to 46.3 Nm. When iNMES was delivered at 10 Hz, contractions were produced mainly though H-reflexes from the $iNMES_{(n)}$ site (first stimulation artifact in Figure 3-5C) and through M-waves from the $iNMES_{(m)}$ site (second stimulation artifact in Figure 3-5C). When iNMES was delivered at 20 Hz, $iNMES_{(n)}$ generated an initially large H-reflex (grey line) but on average H-reflexes were smaller than at 10 Hz; $iNMES_{(m)}$ generated contractions via M-waves but small H-reflexes were present. The TFR described in Figure 3-5D shows that torque produced by iNMES was similar to torque produced by mNMES for all frequencies. Torque produced by nNMES was relatively constant independent of frequency, although there was a small decrease in torque at 40 Hz. There was a gradual increase in torque throughout a train of stimulation for this participant during the three types of mNMES. This effect increased with increases in frequency and was present in 6 out of 9 participants.

The relationship between torque and frequency for the group (n=9) is shown in Figure 3-6A. There were no significant differences in torque produced when each NMES type was delivered at 20 Hz [$p>0.05$]. When iNMES was delivered to produce 20% MVIC at 20 Hz, torque generated from the $iNMES_{(m)}$ site (11.2 ± 2.25% MVIC) was not significantly different from that generated from the $iNMES_{(n)}$ site (12.7 ± 2.66% MVIC) [$t_{(8)}=-1.86$; $p=0.09$]. For torque, there was an interaction between NMES type and frequency [$F_{(3,24)}=7.76$; $p<0.001$; observed power=0.97]. Torque generated by iNMES was significantly larger than mNMES at stimulation frequencies from 60 to 100 Hz [$p<0.01$, single asterisks in Figure 3-6A]; iNMES produced significantly larger torque than nNMES at stimulation frequencies from 30 to 100 Hz [$p<0.01$, double asterisks in Figure 3-6A] and; mNMES generated significantly larger torque than nNMES at stimulation frequencies from 40 to 100 Hz [$p<0.001$, pound signs (#) in Figure 6A]. The average torque increase from 10 to 100 Hz during mNMES, nNMES and iNMES was 22, 9 and 34% MVIC, respectively. Torque generated reached steady state during mNMES at 60 Hz [$p>0.67$], nNMES at 20 Hz [$p>0.34$] and iNMES at 80 Hz [$p>0.99$]. Figure 3-6B shows the comparison between iNMES torque and LS_{torque}. The LS_{torque} was significantly larger than iNMES torque at 20, 40 and 60 Hz [$t_{(8)}=-5.24$; $p<0.001$; $t_{(8)}=-3.1$; $p=0.01$; and $t_{(8)}=-3.47$; $p=0.008$, respectively] but not at 80 Hz [$t_{(8)}=-1.43$; $p=0.19$].

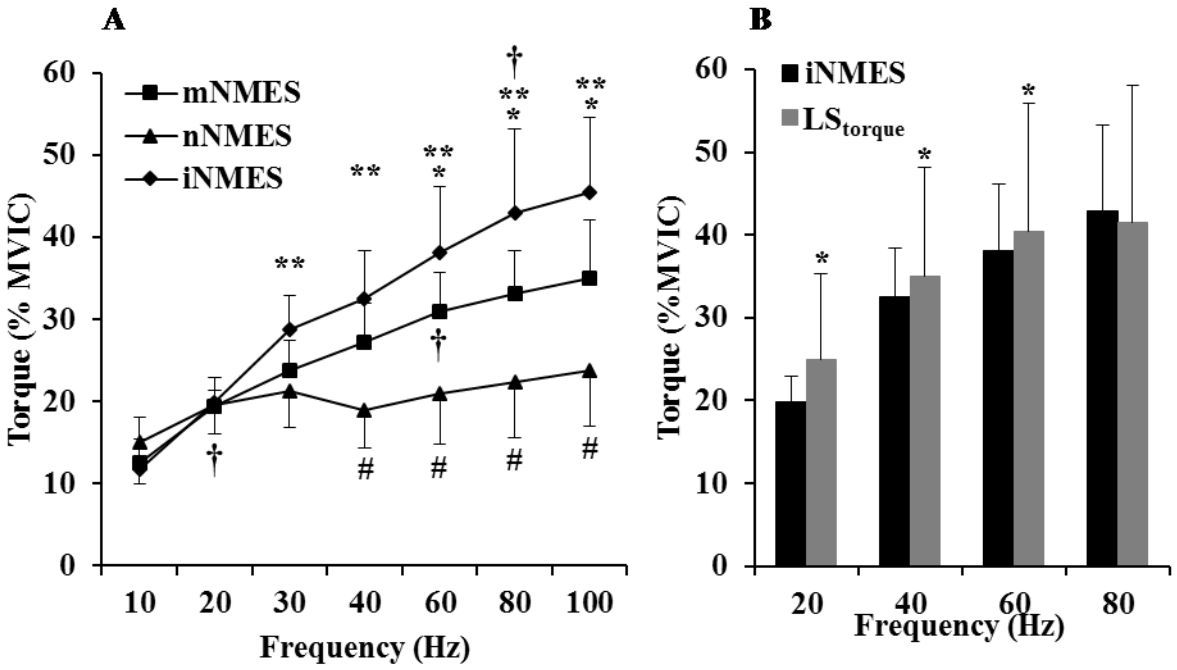

Figure 3-6. Group data from the experiments performed on the TS muscle. TFR recorded from the TS muscle during mNMES, nNMES and iNMES **(A)** († torque steady state: no difference from 100 Hz; * iNMES vs. mNMES; **iNMES vs. nNMES; #mNMES vs. nNMES; $p<0.05$). Comparison between iNMES torque and LS_{torque} **(B)**. (* iNMES vs. LS_{torque}; $p<0.05$).

3.3.2.2 EMG

Similar to the TA results, there was no main effect of frequency on M-wave [$F_{(3,24)}=2.10$; $p=0.12$; observed power=0.47] or H-reflex [$F_{(3,24)}=0.72$; $p=0.55$; observed power=0.18] amplitude generated during mNMES and $iNMES_{(m)}$ at 10 and 20 Hz as shown in Figure 3-7. No main effect of frequency on M-wave amplitude [$F_{(3,24)}=2.80$; $p=0.056$; observed power=0.61] was identified when nNMES and $iNMES_{(n)}$ were delivered at 10 and 20 Hz. There was a main effect [$F_{(3,24)}=14.64$; $p<0.001$; observed power=0.99] on the amplitude of H-reflexes during nNMES and $iNMES_{(n)}$ at 10 and 20 Hz. The H-reflexes produced by $iNMES_{(n)}$ at 10 Hz were significantly larger than the H-reflexes produced by nNMES at 10 Hz [$p=0.01$], nNMES at 20 Hz [$p<0.001$] and $iNMES_{(n)}$ at 20 Hz [$p<0.001$]. On average, the baseline EMG amplitude was 0.022 ± 0.008 mV during mNMES and 0.011 ± 0.008 mV during nNMES.

Taking into account single subject data, the largest evoked M-wave during the TS experiments was recorded when $iNMES_{(m)}$ was delivered at 10 Hz and its amplitude was 4.01 mV. One participant showed consistent H-reflexes during mNMES (0.3 mV) and $iNMES_{(m)}$ (0.5 mV). The same participant also had the largest H-reflex (2.58 mV) and it was recorded during $iNMES_{(n)}$ at 10 Hz.

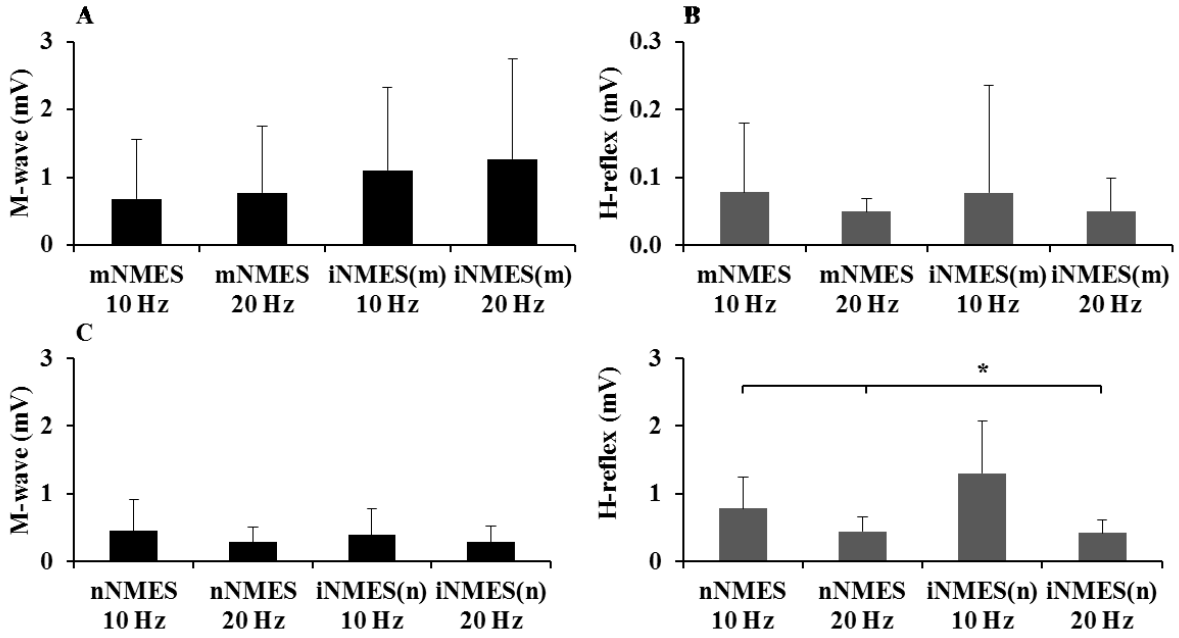

Figure 3-7. Average M-wave (**A** and **C**) and H-reflex (**B** and **D**) amplitudes recorded when NMES was delivered at 10 and 20 Hz to the TS muscle. Panels **(A)** and **(B)** show the M-wave and H-reflex amplitudes when the stimulation was delivered over the triceps surae muscle belly (mNMES or $iNMES_{(m)}$). Panels **(C)** and **(D)** show M-wave and H-reflex amplitudes when the stimulation was delivered over the common peroneal nerve trunk (nNMES or $iNMES_{(n)}$). (*$p<0.05$)

3.4 Discussion

The main objectives of this study were to test the effect of frequency on torque produced when mNMES, nNMES and iNMES were delivered to the TA and TS muscles, and determine whether the pathway used to generate the contractions, central or peripheral, influenced the TFR. In general, at the higher stimulation frequencies iNMES produced more torque than mNMES or nNMES, independent of the muscle tested. For TA, iNMES produced the most

torque at frequencies above 20 Hz and there were no differences in the torque produced by mNMES and nNMES at any frequency. Peripheral pathways were mainly involved in generating contractions of the TA. For the TS muscle, iNMES delivered at frequencies above 40 Hz produced the most torque, mNMES produced more torque than nNMES at frequencies above 30 Hz and torque produced by nNMES was relatively constant across frequencies, reaching a steady state at 20 Hz. Contractions of the TS muscle were driven mainly by peripheral pathways during mNMES/iNMES$_{(m)}$ and by central pathways during nNMES/iNMES$_{(n)}$.

3.4.1 The TFR of NMES applied to the TA

We hypothesized that: 1) torque produced by iNMES (i.e. at 40 Hz) would be the linear summation of torque produced by mNMES (i.e. at 20 Hz) and nNMES (i.e. at 20 Hz) alone, for both the TA and TS muscles; and 2) torque generated by central pathways would reach a steady state at lower frequencies than torque produced by peripheral pathways. Our results showed that, torque produced by iNMES (i.e. 20, 40, 60 and 80 Hz) was not different than the LS_{torque} resulting from the sum of the torque produced by mNMES and nNMES at half the net frequency (i.e. 10, 20, 30 and 40 Hz), confirming our first hypothesis. These results suggest that iNMES$_{(m)}$ and iNMES$_{(n)}$ site were each recruiting different groups of MUs with approximately 0% overlap and as a result, the torque produced by iNMES was the linear summation of the torque produced from the two stimulation sites. If the three NMES types recruited the same MUs (i.e. 100% overlap), one would expect that the torque produced at each frequency throughout the TFR would be the same, which was not the case. The recruitment of different MUs by each stimulation site during iNMES was previously confirmed by (Okuma et al., 2013) which showed that MU recruitment overlap is lowest at low stimulation amplitudes when mNMES and nNMES were delivered to the TA using single pulses of stimulation. The amplitude of the M-waves in the present study was not different between mNMES and iNMES$_{(m)}$ or nNMES and iNMES$_{(n)}$, suggesting that a similar number of MUs were recruited by stimulation over the muscle belly during mNMES and iNMES (i.e. iNMES$_{(m)}$) and by stimulation over the common peroneal nerve during nNMES and iNMES (iNMES$_{(n)}$). However, different groups of MUs were recruited by each stimulation site, resulting in a larger torque at frequencies higher than 20 Hz during iNMES than mNMES and nNMES.

Torque steady state is represented by the point in the TFR at which further increases in frequency do not result in further increases in torque. This steady state frequency is seldom reported in the literature describing the steady state frequency when mNMES and nNMES are delivered to the TA. Torque steady state observed for mNMES (60 Hz) was slightly higher than results reported in the literature for mNMES delivered to the TS muscle (50 Hz) (Sale et al., 1982; Stackhouse et al., 2005) but similar to those obtained in the quadriceps (60 Hz) (Binder-Macleod, 1995; Binder-Macleod et al., 1992). Orizio et al. (2004) recorded TFR during mNMES delivered to the TA but only in frequencies up to 50 Hz. Although these authors did not report a steady state frequency, it seems that torque production stopped increasing at frequencies between 40 and 50 Hz. Torque steady state frequency reported for nNMES agrees with results from other studies on the TS muscle showing that nNMES should reach steady state at frequencies around 30 Hz (Shields et al., 1997). The reasons behind the differences in steady state frequency between mNMES and nNMES are unclear. It is unlikely that MU type is the factor behind these differences since mNMES and nNMES had similar TFR profiles.

Torque production during iNMES reached a steady state at 80 Hz, approximately the sum of the steady state frequencies for mNMES and nNMES (60 Hz + 30 Hz). This result aligns with the increase in torque during iNMES compared to mNMES and nNMES, a consequence of the recruitment of different MUs by $iNMES_{(m)}$ and $iNMES_{(n)}$ sites. If the same MUs were recruited by each NMES type, then the steady state frequency for mNMES, nNMES and iNMES would be the same, which was not the case. The steady state reached at higher frequencies during iNMES is due to the fact that, even though the net frequency is high, each stimulation site is still in the ascending portion of the TFR. For example, the torque produced by mNMES at 60 Hz was not different from the torque produced at 30 Hz since mNMES recruited the same group of MUs over time and reached steady state at 30 Hz. On the other hand, iNMES delivered at 60 Hz, produced a torque not different than the sum of the torque produced by mNMES or nNMES delivered at 30 Hz. In this case, $iNMES_{(m)}$ and $iNMES_{(n)}$ sites were being activated at 30 Hz each; neither mNMES nor nNMES had reached torque steady state yet. Therefore, iNMES can produce more torque than mNMES and nNMES because different MUs are recruited by each stimulation site and the recruited MUs remain on the ascending portion of the TFR even at high net frequencies of iNMES.

3.4.2 Central and peripheral contributions during NMES applied to the TA

To test the extent to which central and peripheral contribute to electrically-evoked contractions elicited by NMES, EMG was recorded when the net frequency of stimulation was 10 and 20 Hz. The EMG data showed a strong contribution of peripheral pathways, regardless of NMES type, to TA contractions. These results were expected since there is a low probability of generating H-reflexes in TA (Zehr, 2002) where H-reflexes are a marker of central contribution to torque generation (Bergquist et al., 2011a; Bergquist et al., 2012b; Collins et al., 2001, 2002; Wegrzyk et al., 2015b). Moreover, the pulse duration was manipulated to minimize the probability of the appearance of H-reflexes. Thus, the expectation of a predominantly peripheral contribution to contractions evoked by NMES delivered to the TA was confirmed. However, some participants showed evidence of central pathways contributing to TA contractions in the present study. An example is provided by the single participant data shown in Figure 3-2 in which there were occasionally small H-reflexes in the EMG and torque increased during NMES trains in some trials, particularly at the higher frequencies. This increase in torque was previously defined as "extra torque" (Collins et al., 2001, 2002; Klakowicz et al., 2006) and its' mechanism was linked to the activation of persistent inward currents in motor neurons (further discussed in the next section) (Collins et al., 2001) and was abolished by nerve block (Collins et al., 2001; Lagerquist et al., 2009a), facts that together indicate that extra torque is modulated by spinal mechanisms. Extra torque was also shown to increase with increases in frequency (Collins et al., 2002). This central contribution when NMES was delivered to the TA was evident in the torque traces of 3 out of 9 participants. Therefore, we cannot attribute the torque generated by mNMES and nNMES delivered to the TA exclusively to peripheral pathways.

3.4.3 The TFR of NMES applied to the TS

The TS results did not support our first hypothesis, as iNMES generated significantly less torque than the linear summation of the torque produced by mNMES and nNMES at half the frequency at 20, 40 and 60 Hz but not at 80 Hz. These results suggest that there was a small amount of overlap between MUs recruited by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites for the TS muscle. The frequency-dependent difference in LS_{torque} and iNMES torque could be attributed to an increase in asynchronous activity during the 80 Hz stimulation (Bergquist et al., 2011a; Collins et al., 2001). During NMES, the recruitment of motor or sensory axons is

time-locked to the stimulation pulse, where M-waves and H-reflexes occur at a set interval after a stimulation pulse. Previous research showed that bursts of high frequency stimulation during nNMES can result in a MU discharge that is not time-locked to the stimulation pulse, referred to as asynchronous activity (Bergquist et al., 2011a; Bergquist et al., 2012b). The asynchronous activity reflects a central pathway contributing to torque generation during NMES and is thought to be mediated in part by the activation of persistent inward currents in motor neurons (Collins et al., 2001). Such an activation of persistent inward currents may result in an increased capacity to produce torque without changing stimulation amplitude (Bergquist et al., 2011a; Bergquist et al., 2012b; Collins et al., 2001, 2002; Lagerquist et al., 2009a; Wegrzyk et al., 2015b) which account for the similar torque between LS_{torque} and iNMES at high but not at low frequencies of stimulation. Independent of the contraction mechanism involved the overlap in MU recruitment between mNMES and nNMES is likely quite low in the TS muscle at the stimulation amplitude tested presently.

The TS's torque steady state frequency during iNMES was reached at similar frequencies as in the TA, but not for mNMES and nNMES. The steady state frequency during mNMES delivered to the TS muscle is in agreement with previous studies which suggested a steady state frequency of 50 Hz during mNMES (Sale 1982). The steady state at 20 Hz during nNMES contradicts previous results from Shields and Chang (1997), which suggested torque steady state at about 40 Hz during nNMES delivered to the TS muscle in subjects with spinal cord injury. The explanation for this is discussed in the next section as it is related to the pathway by which contractions were generated.

3.4.4 Central and peripheral contributions during NMES applied to the TS

The differences in the steady state frequencies between the different types of NMES to the TA and TS muscles could be explained by the mechanisms responsible for generating the contractions. In the TA muscle, the electrically-evoked contractions were generated mostly by peripheral pathways (M-waves) independent of NMES type or frequency. In the TS muscle, mNMES generated contractions similar to the ones generated in the TA muscle, using mainly peripheral pathways. When nNMES was delivered to the TS, alone or during iNMES, H-reflexes larger than M-waves were generated, suggesting a larger contribution of central pathways. However, the effect of central activation was stronger at low frequencies of stimulation. Electrically-evoked contractions driven mainly by peripheral pathways, such as

for the TA muscle and mNMES delivered to the TS muscle, were consistent with TFR profiles previously described in the literature (Binder-Macleod, 1995; Binder-Macleod et al., 1992; Orizio et al., 2004; Sale et al., 1982), since mainly motor axons were activated. Contractions generated with a larger contribution of central pathways can change the TFR profile since H-reflexes are present. The amplitude of H-reflexes is dependent on stimulation frequency, with increases in frequency resulting in smaller H-reflexes (Crone et al., 1989; Hirst et al., 1981; Hultborn et al., 1996). The frequency-dependent changes in H-reflex amplitude are mainly due to homosynaptic depression of Ia afferent terminals resulting in a decreased probability of neurotransmitter release with progressive increases in frequency (Crone et al., 1989; Hultborn et al., 1996). Our results showed that H-reflexes generated during nNMES delivered at 10 Hz were larger than at 20 Hz; we also showed that during iNMES delivered at 10 Hz the H-reflexes generated by the $iNMES_{(n)}$ site, which was actually firing at 5 Hz, where larger than the H-reflexes generated by nNMES delivered alone at 10 Hz. These results demonstrate the effect of homosynaptic depression of H-reflexes with increases in frequency. One would expect that torque would decrease if the main contributor to torque generation also decreased. Since the torque during nNMES or $iNMES_{(n)}$ was produced mainly by large H-reflexes, the depression in H-reflexes with progressive increases in frequency resulted in progressive decreases torque. This explains why nNMES reached a torque steady state at lower frequencies (20 Hz) compared to mNMES, where the contractions were not affected by central pathways. Shields and Chang (1997) suggested a torque steady state frequency of about 40 Hz when nNMES was delivered to the TS muscle of subjects with spinal cord injury, contradicting the present results. Even though these authors do not provide a full description of the EMG analysis, it seems that the contractions were mainly driven by peripheral pathways (large M-waves) resulting in a torque steady state at higher frequencies. It is also likely that the H-reflex depression at the $iNMES_{(n)}$ site reduced the torque produced at high frequencies during iNMES, since $iNMES_{(n)}$ did not increase torque with increases in frequency in a same rate as $iNMES_{(m)}$. Together, these results suggest that contractions driven mainly through central pathways (H-reflexes) are susceptible to enhanced inhibition with increases in stimulation frequencies, resulting in torque steady state at lower frequencies compared to contractions driven mainly through peripheral pathways.

3.5 Conclusion and practical implications

The results of this study demonstrated that iNMES produced approximately double the torque than mNMES or nNMES in both the TA and TS muscles, without changes in stimulation amplitude. The larger torque during iNMES was attributed to the recruitment of different MUs by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites resulting in a summation of the torque produced from the two stimulation sites. Torque steady state was achieved at higher net frequencies when peripheral pathways were mainly involved in generating the contractions, such as when all three types of NMES were delivered to the TA and when mNMES was delivered to the TS muscle. The steady state at lower frequencies when nNMES was delivered to the TS muscle was attributed to the frequency-dependent depression of H-reflexes resulting in lower than expected maximal torque at high stimulation frequencies.

Together, these results are important when choosing the type and frequency of NMES to use for rehabilitation. The iNMES method shows great potential to produce large contractions in the TA and TS muscles, while using a lower net frequency than mNMES and nNMES. Torque during iNMES was not only large but also produced by the recruitment of different MUs by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites. The lower frequency delivered to each stimulation site and the low overlap in MU recruitment between stimulation sites are important to reduce the rapid fatigability associated with the use of NMES.

CHAPTER 4. TORQUE, CURRENT, AND DISCOMFORT DURING THREE TYPES OF NEUROMUSCULAR ELECTRICAL STIMULATION OF TIBIALIS ANTERIOR[4]

4.1 Introduction

The capacity to generate adequate torque with minimal fatigability and discomfort are challenges for using neuromuscular electrical stimulation (NMES) for rehabilitation (Maffiuletti, 2010; Sheffler et al., 2007). Interleaved NMES (iNMES) may be one way to overcome these challenges for stimulating the ankle dorsiflexor tibialis anterior (TA; Lou et al., under review). Instead of delivering NMES though a single pair of electrodes over either the TA muscle belly (mNMES) or the common peroneal nerve (nNMES), such as during traditional NMES, iNMES involves alternating stimulation pulses between the mNMES ($iNMES_{(m)}$) and nNMES ($iNMES_{(n)}$) sites. Given that mNMES and nNMES recruit different pools of motor units [MUs; (Okuma et al., 2013)], iNMES recruits different MUs with every other stimulus pulse. As shown in the results from the experiments described in Chapter 3, iNMES produces more torque at a given frequency, than mNMES or nNMES delivered alone. Moreover, iNMES generates torque that is "adequate" for rehabilitation and 5 times more than what is necessary to dorsiflex the ankle during the swing phase of walking, with little overlap between MUs recruited from the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites (Chapter 2). A previous study also shown that iNMES results in less fatigability over the course of a fatigability protocol than mNMES and nNMES (Lou at al. 2015, under review). The present study is the first specifically designed to compare discomfort between mNMES, nNMES and iNMES.

Discomfort during NMES is thought to be due to the activation of afferents from nociceptors in the skin and musculotendinous structures (Delitto et al., 1995; Matthews et al., 1997) and/or ischemia, metabolite accumulation and musculotendinous stress (Delitto et al., 1995; Matthews et al., 1997). Although there are many studies of discomfort during NMES of the quadriceps (Alon, 1985; McNeal et al., 1988; Patterson et al., 1991), only a few studies

[4] The contributing authors to the work presented in this chapter were: Austin J Bergquist, and David F Collins.

have addressed discomfort during NMES of the muscles that dorsiflex the ankle. In one study, larger electrodes induced more discomfort than smaller electrodes during mNMES over the TA. It was suggested that this was due to the recruitment of more nociceptor afferents with the larger electrodes (Milner et al., 1969). Gracinin and Trnkiczy (1975) found that there was less discomfort when mNMES was delivered over TA using relatively short pulse durations (300 μs) than when longer pulses (1 ms) were used. Naaman et al. (2000) reported that nNMES produced less discomfort than mNMES when both were set to produce the same dorsiflexion movement and attributed the difference to the greater current and larger electrode size used for mNMES. Recently, 9 participants were asked to assess discomfort midway through fatigability protocols delivered using mNMES, nNMES or iNMES (Lou et al., under review). In that study, although iNMES used 10-20% less current than mNMES or nNMES, respectively, and discomfort scores were ~25% lower than during both mNMES and nNMES, there were no significant differences between the three types of NMES for either current or discomfort scores. This trend towards less current and discomfort during iNMES, however, led us to conduct the present study to more rigorously test discomfort between these three types of NMES.

Accordingly, the present study was designed to compare discomfort and related variables between mNMES, nNMES and iNMES during contractions that generated 5-30% of the torque produced during a maximal voluntary isometric contraction (MVIC). We hypothesised that at a same torque amplitude, iNMES would require less current and produce less discomfort than mNMES and nNMES. We also wanted to identify the maximal torque that could be produced by each type of NMES before it was limited by discomfort and hypothesized that in these trials discomfort would be similar between NMES types; however, torque would be larger during iNMES than during mNMES or nNMES. To provide a more complete assessment of the three types of NMES, current, current density and stimulation efficiency were also compared. Current and current density were included in the analysis because they are known to be major contributing factors to discomfort during NMES (Delitto et al., 1992; Gracanin et al., 1975; Naaman et al., 2000; Vanderthommen et al., 2007). Stimulation efficiency was calculated because it describes the relationship between torque and current, with a greater efficiency being associated with more torque being produced per unit of current. The ability to optimize torque generating capacity during NMES while minimizing

discomfort and the demands on battery power (i.e. current) may lead to a more successful use of NMES for rehabilitation.

4.2 Methods

4.2.1 Participants

Sixteen volunteers (9 females and 6 males; 28.8 ± 7.3 years; mean ± standard deviation) participated in one experimental session that lasted approximately 2 hr. No participant had any known neuromusculoskeletal impairment. The experimental protocols were approved by the Human Research Ethics Board at the University of Alberta.

4.2.2 Torque

Isometric dorsiflexion torque at the right ankle was measured using a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York). Participants were seated with the hip and knee positioned at approximately 90° and ankle at approximately 100°. The centre of rotation of the ankle joint was aligned with the axis of the dynamometer.

4.2.3 Maximal voluntary isometric contractions (MVICs)

Three to five MVICs of the dorsiflexors were recorded at the beginning of each experiment. Participants performed the MVICs while receiving verbal encouragement to perform maximally. Each contraction was separated by a 2-minute rest period.

4.2.4 Neuromuscular Electrical Stimulation (NMES)

Ankle dorsiflexion torque was generated by delivering NMES over the TA muscle belly (mNMES), the common peroneal nerve trunk (nNMES), or in an alternating or "interleaved" pattern between both (iNMES). Two constant-current stimulators (Digitimer, Welwyn Garden City, UK) were used to deliver the stimulation: one for mNMES (DS7AH), one for nNMES (DS7A) and both were used for iNMES. Custom written Labview software was used to control the timing of the stimulus pulses.

At the mNMES site, a pair of adhesive gel electrodes (5.08 x 5.08 cm or 25.8 cm^2; model 400-899, Richmar, Chattanooga, TN, USA) were placed over the main motor point of TA (Botter et al., 2011), with the anode approximately 1 cm distal to the cathode. At the nNMES site, a pair of adhesive gel electrodes (3.2 cm round, model 400-864, Richmar,

Chattanooga, TN, USA) were placed over the common peroneal nerve. The cathode was placed distal to the fibular head while the anode was positioned ~1 cm anterior to the cathode along the anticipated path of the nerve trunk. The exact location of the electrodes at the nNMES site was adjusted to produce dorsiflexion with minimal or no eversion. During iNMES, stimulation pulses were alternated between the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites.

The trains of NMES consisted of 12 pulses, each with 0.1 ms duration, delivered at 40 Hz. Stimulation amplitudes for mNMES and nNMES were set by delivering trains every 5 to 10 s and adjusting the amplitude until the desired torque was achieved (below). To set stimulation amplitude for iNMES, 6 pulses at 20 Hz were delivered to $iNMES_{(m)}$ and $iNMES_{(n)}$ separately to generate half of the desired torque for iNMES. If these amplitudes did not result in the desired net torque during iNMES, amplitudes were adjusted at each site until the desired torque was achieved, with similar torque being produced when the two sites were stimulated separately. The current delivered during each pulse was measured using a current probe (mA 2000 Noncontact Milliammeter; Bell Technologies, Orlando, Florida).

Stimulation amplitude was manipulated to generate 5, 10, 20 and 30% MVIC for each NMES type. In addition, in separate trials, stimulation amplitude was increased to the maximum that the participant could tolerate (MAX_{torque} trials), to identify the largest torque that could be produced by each type of NMES before limited by discomfort. During these MAX_{torque} trials for mNMES, stimulation amplitude was increased until participants indicated that they could not tolerate any further increases. The initial intention was to use the same method to assess maximal torque during nNMES; however, for 6 participants torque began to decrease with increases in stimulation amplitude before the maximal tolerable amplitude was reached. This decrease in torque occurred because nNMES over the common peroneal nerve activates not only motor axons innervating the dorsiflexor muscles but also motor axons innervating the evertors (peroneus brevis and longus) and, in some participants, increasingly high stimulation amplitudes result in decreases in dorsiflexion torque. To account for this issue, two maximal trials were recorded for nNMES in these 6 participants. The MAX_{torque} trials were recorded during nNMES for all participants and corresponded the maximal torque that could be produced regardless of whether it was limited by discomfort (n=5 participants) or by activation of the evertors (n=6 participants). A second nNMES MAX trial ($MAX_{amplitude}$) was recorded for the 6 participants in whom maximal torque was limited by activation of the

evertors and not by discomfort. For this $MAX_{amplitude}$ trial, nNMES amplitude was increased until participants indicated that they could not tolerate any further increases, independent of the torque generated. For the iNMES MAX_{torque} trials, the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites were stimulated at the amplitudes identified during the mNMES MAX_{torque} trials and nNMES MAX_{torque} trials, respectively, to identify the maximal torque that could be produced by iNMES regardless of whether it was limited by discomfort or evertors activation.

4.2.5 Visual analog scale (VAS)

The VAS was used to assess the level of discomfort experienced by subjects during the NMES protocols (Broderick et al., 2011; Naaman et al., 2000; Verhoeven et al., 2006). The VAS consisted of a 100 mm horizontal line that was labeled at each of the two ends. The left end was labeled as "no pain" and the right end as "maximum pain tolerable". Participants were told that there is no wrong answer when using the VAS (Philip, 1990). They were instructed to make a mark on the line that represented the VAS which corresponded to their level of discomfort with respect to the labels at each end of the scale.

To assess discomfort, procedures were conducted in the following order: 1) the stimulation amplitude was set for one NMES type and torque amplitude (i.e. mNMES was set to generate 10% MVIC); 2) participants were instructed to attend to their discomfort level during three trains of NMES that were delivered 5 s apart at this stimulation amplitude; 3) they were then instructed to indicate their discomfort during the three NMES trains using one VAS score. The same process was repeated for each NMES type and each torque amplitude. The amplitude of stimulation and the site of stimulation were randomized for each participant.

4.2.6 Data Acquisition and Analyses

Data were acquired at 25 kHz using custom-written Labview software (National Instruments, Austin, Texas). The high sampling rate was required to adequately capture the 0.1 ms current pulse. All data were stored on a computer for analyses that was performed *post-hoc* using custom-written Matlab software (The Mathworks, Natick, Massachusetts).

4.2.6.1 Torque

To calculate the torque generated during each MVIC, a 500-millisecond window was selected centered on the region of peak torque and the average torque was calculated. The largest MVIC was used to normalize the torque generated during the NMES protocols. Torque

generated during the NMES protocols was calculated using a similar procedure, however, torque was measured over a 50 ms window.

4.2.6.2 Current and current density

Current amplitude was measured from baseline to peak of the pulse recorded by the current probe. Current density was calculated by dividing the area of one electrode by the current used in a given protocol (mA/cm^2); this was done to account for the differences in electrode size between the mNMES and nNMES sites (Lieber et al., 1991; Maffiuletti et al., 2014). Current and current density were calculated for the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites separately when iNMES was delivered.

4.2.6.3 Stimulation efficiency

Stimulation efficiency was calculated by dividing torque (% MVIC) by current (mA) and also by current density (mA/cm^2) (Alon et al., 2013; Lieber et al., 1991; Maffiuletti et al., 2014).

4.2.6.4 Visual analogue scale (VAS)

The VAS analysis consisted of measuring, with a ruler, the distance in millimetres between the mark made by the participant and the start of the line on the left side.

4.2.6.5 Statistical analysis

Although 16 participants took part in these experiments, statistical analyses were only performed on data from 11 participants (6 females and 5 males; 29.9 ± 8.6 years). Two participants (1 female, 23 years old and 1 male, 26 years) were excluded from the analysis because they could not tolerate the stimulation amplitudes required to generate torque higher than 20% MVIC during mNMES. Three other participants were excluded because of technical problems that resulted in the loss of data. For one of the 11 participants included in the statistical analysis, torque and current were not recorded for the nNMES MAX_{torque} trial. For a second participant, also included in the statistical analysis, the VAS score was not recorded when mNMES was delivered to generate 5% MVIC. The missing data for these 2 participants was imputed using the k-Nearest Neighbors algorithm for multiple imputation (Rubin, 1996).

All statistical analyses were performed on group data using Statistica 12.0 software (StatSoft, Tulsa, OK). The data for torque, current, current density and stimulation efficiency followed a normal distribution as determined using Shapiro-Wilk's tests. Two main statistical analyses were performed, one for full group data (n=11) where current, current density,

stimulation efficiency and VAS scores were compared between NMES types at each torque amplitude (5-30% MVIC and MAX_{torque}). These data are shown in panel A of all figures. The second analysis compared data between the MAX_{torque} and $MAX_{amplitude}$ trials for the 6 participants in whom torque during the nNMES MAX_{torque} trials was limited by activation of the evertors, these data are displayed in panel B of all figures.

Torque and stimulation efficiency were compared between NMES types (mNMES, nNMES and iNMES) at each torque amplitude (5, 10, 20, 30% MVIC and MAX_{torque}) using a 3x5 repeated measures analysis of variance (rmANOVA) test. Current and current density were compared using the same procedures used for torque data; however, current and current density results during iNMES were compared for both $iNMES_{(m)}$ and $iNMES_{(n)}$ (4x5 rmANOVA).

The VAS scores did not follow a normal distribution. Therefore, the VAS data were transformed using a logarithm with base 10. This transformation resulted in a normal distribution, thus a 3x5 rmANOVA was used to compare the transformed VAS results between NMES types (mNMES, nNMES and iNMES) and at each torque amplitude (5, 10, 20, 30% MVIC and MAX_{torque}). Tukey post-hoc tests were used, when appropriate, to identify specific differences when the rmANOVAs identified significant main effects or interactions. Dependent t-tests for paired-samples were used to compare the results of torque, current, current density, stimulation efficiency and transformed VAS scores between the nNMES MAX_{torque} and $MAX_{amplitude}$ trials. Pearson product-moment correlation coefficient tests were used to correlate the transformed VAS scores with torque, current and current density. The classification of the level of correlation, i.e. 0.1 small, 0.3 moderate, 0.5 strong, was made according to Cohen's classification (Cohen, 1992). For all tests, the level of significance was set at 0.05. All data are reported as mean ± standard deviation.

4.3 Results

4.3.1 Torque

For all 11 participants, stimulation amplitude was set to generate 5, 10, 20 and 30% MVIC and the maximal possible (MAX_{torque}) during mNMES, nNMES and iNMES (Figure 4-1A). For torque, there was an interaction between NMES type and torque [$F_{(8,80)}$=32.47; $p<0.001$; observed power=1.00]. There were no significant differences between NMES types

regarding the torque produced at a given submaximal torque amplitude (5 [p>0.255], 10 [p=1.000]; 20 [p>0.999] and 30% MVIC [p>0.999]). During the MAX_{torque} trials, which represented the maximal torque that could be generated by each NMES type, independent whether it was limited by discomfort or by the action of evertors, there were significant differences between torque produced by all three NMES types [p<0.001]. Maximal torque generated by nNMES was 1.7 times larger than iNMES and 1.9 times larger than mNMES and iNMES generated 1.4 times more torque than mNMES. The average torque over all the trials during mNMES ranged from 1.7 (at 5% MVIC) to 11.4 Nm (at MAX_{torque}), 1.7 to 21.7 Nm during nNMES and 1.7 to 17.1 Nm during iNMES.

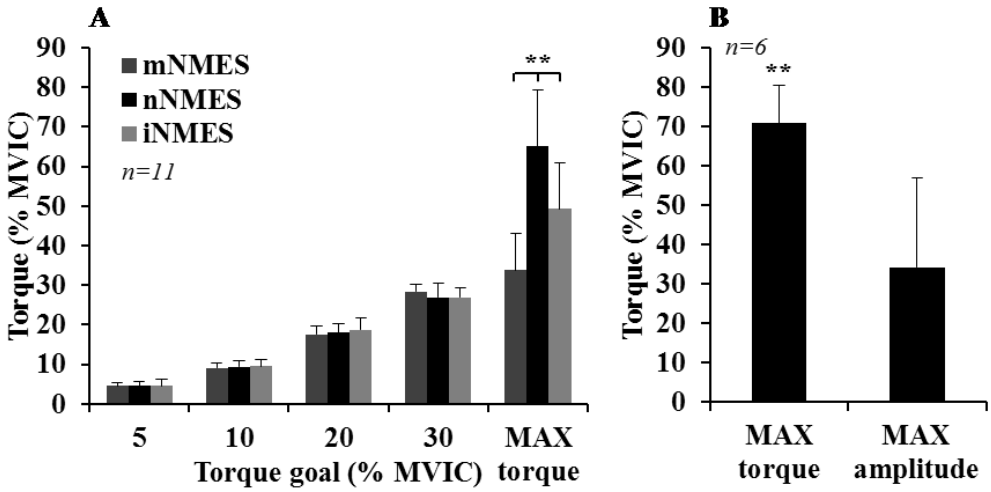

Figure 4-1. Mean torque produced by each type of NMES across the group of 11 participants at submaximal stimulation amplitudes and at the stimulation amplitude that resulted in maximal torque (MAX_{torque}) **(A)**. Torque generated by nNMES in 6 participants when the goal was to generate the maximal torque possible (MAX_{torque}) or to identify the maximal tolerable stimulation amplitude ($MAX_{amplitude}$) **(B)**. *p<0.05; **p<0.01

Our initial intention for the MAX_{torque} trials was to identify the largest torque that could be produced before it was limited by discomfort. However, as described in the Methods, for 6 participants during nNMES, maximal dorsiflexion torque (MAX_{torque}) was generated at a stimulation amplitude that was lower than the maximal amplitude tolerable, due to the activation of the evertors. For these 6 participants an extra trial was recorded at the maximal amplitude tolerable ($MAX_{amplitude}$). Torque decreased by 2.1 times in these 6 participants [$t_{(5)}$=5.19; p=0.003] when nNMES amplitude was increased from that which generated the

maximal torque (MAX_{torque}) to the maximum tolerable ($MAX_{amplitude}$) as shown in Figure 4-1B.

4.3.2 Current

For current (n=11, Figure 4-2A), there was an interaction between torque and NMES type [$F_{(12,120)}$=11.52; p<0.001; observed power=1.00]. The results of the post-hoc analysis showed that there was no difference in current between NMES types when torque was 5 [p>0.998] or 10% MVIC [p>0.928]. To generate 20% MVIC, mNMES required more current than nNMES [p=0.005]. At 30% MVIC and at MAX_{torque}, mNMES required more current than nNMES [p<0.001] and $iNMES_{(m)}$ required more current than $iNMES_{(n)}$ [p<0.001]. There were no significant differences between the current delivered to the mNMES vs. $iNMES_{(m)}$ or nNMES vs. $iNMES_{(n)}$ at all torque amplitudes tested [p>0.05]. To generate 30% MVIC, mNMES required 4 times more current than nNMES. To generate MAX_{torque}, mNMES required 3.4 times more current than nNMES and generated 1.9 times less torque than nNMES. When current data were compared for 6 participants during the two nNMES maximal trials, the current used to induce the maximal discomfort during $MAX_{amplitude}$ trial was 1.9 times larger than the one necessary to produce the maximal torque [$t_{(5)}$=-4.43; p=0.006] (Figure 4-2B).

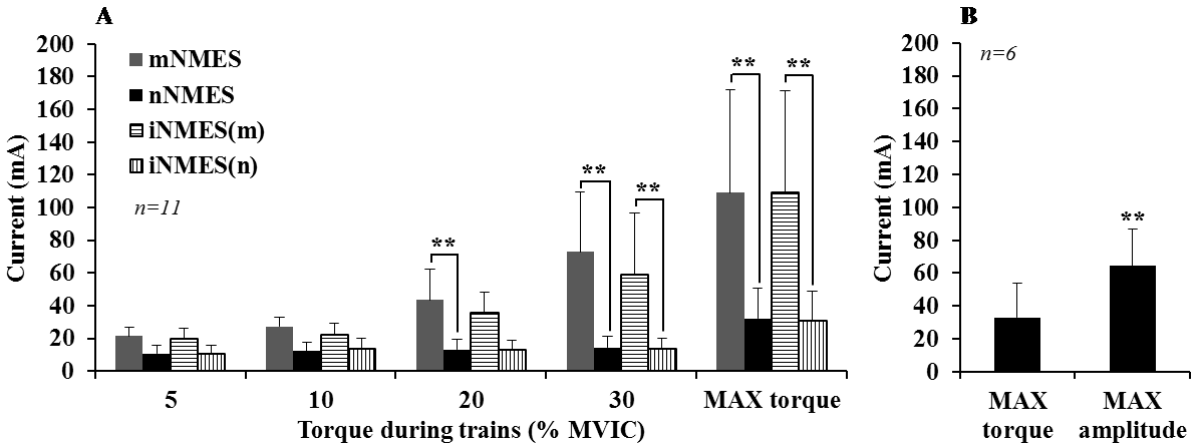

Figure 4-2. Current delivered during mNMES, nNMES and iNMES over a range of torque amplitudes. The current during iNMES was calculated separately for the $iNMES_{(m)}$ and $iNMES_{(n)}$ **sites (A)**. Current recorded during the nNMES MAX_{torque} trials and the nNMES $MAX_{amplitude}$ trials in 6 participants **(B)**. *p<0.05; **p<0.01

4.3.3 Current density

Current density (n=11) was calculated to account for the different size of electrodes used at the mNMES and nNMES sites (Figure 4-3A). There was an interaction between torque and NMES type [$F_{(12,120)}$=2.03; p=0.027; observed power=0.908]. Post-hoc analysis indicated that there were no significant differences in current density between the three types when delivered to generate 5, 20 or 30% MVIC [p>0.211]. To generate 10% MVIC, current density was significantly larger during nNMES compared to mNMES [p=0.006] and $iNMES_{(n)}$ compared to $iNMES_{(m)}$ [p=0.005]. At MAX_{torque}, (Figure 4-3A), current density during nNMES was significantly larger than mNMES [p<0.001] and during $iNMES_{(n)}$ compared to $iNMES_{(m)}$ [p=0.003]. There were no significant differences in current density between mNMES and $iNMES_{(m)}$ [p>0.05], and nNMES and $iNMES_{(n)}$ [p>0.05] at any torque amplitude. Figure 4-3B (n=6) shows that current density was larger during the nNMES $MAX_{amplitude}$ trial compared to MAX_{torque} trial [$t_{(5)}$=-4.43; p=0.006].

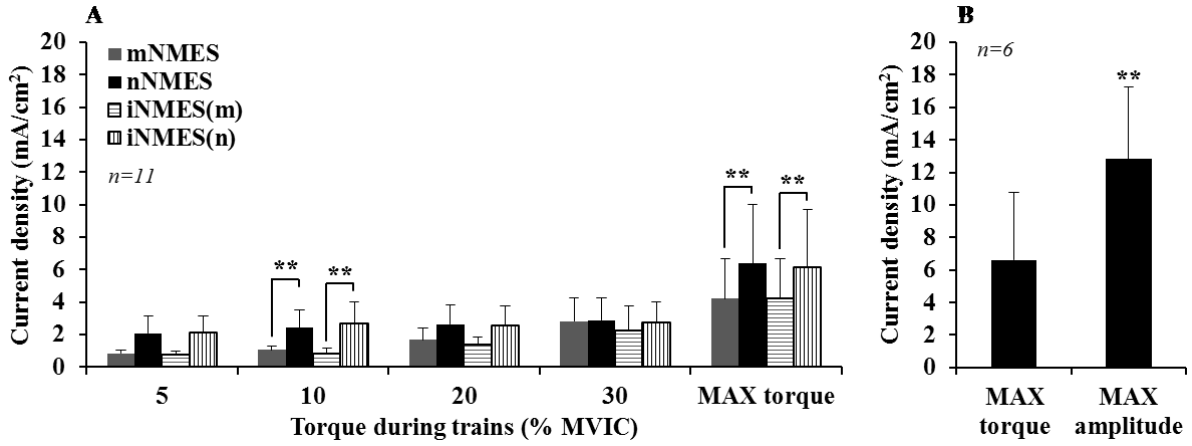

Figure 4-3. Current density calculated when mNMES, nNMES and iNMES were delivered over a range of torque amplitudes **(A)**. Current density recorded during the nNMES MAX_{torque} trials and the nNMES $MAX_{amplitude}$ trials in 6 participants **(B)**. *p<0.05; **p<0.01

4.3.4 Stimulation efficiency

Stimulation efficiency was calculated to identify the effect of NMES type and amplitude on the capacity to produce torque (Figure 4-4). Stimulation efficiency was only calculated for mNMES and nNMES since torque could not be recorded for $iNMES_{(m)}$ and $iNMES_{(n)}$ separately. There was an interaction between NMES type and torque [$F_{(4,40)}$=16.977; p<0.001;

observed power=1.00] (n=11, Figure 4-4A). There were no differences in stimulation efficiency between mNMES and nNMES when they were delivered to generate 5 [p=0.723] and 10% MVIC [p=0.480]. However, nNMES efficiency was higher than mNMES when they were delivered to generate 20% MVIC [p<0.001], 30% MVIC [p<0.001], and MAX_{torque} [p<0.001]. During the nNMES maximal trials (n=6), efficiency was significantly lower [$t_{(5)}$=3.47; p=0.017] during the $MAX_{amplitude}$ trial compared to MAX_{torque} trial, as shown in Figure 4-4B.

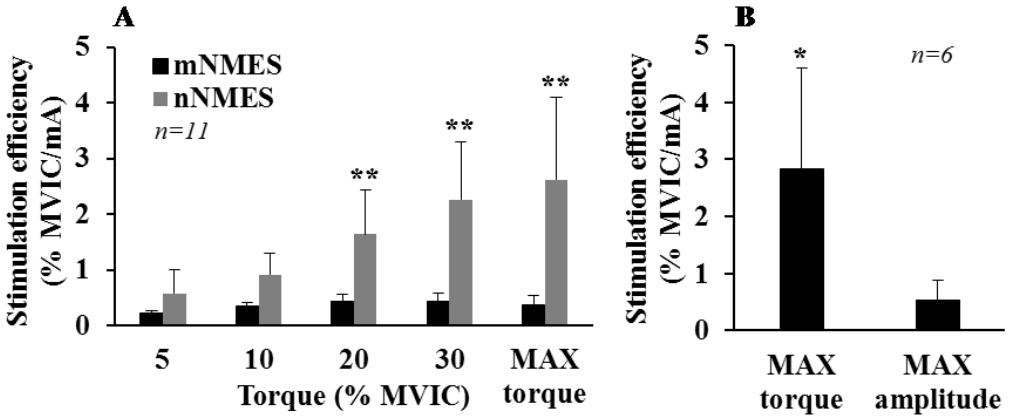

Figure 4-4. Stimulation efficiency calculated by dividing torque and current used during mNMES and nNMES over a range of torque amplitudes **(A)**. Stimulation efficiency calculated for the nNMES MAX_{torque} trials and the nNMES $MAX_{amplitude}$ trials in 6 participants **(B)**. *p<0.05; **p<0.01

Stimulation efficiency was also calculated using current density (instead of current) and torque to account for the different electrode sizes used during mNMES and nNMES (n=11, Figure 4-5A). There was an interaction between torque and NMES type [$F_{(4,40)}$=8.248; p<0.001; observed power=0.996]. The only significant difference identified was for when stimulation amplitude was set to generate 10% MVIC, where mNMES was more efficient than nNMES [p=0.002]. Stimulation efficiency during the nNMES $MAX_{amplitude}$ trials (n=6) was less than during the MAX_{torque} trials [$t_{(5)}$=3.47; p=0.017] (Figure 4-5B).

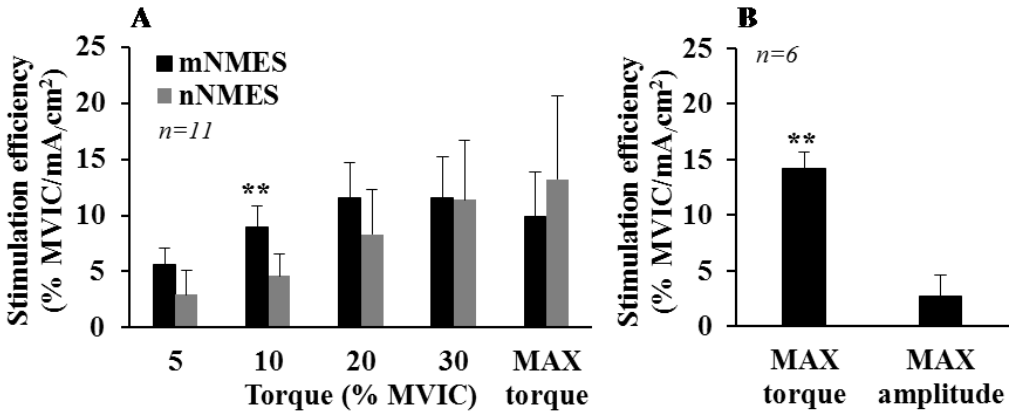

Figure 4-5. Stimulation efficiency calculated by dividing torque and current density **(A)**. Stimulation efficiency calculated for the nNMES MAX_{torque} trials and the nNMES $MAX_{amplitude}$ trials in 6 participants **(B)**. *p<0.05; **p<0.01

4.3.5 VAS scores

The log transformed VAS scores were used to evaluate discomfort. There was an interaction between torque and NMES type [$F_{(8,80)}$=4.78; p<0.001; observed power=0.996] (n=11, Figure 4-6A). There were no differences in discomfort between NMES types when they were delivered to produce 5 [p>0.947], 10 [p>0.999] or 20% MVIC [p>0.277]. In contrast, when torque was 30% MVIC, mNMES generated more discomfort than nNMES [p<0.001] and iNMES [p<0.001]. When the stimulation amplitude was set to generate maximum torque (i.e. MAX_{torque} trials; Figure 4A), mNMES generated more discomfort than nNMES [p=0.03] but was not different than the discomfort produced during iNMES [p=0.911]. Figure 4-6B shows that the discomfort produced during the nNMES $MAX_{amplitude}$ trials (n=6) was higher than during MAX_{torque} trial [$t_{(5)}$=-4.11; p=0.009].

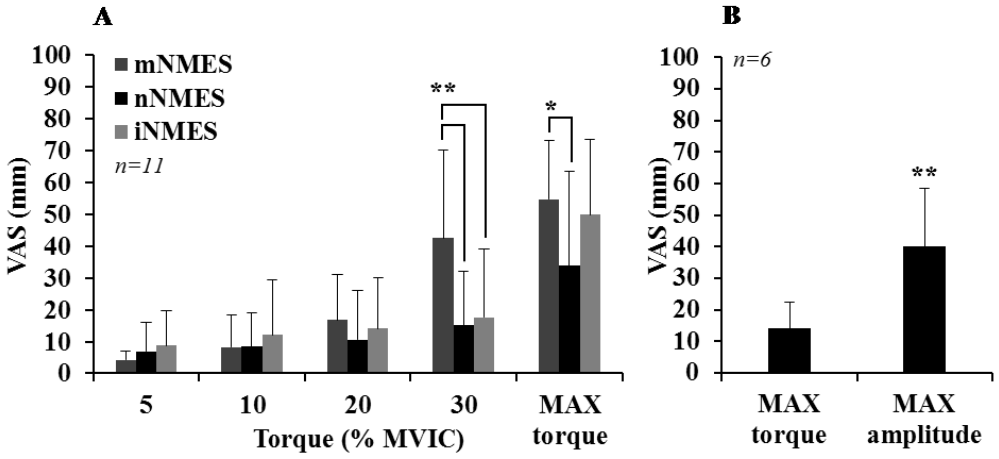

Figure 4-6. Discomfort scores as assessed using the VAS when mNMES, nNMES and iNMES were delivered over a range of torque amplitudes. The MAX_{torque} corresponds to the stimulation amplitude that produced the maximal torque independent of whether it was limited by discomfort or activation of the evertors (n=11). **(A)**. Discomfort scores for the nNMES MAX_{torque} trials and the nNMES $MAX_{amplitude}$ trials for 6 participants **(B)**. These data are the original not transformed VAS scores. *p<0.05; **p<0.01

4.3.6 Correlations

Table 4-1 shows the correlations between log transformed VAS and torque, current and current density. Strong correlations were identified between VAS scores and torque produced during mNMES, nNMES and iNMES. The correlation between VAS scores and current during mNMES and nNMES was moderate to strong. The correlation between VAS scores and current density during mNMES and nNMES was also moderate to strong.

Table 4-1. Correlations between transformed VAS scores and torque, current and current density. Torque results compared with transformed VAS scores for mNMES, nNMES and iNMES, while for current and current density only for mNMES and nNMES.

	N	r	p<
Torque			
mNMES	55	0.706	0.001
nNMES	55	0.453	0.001
iNMES	55	0.552	0.001
Current (mA)			
mNMES	55	0.502	0.001
nNMES	55	0.483	0.001
Current density (mA/cm^2)			
mNMES	55	0.491	0.001
nNMES	55	0.479	0.001

4.4 Discussion

This study was designed to compare discomfort and related variables between mNMES, nNMES and iNMES. In general, at low torque amplitudes (5-20% MVIC), there were no differences in discomfort between NMES types, and current, current density and efficiency were similar between NMES types, with few exceptions. To generate contractions of 30% MVIC, mNMES produced more discomfort than iNMES and nNMES. When the stimulation was delivered to produce the maximal torque (MAX_{torque} trials, n=11), mNMES required the highest current and produced the least torque with the most discomfort; nNMES used the lowest current and produced the largest torque and the least discomfort; iNMES generated more torque than mNMES but was not different than nNMES.

There were no differences in the discomfort produced by the three types of NMES when delivered to produce 5 to 20% MVIC, contrary to our hypothesis. This hypothesis was based on the preliminary results of Lou et al. (under review) which showed a trend (but no significant differences) for iNMES to use less current and produce less discomfort than mNMES and nNMES. There were significant differences at these torque amplitudes in current and current density, for example, current was largest during mNMES at 20% MVIC and current density was largest at 10% MVIC during nNMES. However, these differences in these

parameters did not play a large enough role to alter discomfort between NMES types, as there were no differences in the VAS scores.

When stimulation amplitudes were increased to generate 30% MVIC, mNMES produced more discomfort than nNMES and iNMES. The current necessary to generate this torque was 3 times larger during mNMES than nNMES, but there was no significant difference in current density. Naaman et al. (2000) showed that, for TA, nNMES produced less discomfort than mNMES, consistent with the current results, although torque was not reported in that study. These differences in discomfort suggest that mNMES recruits a greater number of nociceptive afferents than nNMES. For mNMES to produce progressively larger torque, current has to be increased to recruit motor axons in progressively deeper portions of the muscle belly (Adams et al., 1993; Okuma et al., 2013). It is probable that these larger currents recruit progressively more nociceptive afferents throughout the muscle belly and in the surrounding skin that is innervated not only by the common peroneal nerve but also by lateral and medial portions of the sural nerve, which together produced more discomfort than nNMES. On the other hand, nNMES activates axons bundled inside the common peroneal nerve trunk which has a vastly smaller diameter than the muscle belly and is directly under the stimulating electrodes. Therefore, the lower current used during nNMES likely resulted in the depolarisation of fewer nociceptor afferents than during mNMES and, consequently, less discomfort. These differences in how axons are recruited between mNMES and nNMES accounts for the fact that nNMES was 5 times more efficient (efficiency calculated based on current) while producing less discomfort than mNMES to generate 30% MVIC.

Discomfort during contractions of 30% MVIC during iNMES was less than during mNMES but not different from during nNMES. Current and current density used to generate 30% MVIC during $iNMES_{(m)}$ and $iNMES_{(n)}$ were not significantly different from mNMES and nNMES, respectively. However, the current delivered to mNMES and $iNMES_{(m)}$ was higher than during nNMES and $iNMES_{(n)}$, respectively. It is probable that discomfort during iNMES was not as high as during mNMES because stimulation was delivered at both sites ($iNMES_{(m)}$ and $iNMES_{(n)}$) at only half of the net frequency. Consequently, the total amount of current delivered over time to each NMES site was reduced, resulting in less discomfort during iNMES than mNMES.

Based on data reported by Lou et al. (under review), we hypothesised that when the stimulation was set to generate the maximal torque possible with each NMES type (MAX_{torque} trials, n=11), all three types of NMES would result in similar discomfort but that iNMES would produce the most torque. However, we found that nNMES produced contractions of 65% MVIC on average, 1.7 times larger than the maximal torque produced by iNMES (49% MVIC) and 1.9 times larger than the maximal torque produced by mNMES (33% MVIC). Further, discomfort during nNMES was less than during mNMES and was not different than during iNMES. Thus, nNMES and not iNMES generated the most torque and VAS scores were not equal between NMES types. We believe that the torque produced by iNMES was not as large as during nNMES due to the high discomfort at the $iNMES_{(m)}$ site, which reduced the contribution to torque made by the $iNMES_{(m)}$ site. The relatively low discomfort scores during the nNMES MAX_{torque} trials reflect the fact that for 6 out of 11 participants, nNMES was delivered at the amplitude that produced the maximal torque which was 2.1 times less than the amplitude that produced the maximal discomfort for these individuals which contributed to lower VAS scores for nNMES and to a lesser extent iNMES. The reduction in torque during the nNMES $MAX_{amplitude}$ trials compared to the MAX_{torque} trials occurred because the common peroneal nerve innervates not only the ankle dorsiflexors but also evertors. Thus, at high stimulation amplitudes, dorsiflexors and evertors were concomitantly activated resulting in a decrease in dorsiflexion. Thus, in most individuals, maximal torque during nNMES can be generated before discomfort becomes a limiting factor, explaining the lower discomfort and larger torque during the nNMES MAX_{torque} trial than the $MAX_{amplitude}$ trial.

4.5 Clinical Implications

Each of the three types of NMES investigated in the present study has advantages and disadvantages for their use in rehabilitation. The main advantage of mNMES is the simple application of electrodes, since the TA muscle belly is easily identifiable. Further, mNMES typically produces “pure” dorsiflexion due to the relatively selective activation of TA motor axons only. However, mNMES produced more discomfort and required more current than nNMES when stimulation amplitude was set to generate torque larger than 20% MVIC. In fact, two participants were excluded from the statistical analyses because they could not tolerate mNMES amplitudes necessary to generate torque larger than 20% MVIC. Therefore,

mNMES should be chosen if the goal of a rehabilitation program is to generate low torque for short periods, where discomfort is less relevant, since it is the easiest NMES method.

The main advantages of nNMES are that it requires less current to generate similar torque (i.e. better efficiency) than mNMES and iNMES, which could reduce the demand on battery power of personal stimulators. However, it is more difficult to locate the common peroneal nerve than the TA muscle belly, making electrode placement problematic and the exclusive activation of motor axons that produce "pure" dorsiflexion unlikely, especially at higher stimulation amplitudes. If the goal of NMES training is to generate large torque to produce strength training-like adaptations, nNMES should be used since it generated contractions of 65% MVIC.

Although iNMES has advantages in terms of producing torque (Chapter 2 and 3) and reducing fatigability (Lou et al., under review), it suffers from limitations inherent to both mNMES and nNMES. For example, VAS scores during iNMES were not, as was expected, lower than during the mNMES and nNMES, likely due to the relatively high current required at the $iNMES_{(m)}$ site. Further, during $iNMES_{(n)}$ the activation of evertors concurrently with the activation of the dorsiflexors may limit the torque that can be produced by iNMES. Interleaved NMES should be considered if the goal of a rehabilitation program is to produce submaximal torque for long periods resulting in aerobic/endurance adaptations, or to enable users to engage in functional tasks for long periods, since iNMES results in less fatigability than mNMES or nNMES (Lou et al., under review).

The recommendations described above are relevant for individuals with intact sensation. For individuals who lack sensation, for example due to a complete spinal cord injury, mNMES may be more suitable than nNMES, as torque would not be limited by discomfort and it produces dorsiflexion with no eversion, however, it still requires higher current. For individuals with hypersensitivity, nNMES may be the most appropriate method since torque is produced more efficiently while reducing the activation of nociceptor afferents and, consequently, discomfort.

4.6 Conclusions

Herein we show that the maximal torque produced by mNMES, nNMES and iNMES is more than adequate for generating functional movements such as dorsiflexing the ankle during

walking. Further, there were no differences in discomfort and minimal differences in current and current density between the three NMES types for contractions in this "functional" range, equal to and lower than 20% MVIC. Differences in discomfort between the three types of NMES emerged at stimulation amplitudes that generated 30% MVIC; mNMES produced the most discomfort and required the most current. In separate trials, we found that nNMES generated the most torque and required the least current, resulting in higher efficiency (based on current) than mNMES. The maximal torque produced by iNMES was larger than mNMES but smaller than nNMES and this was likely limited by the discomfort produced at the $iNMES_{(m)}$ site. The differences in discomfort between NMES types were attributed to the differences in current required at the mNMES and nNMES sites and to differences in the locations of the axons being stimulated in the TA muscle belly and common peroneal nerve trunk. The results suggest that the choice of NMES type should be based on the goal of the rehabilitation program, be that to produce large torque or to minimize fatigability or discomfort.

CHAPTER 5. GENERAL DISCUSSION

The experiments described in this thesis were designed to address gaps in knowledge about iNMES to gain a better understanding about how best to use iNMES for rehabilitation. These gaps include: 1) the effects of the *amplitude of stimulation* on the overlap of MUs recruited by the mNMES and nNMES sites; 2) the influence of *frequency of stimulation* and *pathway* on torque; and 3) how does *discomfort* and *maximal torque* during iNMES compare to that during mNMES and nNMES. The work described in Chapter 2 characterized the overlap between motor units (MUs) recruited by mNMES over the tibialis anterior (TA) muscle belly and nNMES over the common peroneal nerve trunk, across a full range of *stimulation amplitudes* and quantified the torque produced when iNMES was delivered at the stimulation amplitude that had the lowest overlap. The experiments described in Chapter 3 identified the effect of different *stimulation frequencies* and *pathways* on torque (i.e. torque-frequency relationship, TFR) during mNMES, nNMES and iNMES delivered to the TA and triceps surae (TS) muscles. The experiments described in Chapter 4 compared *discomfort* between mNMES, nNMES and iNMES during contractions that generated 5-30% of the torque produced during maximal voluntary isometric contractions (MVIC) and quantified the *maximal torque* that could be produced by each NMES type. Section 5.1 of this General Discussion provides an overview of the main results of each research project. The main limitations and future directions of each project are described in Section 5.2. Section 5.3 provides an overview of the clinical implications of the work as a whole and incorporates suggestions about ways to overcome some of the challenges of using NMES for rehabilitation.

5.1 Overview

5.1.1 Interleaved neuromuscular electrical stimulation: motor unit recruitment overlap

The experiments described in Chapter 2 were designed to estimate the overlap between MUs recruited by mNMES and nNMES delivered to activate TA and quantify the torque produced by iNMES delivered at the stimulation amplitude that produced the lowest overlap. The lowest overlap was produced when twitches evoked by mNMES and nNMES were 10% of the peak twitch torque (PTT), which was the lowest amplitude tested. Overlap increased progressively with increases in stimulation amplitude up to that which produced 50% PTT or

72% overlap. These results are consistent with the results of Okuma et al. (2013) which showed, using EMG fine wires inserted into the muscle, that MUs in different portions of the TA muscle can be recruited by mNMES and nNMES. Our results take the work of Okuma et al. (2013) further by showing how changes in the amount of overlap of MUs recruited by the two stimulation sites influence the torque produced, a more functionally relevant outcome measure for using NMES for rehabilitation than was obtained using EMG in the Okuma study. When trains of iNMES were delivered at the lowest amplitude tested, at which single pulses delivered at the mNMES and nNMES sites produced twitches of 10% PTT and the lowest overlap, contractions equivalent to 25% MVIC were generated, 5 times more torque than what is necessary to dorsiflex the ankle during the swing phase of walking (Bogey et al., 2010; Davy et al., 1987). Overall, the results of Chapter 2 showed that mNMES and nNMES delivered to the TA can recruit different MUs (low overlap) at low stimulation amplitudes. Further, trains of iNMES can generate functionally significant torque even when delivered at low stimulation amplitudes that produce low overlap.

5.1.2 Tibialis anterior and triceps surae torque-frequency relationship when electrical stimulation is delivered over the muscle belly, nerve trunk or interleaved between both

The experiments described in Chapter 3 were designed to test the effect of stimulation frequency on torque generated by mNMES, nNMES and iNMES delivered to the TA and TS muscles, and to identify whether the pathway responsible for generating the contractions has an effect on the TFR. During these experiments, stimulation amplitude was set to generate 20% MVIC at 20 Hz for all three NMES types and then frequency was varied over seven frequencies from 10 to 100 Hz. Torque reached a "steady state" when there were no significant increases in torque with further increases in frequency. The presence of M-waves in the electromyographic (EMG) recordings provided evidence that contractions were generated through peripheral pathways and the presence of H-reflexes indicated that contractions were generated, at least in part, through central pathways.

In TA, iNMES produced an average torque of 50% MVIC at 100 Hz, approximately double the torque produced by mNMES and nNMES at the same frequency. Moreover, torque generated by iNMES reached a steady state at a higher frequency than mNMES and nNMES. These two results were attributed to the recruitment of different MUs by the $iNMES_{(m)}$ and

$iNMES_{(n)}$ sites during iNMES. If the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites recruited the same MUs, the torque at all frequencies and the torque steady state frequency would be expected to be the same between NMES types, which was not the case. The recruitment of different MUs by $iNMES_{(m)}$ and $iNMES_{(n)}$ is supported by the results of Chapter 2 and Okuma et al. (2013), which showed low MU recruitment overlap at low to moderate stimulation amplitudes. Contractions generated by NMES of TA were predominantly driven by peripheral pathways (M-waves) independent of frequency or NMES type. This result was expected since there is a low probability of generating H-reflexes in the TA (Zehr, 2002) and a short pulse duration was used to favour the recruitment of motor axons (Brooke et al., 1997; Lagerquist et al., 2009a; Pierrot-Deseilligny et al., 1981; Veale et al., 1973; Zehr, 2002).

There is a higher probability of generating H-reflexes in the TS muscle than in the TA (Zehr, 2002), thus there can be a larger central contribution to electrically-evoked contractions in the TS muscle compared to TA. In the TS, iNMES produced an average torque of 45% MVIC at 100 Hz, 1.2 times and 1.8 times larger than the average torque generated by mNMES and nNMES at 100 Hz, respectively. As proposed for the TA muscle, the larger torque during iNMES of the TS muscle was attributed to the recruitment of different MUs by $iNMES_{(m)}$ and $iNMES_{(n)}$ sites. However, the data indicated that there was more overlap in MUs recruited by each site for the TS muscle than was found for TA as less torque was produced by iNMES over the TS muscle than the linear summation of the torque produced by mNMES and nNMES alone. For the TS muscle, mNMES produced more torque than nNMES at 40 Hz and higher and the torque steady state frequencies were 60, 20 and 80 Hz during mNMES, nNMES and iNMES, respectively. The mechanism behind the differences in torque produced at high frequencies and the differences in torque steady state frequency between mNMES, nNMES and iNMES were attributed to the different pathways driving the contractions. Contractions were primarily driven by M-waves during mNMES and $iNMES_{(m)}$ (peripheral pathway) and by H-reflexes (central pathway) during nNMES and $iNMES_{(n)}$. During nNMES or $iNMES_{(n)}$ the H-reflexes were larger when the net frequency was 10 Hz compared to 20 Hz. H-reflex depression with increases in frequency has been attributed to increased homosynaptic depression at Ia afferent terminals (Crone et al., 1989; Hultborn et al., 1996). Therefore, increases in NMES frequency produced progressively smaller H-reflexes resulting in a steady state at lower frequencies during nNMES compared to mNMES. Torque steady state at low

frequencies during nNMES resulted in a lower than expected torque when frequency was high, where nNMES generated only 24% MVIC at 100 Hz and a reduced torque during iNMES due to the limitation in the torque produced by the $iNMES_{(n)}$ site.

Together, the results of the experiments described in Chapter 3 indicated that: 1) for both the TA and TS muscles, at this stimulation amplitude (i.e. necessary to generate 20% MVIC at 20 Hz), the larger torque produced by iNMES compared to mNMES and nNMES was a consequence of the recruitment of different MUs by the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites; and 2) electrically-evoked contractions driven mainly by central pathways (nNMES and $iNMES_{(n)}$) delivered to the TS muscle are susceptible to homosynaptic depression with increases in frequency, resulting in smaller H-reflexes and consequently, a steady state in torque is reached at lower frequencies and maximal torque is lower than contractions driven by peripheral pathways.

5.1.3 Torque, current, and discomfort during three types of neuromuscular electrical stimulation of tibialis anterior

The experiments described in Chapter 4 were designed to compare discomfort and related NMES variables between mNMES, nNMES and iNMES during contractions that generated 5-30% MVIC. This project was also originally designed to identify the maximal torque that could be produced by each NMES type before it was limited by discomfort.

No differences in discomfort between NMES types were identified during contractions from 5-20% MVIC. There were some differences in current and current density between NMES types but those did not result in different visual analogue scale (VAS) scores between NMES types. Therefore, there is no advantage in using any of the three NMES types at these torque amplitudes based on discomfort.

To generate 30% MVIC, nNMES required less current, was more efficient and generated less discomfort than mNMES. The discomfort produced by nNMES was not different from that during iNMES. Therefore, nNMES has the advantage over mNMES when delivered to generate 30% MVIC by requiring less battery power to generate the same torque (higher efficiency) and by producing less discomfort. The differences in discomfort produced by the three NMES types at 30% MVIC were attributed to differences in how MUs are recruited between mNMES and nNMES. During mNMES, larger electrodes are used than during nNMES and motor axons are distributed throughout the muscle belly, both of which

contribute to the high currents that are required to generate large torque (Adams et al., 1993). These high currents result not only in the activation of motor axons but also nociceptor afferents in skin, musculotendinous and vascular structures (Delitto et al., 1995; Matthews et al., 1997), producing high discomfort. On the other hand, nNMES electrodes are smaller and are located directly over the common peroneal nerve trunk, which has a much smaller diameter than the muscle belly, accordingly all axons are enclosed in a small area. Thus, the current required to produce contractions of 20% MVIC or higher during nNMES was lower than during mNMES, resulting in less discomfort likely due to of the recruitment of fewer nociceptor afferents.

We hypothesized that during the maximal trials iNMES would produce the largest torque and that discomfort would be similar between NMES types, however, our results did not support this hypothesis. During the maximal trials, nNMES generated contractions of 65% MVIC on average, which was 1.9 times larger than mNMES and 1.7 times larger than iNMES. Maximal torque was not only largest during nNMES but the current needed to produce it was lower than mNMES. The lowest maximal torque (33% MVIC) was produced by mNMES which also required the most current and produced the most discomfort. Although reasonably large torque was produced by iNMES (49% MVIC), this torque was likely limited by the high discomfort produced at the $iNMES_{(m)}$ site, since the same current was delivered as during mNMES alone (which produced the highest discomfort). The main reason for the lower discomfort during nNMES during these maximal trials was the amplitude of stimulation used. For 6 participants out of 11, the maximal torque during nNMES was produced at a current that was below that which resulted in maximal discomfort. This effect was attributed to the co-activation of dorsiflexors and evertors reducing the maximal dorsiflexion torque.

Together, the results of the experiments described in Chapter 4 showed that all three types of NMES can produce up to 20% MVIC with no difference in discomfort between NMES types. The largest torque during the maximal trials was produced by nNMES followed by iNMES and mNMES. Torque was not only larger during nNMES but was also produced using lower current and generating less discomfort than mNMES. The maximal torque produced by iNMES was likely limited by the high discomfort produced at the $iNMES_{(m)}$ site.

5.2 Limitations and future directions

5.2.1 Interleaved neuromuscular electrical stimulation: motor unit recruitment overlap

5.2.1.1 Limitations

The technique used to estimate overlap in MU recruitment in the experiments described in Chapter 2 has been used to identify the selectivity of MU recruitment between different pairs of electrodes in an implanted array. Two main factors could potentially affect the overlap estimation when using surface electrodes: 1) improper timing of stimulation between mNMES and nNMES sites will influence twitch amplitude and underestimate overlap, and; 2) the presence of H-reflexes could affect the amplitude and duration of the recorded twitch. When using the overlap estimation technique described in Chapter 2, 100% overlap was indicated if m+nNMES produced the same twitch amplitude as mNMES and nNMES, and no overlap was indicated if m+nNMES was double the amplitude of mNMES and nNMES. Two maximal twitches, one originating from mNMES and one from nNMES, arriving to the muscle at different delays could result in a twitch longer and larger than if both stimuli had arrived to the muscle at the same time. In this case, torque summation would result in an underestimation of overlap. In pilot experiments, two methods were tested to identify the proper delay to ensure that mNMES and nNMES activate the muscle quasi-simultaneously. These methods included adjusting the delay to align the onset or first peak of M-waves from the two Stimulation sites, or to align the onset or first peak of the torque response produced by mNMES and nNMES. Both of these methods resulted in torque summation that was evident when both mNMES and nNMES were set to generate the largest twitch possible. Therefore, the delay was based on the latency that produced no torque summation when mNMES and nNMES were delivered at the amplitude that produced the largest twitch, in other words, the twitch produced by m+nNMES was not larger than the maximal twitch evoked by mNMES of NMES alone. This issue with the timing of the stimulation delivered to each site is a limitation of the method that needs to be considered when using this technique; however, we do not believe that this was a complication throughout the experiments described in Chapter 2.

The pathway involved in generating an electrically-evoked muscle contraction when data are recorded to construct a twitch torque recruitment curve is an important factor to be taken into consideration when identifying overlap. Such twitches can be a consequence of the

activation of motor and/or sensory axons. The activation of both motor and sensory axons would result in two twitches at different latencies (M-wave + H-reflex), increasing the duration and amplitude of the total twitch. To minimize the effect of H-reflexes on the twitch torque characteristics, experiments were performed on the TA, a muscle where the probability of generating H-reflexes is low (Zehr, 2002), and by using a short pulse duration which is known to preferentially recruit motor axons (Brooke et al., 1997; Lagerquist et al., 2009a; Pierrot-Deseilligny et al., 1981; Veale et al., 1973; Zehr, 2002). During our experiments, only small and infrequent H-reflexes were identified. However, future studies using the overlap estimation technique proposed here should be aware of this limitation, especially when muscles such as the TS or quadriceps are tested, since there is a larger probability of generating H-reflexes in these muscles (Zehr, 2002).

5.2.1.2 Future directions

Interleaved NMES can produce functionally significant torque with minimal fatigability when overlap between stimulation sites is low. Future research could explore the effect of stimulation amplitude on fatigability during iNMES. Previous studies showed that fatigability is reduced during iNMES compared to traditional NMES (Lou et al., under review). One stimulation amplitude was used to deliver trains of iNMES in those experiments (~15% MVIC) and in the experiments described in Chapter 2 (~25% MVIC). Future studies addressing this topic could identify the torque produced at multiple stimulation amplitudes during trains of iNMES to test the relationship between overlap and fatigability over a range of stimulation amplitudes. I expect that the larger the overlap, the higher the fatigability during iNMES delivered to the TA. The reduced fatigability resulting from the reduced overlap during iNMES would be of interest if the goal of the program is to produce functional movements for long periods such as during walking or during FES-biking sessions.

5.2.2 Tibialis anterior and triceps surae torque-frequency relationship when electrical stimulation is delivered over the muscle belly, nerve trunk or interleaved between both

5.2.2.1 Limitations

Three limitations of the experiments described in Chapter 3 may have influenced our measurements. The first limitation was that M-waves and H-reflexes were not normalized by the maximal M-wave. This lack of normalisation may have increased the variability in the

response amplitudes across the group when reported in mV, which may have made it more difficult to identify frequency-dependent changes in the amplitudes of M-waves and H-reflexes in the present study. Despite this limitation, I believe the pathways involved in generating the muscle contractions by each type of NMES are still well represented by the data and that our understanding of these pathways would not have changed if EMG data were normalized.

The second limitation was that torque was likely limited during iNMES resulting from H-reflex frequency-dependent depression at the $iNMES_{(n)}$ site. During iNMES, $iNMES_{(m)}$ and $iNMES_{(n)}$ were intentionally manipulated to generate equal torque, where each site generated half the desired torque of 20% MVIC at 20 Hz. However, the torque generated by $iNMES_{(n)}$ was likely influenced by the presence of large H-reflexes at low frequencies. This resulted in a torque steady state at low frequencies during $iNMES_{(n)}$ but not for $iNMES_{(m)}$. Therefore, it is possible that when iNMES was delivered at progressively higher frequencies, the $iNMES_{(n)}$ site was not generating equal torque as the $iNMES_{(m)}$ site, compromising the total torque produced by iNMES.

A third limitation was that contractions generated in the TA were predominantly but not exclusively produced by peripheral pathways. The central contribution to the torque generated by NMES delivered to the TA was represented by the presence of small H-reflexes and extra torque in 3 out of 9 participants. Extra torque is represented by an augmentation in torque throughout the stimulation train with no changes in frequency or stimulation amplitude (Collins et al., 2001; Klakowicz et al., 2006; Lagerquist et al., 2009a). Extra torque has been suggested to be related to the activation of persistent inward currents in motor neurons in the spinal cord and/or post-tetanic potentiation of neurotransmitter release from afferent terminals (Collins et al., 2001). Extra torque was present even though the experiments were performed in the TA and using short pulse duration. Both of these factors likely minimised but did not eliminate the central contribution to the evoked contractions.

5.2.2.2 Future directions

It would be of interest to describe the TFR in the TA and TS muscles over a range of stimulation amplitudes. In the study described in Chapter 3, only one stimulation amplitude was tested (20% MVIC at 20 Hz). Future experiments could be designed to identify the range of stimulation amplitudes at which iNMES would still produce more torque than mNMES and

nNMES when only frequency was manipulated. I expect that, based on the results described on Chapter 2, a progressively larger overlap in the MUs recruited by $iNMES_{(m)}$ and $iNMES_{(n)}$ can be expected with progressive increases in stimulation amplitude, which would result in a progressively smaller differences in the torque produced at high stimulation frequencies by iNMES than mNMES and nNMES. Results from such experiments would help to identify the range of amplitudes over which iNMES has an advantage over mNMES and nNMES in the capacity to generate torque.

It is also presently unclear which frequency of iNMES would reduce fatigability compared to mNMES and nNMES. I expect that fatigability would be lower during iNMES than mNMES and nNMES at the lower range of frequencies due to the lower metabolic demand imposed to each stimulation site; however, torque at the low range of frequencies would be compromised. This information could be used by clinicians/researchers when choosing which NMES method to adopt: iNMES is thought to be more efficient in producing more torque with lower fatigability while using lower net frequencies than mNMES and nNMES, but only at stimulation amplitudes that result in low overlap. If the same MUs were recruited by each stimulation site, there would be no difference in torque and fatigability during mNMES, nNMES and iNMES, independent of frequency.

It was previously shown that H-reflex pathways recruit motor neurons following Henneman's size principle (Buchthal et al., 1970; Trimble et al., 1991). It was also shown that generating contractions through central pathways results in less fatigability than generating contractions via peripheral pathways when recruitment is thought to be random (Bergquist et al., 2014). The effect of central pathways on reducing fatigability during iNMES has not been tested. Theoretically, iNMES would be more resistant to fatigability than mNMES and nNMES as long as each stimulation site recruits different MUs. This resistance to fatigability during iNMES could be further increased if at least one of the stimulation sites recruited MUs through central pathways. This central effect on reduced fatigability would be stronger at low stimulation frequencies where H-reflexes were shown to be larger.

5.2.3 Torque, current, and discomfort during three types of neuromuscular electrical stimulation of tibialis anterior

5.2.3.1 Limitations

The main limitations of the study described in Chapter 4 were that different electrode sizes were used during mNMES and nNMES and that maximal torque was not limited by discomfort in 6 participants but rather it was limited by activation of the evertors. The electrodes used for mNMES were larger than the electrodes used for nNMES. The use of different electrode sizes confounds the comparison between the mNMES and nNMES sites because current and discomfort is known to be influenced by electrode size (Alon, 1985; Forrester et al., 2004; McNeal et al., 1988; Patterson et al., 1991). The choice of using different electrode sizes was based on its ecological validity since the choice was based on the way that mNMES and nNMES are delivered in the clinical setting during FES-cycling or when NMES is used to correct foot drop resulting from central nervous system injuries (Sheffler et al., 2007; Sheffler et al., 2006).

The second main limitation was related to how nNMES over the common peroneal nerve produces contractions which limited the maximal amount of torque that could be produced and led to an underestimation of VAS scores during the nNMES maximal trials. The limitation in torque produced and underestimation of the VAS score resulted from the fact that for 6 out of 11 participants the stimulation amplitude that produced the maximal torque was lower than the amplitude that produced the maximal discomfort. For the other 5 participants the amplitude that produced the highest discomfort also produced the largest torque. Therefore, the group (n=11) VAS scores reported for the nNMES maximal trials did not represent the amplitude where torque is limited by discomfort but they represented the discomfort produced while generating the maximal torque. We could have grouped the results based on stimulation amplitude that produced the highest discomfort during nNMES since these data was also recorded for the 11 participants. This would have resulted in higher VAS scores which would have represented the stimulation amplitude that produced the highest discomfort, and this may have supported our original hypothesis. However, this result would have only indicated that the amplitude that produces the most discomfort is higher than the amplitude that produces the most torque, resulting in an underestimation of the maximal torque that could be produced by nNMES. Both results have clinical significance, but we

opted to describe our group results (n=11) using the first method to be able to identify the maximal torque that could be generated and only describing the amplitude that produced the maximal discomfort for the 6 participants. This choice was made mainly because the amplitude to produce the maximal discomfort was higher than the amplitude to produce the maximal torque.

5.2.3.2 Future directions

Future experiments could test whether using electrodes of same size for mNMES and nNMES would have altered the results reported in Chapter 4, as this would provide a clearer idea of whether it is electrode size or stimulation site that is responsible for the differences in discomfort between mNMES and nNMES. I expect that lower maximal torque would be identified if the large electrodes that were used for mNMES were also used for nNMES. The reduced torque in this scenario would be a consequence of a larger activation of evertors (resulting in plantarflexion torque) since these electrodes would be positioned partially over the muscle belly and nerve branches of the peroneus longus muscle. I also expect that, electrodes of same sizes positioned over the muscle belly or the nerve trunk and stimulated using the same current would produce similar discomfort since both would likely recruit similar numbers of nociceptor afferents by covering the same skin areas. On the other hand, electrodes of same sizes positioned over the muscle belly or the nerve trunk and stimulated to generate a similar torque, a comparison that is more relevant between NMES types than current, would generate more discomfort during mNMES than nNMES. Thus, I believe that stimulation site is more important than electrode size in producing discomfort during NMES of the dorsiflexors.

5.3 Clinical implications

In the previous sections, I discussed the main results, limitations and future directions for each of the three experimental chapters separately. The purpose of this section is to integrate the results of these three chapters and provide suggestions for ways to overcome challenges inherent to the use of NMES, such as discomfort and fatigability.

5.3.1 mNMES

The main advantages of mNMES are: 1) it is easy to use in the clinical setting since the bellies of superficial muscles are easily identifiable; 2) "pure" torque can be generated by carefully positioning the stimulating electrodes only over the target muscle; 3) contractions are driven primarily by M-waves resulting in more consistent torque than contractions driven mainly through central pathways (Baldwin et al., 2006; Bergquist et al., 2012b) and 4) mNMES is a good method to produce at low torque amplitudes with low discomfort. However, disadvantages of mNMES include: 1) maximal dorsiflexion torque is limited to about 30-40% MVIC by discomfort; and 2) the spatial recruitment of MUs is more limited than during nNMES and iNMES.

The mNMES method has proven to be effective for rehabilitation but further research is necessary to improve its efficacy and overcome the challenges proposed earlier. I suggest that mNMES is the best method to be used if the training program requires an easy method of stimulation to generate low and consistent torque, for short periods of time, such as, when the goal of NMES is to produce flexion and extension finger movements for grasping (Prochazka et al., 1997). The main limitations of mNMES delivered to the TA are that discomfort limits the production of large torque and that single channel mNMES results in rapid fatigability. Discomfort, however, may not be a problem for people with reduced or no sensation, for example, patients with incomplete or complete spinal cord injury.

Reducing fatigability during mNMES has been one of the goals of the development of sequential NMES, where multiple channels of mNMES are used over the same muscle belly (Downey et al., 2014; Nguyen et al., 2011; Popovic et al., 2009; Sayenko et al., 2014a; Sayenko et al., 2014b; Sayenko et al., 2013). The hardware necessary to deliver sequential NMES is still restricted to the laboratory environment but this method shows potential to reduce the rapid fatigability intrinsic to NMES (Downey et al., 2014; Nguyen et al., 2011; Popovic et al., 2009; Sayenko et al., 2014a; Sayenko et al., 2014b; Sayenko et al., 2013). The reduced fatigability associated with sequential NMES was suggested to be due to the recruitment of different MUs by each pair of electrodes, similar to the results reported for iNMES (Chapters 2 and 3), but the extent of overlap between MUs recruited by the different sequential NMES electrode pairs has not been tested. Sequential NMES could be improved by using the overlap estimation technique to increase its efficiency and reduce fatigability by

optimizing electrode location based on the location that resulted in lowest overlap. The relation between electrode size and overlap could be also investigated to improve the use of sequential NMES in muscles with different sizes. I expect that the closer proximity between electrodes, the larger the overlap and the lower the advantage of sequential NMES over traditional single channel mNMES. Discomfort during sequential NMES has yet to be tested, but I expect that sequential NMES would produce less discomfort than mNMES to generate a similar torque. The lower discomfort would be a result of the lower current necessary to generate a given torque during sequential NMES compared to mNMES, similar to the results reported in Chapters 3 and 4 for iNMES. Therefore, traditional mNMES could be improved by further developing the sequential NMES method since it has the same advantages of traditional mNMES related to higher resistance to fatigability, and potentially lower discomfort.

5.3.2 nNMES

The main advantages of nNMES are: 1) torque amplitudes of up to 65% MVIC can be generated using much lower current than during mNMES; 2) it produces less discomfort than mNMES when producing relatively large torque; 3) it can recruit all the MUs innervating a given muscle since their axons are grouped inside the nerve trunk; and 4) it has the potential to drive muscle contractions through central pathways (Chapter 3; Appendix A), which, improves resistance to fatigability (Bergquist et al., 2014). Torque produced by nNMES can be limited by: 1) increased homosynaptic depression with increase in stimulation frequency, when contractions are driven by central pathways; and 2) the activation of muscles other than the target muscle, compromising the specificity of muscle activation, such as the concomitant activation of evertors and dorsiflexors when nNMES is delivered to the common peroneal nerve.

The nNMES method has also been used in clinical settings for many years, but to a lesser extent than mNMES mainly due to difficulty in placement of the electrodes over the nerve trunk or inconsistencies in torque resulting from electrode movement (Bergquist et al., 2012b). Despite these issues, our results showed that nNMES has the potential to overcome some of the limitations of using NMES for rehabilitation. More torque can be generated by nNMES than mNMES and iNMES using less battery power while producing less discomfort. Rapid fatigability can be reduced if central pathways are involved in generating contractions

during nNMES. The nNMES efficiency in producing torque can be compromised by the recruitment of MUs other than the ones innervating the target muscle which will compromise the ability to produce the desired torque. The recruitment of the same MUs over time can also reduce the efficiency of nNMES in reducing fatigability, mainly when contractions are driven by peripheral pathways. Even though contractions generated by central pathways have higher resistance to fatigability than those produced through peripheral pathways, the resulting torque is less consistent (Baldwin et al., 2006). Aside from these limitations, the large torque generated by nNMES can be used during long-term training protocols designed to produce muscle adaptations similar to the ones produced by strength training where high amplitude/load is used for short bouts of exercise. This type of training was shown to increase muscle mass (Gerovasili et al., 2009; Sillen et al., 2013), cardiovascular capacity (Deley et al., 2015; Lake, 1992; Sillen et al., 2013) and bone mineral density (Belanger et al., 2000; Deley et al., 2015) which are all diminished with disuse resulting from paralysis (Deley et al., 2015). Clinicians and researchers should consider the use of nNMES over mNMES or iNMES if the goal is to generate the largest torque possible, reduce the battery power, and/or to reduce the discomfort inherent to NMES when producing large torque.

5.3.3 iNMES

Based on the results reported on this thesis, iNMES is the most advantageous of the three tested method since: 1) different MUs are recruited from the $iNMES_{(m)}$ and $iNMES_{(n)}$ sites reducing by half the discharge rate on the MU recruited in each site; 2) in the TA and TS muscles it produces more torque at a given frequency than mNMES and nNMES, but only if the overlap in MU recruitment between stimulation sites is low; and 3) it can generate contractions at least partially driven by central pathways in the TS muscle. However, contractions induced by iNMES are affected by the same limitations described for mNMES and nNMES: 1) contractions generated by central pathways may be affected by homosynaptic depression with increases in stimulation frequency resulting in no further increases in torque; 2) torque is limited by the discomfort produced by the $iNMES_{(m)}$ site in the TA muscle; and 3) eversion can be caused by the $iNMES_{(n)}$ site in the TA muscle.

The iNMES method is suitable to be used in clinics although user-friendly hardware still has to be developed. Three main lines of research could be pursued to determine the extent to which iNMES will be effective in overcoming the main challenges intrinsic to NMES. The

first line of research would be to test whether iNMES has any advantage in reducing fatigability compared to mNMES or nNMES in a population with neuromuscular impairments, where central and peripheral pathways may have different contributions to the torque generated by each NMES site. The second line of research would be to test whether iNMES can generate appropriate volumes of overload (torque) to cause improvements in muscle or bone after a chronic spinal cord injury. The third would be to integrate iNMES into functional electrical stimulation systems to identify the reliability of the method in generating consistent contractions with low discomfort during exercises such as biking or rowing.

Initial results show that iNMES, similar to sequential NMES, can reduce the rapid fatigability associated with NMES by recruiting different MUs from each NMES site. This reduces the firing rate of the recruited MUs, without compromising torque. However, iNMES produces more discomfort and less maximal torque than nNMES. Interleaved NMES should be used if the goal of a rehabilitation program is to produce adaptations similar to endurance training where exercises are performed at low to medium amplitudes/loads for long bouts of exercise. The iNMES method has also the potential to be more suitable if the goal is to increase the distance of walking generated by NMES systems or to increase the duration of NMES-cycling or rowing sessions.

A hybrid of iNMES and sequential NMES that involves rotating stimulus pulses between electrodes over the muscle belly and the nerve trunk could further increase resistance to fatigability and reduce discomfort without compromising torque. The main advantage of this integration would be that, the activation of MUs in the nerve trunk allows the recruitment of MUs located not only in the surface of the muscle, such as during mNMES, but also MUs located in deeper portions. This would likely result in: 1) more torque at lower current when compared to NMES delivered only by electrodes located over the muscle belly (sequential NMES and the $iNMES_{(m)}$ site), and 2) the recruitment of an extra group of MUs by the nNMES site, in addition to MUs recruited by each mNMES channel, reducing fatigability. Moreover, stimulation delivered to the nNMES site can increase the probability of recruiting MUs through central pathways which favours the recruitment of MUs following Henneman's size principle, further reducing fatigability. I expect that a combined interleaved/sequential NMES method would be the ideal method to overcome fatigability and discomfort while

producing torque that has functional significance, especially to produce medium range torque amplitudes necessary to generate walking, standing or rowing.

5.4 Summary

The results of the experiments described in this thesis contribute to a better understanding about how iNMES may be beneficial for rehabilitation. Utilizing different measures, mNMES, nNMES and iNMES delivered to lower leg muscles were characterized and compared. The results of this thesis provide evidence that mNMES is effective for producing low torque with low discomfort and it is the easiest method to use in the clinic. However, at least for TA, the maximal torque produced during mNMES is limited by discomfort. The largest torque was produced by nNMES, with the lowest current and discomfort. However, the torque produced by nNMES may have been limited homosynaptic depression when contractions were generated by central pathways in the TS muscle and plantarflexion/eversion torque when delivered to the TA muscle. The iNMES method was shown to be the most advantageous to be used for rehabilitation. The iNMES method can produce a wide range of torque amplitudes while recruiting different MUs from each NMES site resulting in the need for lower net frequencies to generate similar torque as mNMES and nNMES. However, the capacity to generate large torque during iNMES is limited by the discomfort produced at the $iNMES_{(m)}$ site in the TA and by homosynaptic depression when the $iNMES_{(n)}$ site drives contractions through central pathways in the TS muscle. The results of the experiments described in this thesis addressed some of the gaps in knowledge about the use of iNMES for rehabilitation. In conclusion, iNMES can reduce MU discharge rate without compromising the capacity to generate functionally significant torque. These characteristics of iNMES make it a valuable new option for restoring movement and/or reducing the secondary complications of paralysis.

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APPENDIX A - USING NMES TO GENERATE CONTRACTIONS THROUGH CENTRAL PATHWAYS: EFFECT OF STIMULATING ELECTRODE POSITION AND MUSCLE LENGTH

A.1 Introduction

It is well-known that neuromuscular electrical stimulation (NMES) can generate contractions by depolarising motor axons beneath the stimulating electrodes, thereby generating contractions mainly through a peripheral pathway (Maffiuletti, 2010). In recent years, we have shown that NMES can also generate contractions through central pathways by depolarising sensory axons and recruiting motor units (MUs) via reflex pathways through the spinal cord (Bergquist et al., 2011b; Collins, 2007). This central contribution to the evoked torque can be augmented when stimulation at 20 Hz is interspersed with a brief "burst" of stimulation at 100 Hz using long pulse durations of 1 ms (Collins et al., 2001; Klakowicz et al., 2006; Lagerquist et al., 2009a), resulting in what we have termed "*extra torque*" (Collins et al., 2001). The extra torque is caused by the augmentation of the central contribution and can result in up to a 4-fold increase in torque (Collins, 2007), after the brief burst of high-frequency wide-pulse stimulation. The central origin for this extra torque has been confirmed by the findings that it is accompanied by enhanced H-reflexes (Bergquist et al., 2011a; Klakowicz et al., 2006) and electromyographic (EMG) activity that is "asynchronous" from the stimulus pulses (Bergquist et al., 2011a) and was abolished when the nerve to the muscle was blocked with anesthetic (Collins et al., 2001; Lagerquist et al., 2009a).

In marked contrast to our work, Frigon et al. (2011) found no differences in the extra torque generated by the ankle plantarflexor muscles when burst-like patterns of NMES were delivered before and after a peripheral nerve block. This result suggested no involvement of central pathways on extra torque generation. The authors also reported increased extra torque when the muscle was shortened which led to the idea that extra torque was due to intrinsic properties of the muscle such as staircase (Rassier et al., 2002) or doublet potentiation (Binder-Macleod et al., 1989; Burke et al., 1970). However, EMG was not recorded and the extra torque reported was not larger than 50% (estimated).

What is presently unclear is why we routinely generate large extra torque with a demonstrable central contribution and Frigon et al. (2011) did not. It is possible that the

differences in the protocols used by Frigon et al. and our own account for the differences between the results obtained between the two groups. The main difference between protocols is that Frigon et al. (2011) placed the stimulation electrodes over the muscle belly of the triceps surae (mNMES) while we showed larger central contribution when the stimulation electrodes were placed over the tibial nerve trunk (nNMES) (Bergquist et al., 2011a; Klakowicz et al., 2006; Lagerquist et al., 2009a), which increases the probability of activation of sensory axons (central contribution). Another factor that may be influencing the differences in the results are the differences between responders and non-responders, which has been addressed recently (Wegrzyk et al., 2015a; Wegrzyk et al., 2015b).

Identifying the origin of these differences may clarify the factors that contribute to extra torque generation. Thus, the aim of the present study was to compare extra torque generated by mNMES (Frigon et al., 2011) and nNMES (Bergquist et al., 2011a; Klakowicz et al., 2006; Lagerquist et al., 2009a) and the effects of muscle length. Based on Frigon's original hypothesis we expect to see larger extra torque when the muscle is lengthened due to persistent inward current activation (Hyngstrom et al., 2007). Larger M-waves are expected during mNMES compared to nNMES and larger H-reflexes during nNMES when compared to mNMES (Bergquist et al., 2011a). Larger extra torque is expected during nNMES when compared to mNMES, mainly resulting from a larger contribution of central pathways.

A.2 Methods

A.2.1 Participants

Data from 13 participants (4 females, 9 males; age range: 18-50 years) was collected in one experimental session (~2 h). The experimental protocol was approved by the Human Research Ethics Board at the University of Alberta in accordance with the Declaration of Helsinki.

A.2.2 Experimental procedure

A Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, NY) was used to measure isometric plantarflexion torque during maximal voluntary isometric contractions (MVICs) and NMES with the hip at ~90°, right knee at 170°-180° and ankle at 90° (A90; TS lengthened) and 120° (A120; TS shortened) (Frigon et al., 2011). The neutral position of the

ankle was at 90º. The axis of the dynamometer was aligned with the axis of rotation of the right ankle joint. Foot and leg were firmly secured using Velcro straps.

A.2.3 NMES protocol

Stimulation was delivered or over the muscle belly (mNMES) of the TS or over the tibial nerve (nNMES) using a constant-current stimulator and 1-ms rectangular pulses (DS7A Digitimer, Welwyn Garden City, UK). A current probe (mA 2000 Non-contact Milliammeter; Bell Technologies, Orlando, FL) was used to measure the stimulation current.

During mNMES the stimulation electrodes (7.5 x 13 cm; model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) were placed proximally over the largest circumference of the gastrocnemius muscles (anode) and distally over the soleus muscle (cathode) just distal to the gastrocnemius (Bergquist et al., 2011a; Frigon et al., 2011). nNMES was delivered through two adhesive gel electrodes (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) placed on the skin of the popliteal fossa over the tibial nerve with an interelectrode distance of 1 cm.

A.2.4 EMG

Electromyography was recorded from the soleus using bipolar surface electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT). Electrodes were placed 1 cm apart, parallel to the predicted direction of the muscle fibres. A reference electrode was placed over the tibia. The EMG signals were amplified 500 times and band-pass filtered at 10-1,000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

A.2.5 Experimental protocol

Initially, each participant performed three maximal voluntary isometric contractions (MVICs), 2 min apart, and at each of the 90 and 120 degrees ankle positions.

Trains of NMES consisted of 20-100-20 Hz for 3-2-3 s. Three trains were delivered 60 s apart in both ankle positions and with each NMES type. The stimulation amplitude was set based on the torque produced by "test" stimulation trains (Frigon et al., 2011). The test train consisted of 5 pulses at 100 Hz using between 50-100% of the maximal output of the stimulator (100 mA) to generate the largest torque possible. Stimulation amplitude during the first 3 s of the NMES train was set to generate 10-15% of the torque produced by the test train.

This stimulation amplitude resulted in contractions averaging 4% MVIC at the first 3 s of stimulation.

A.2.6 Data acquisition and analysis

Data were acquired at 5 KHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for post-hoc analysis using custom-written Matlab software (The Mathworks, Natick, MA). Torque during MVICs was quantified by positioning a 500-ms average calculation window over the area where the peak torque was identified. Torque generated during the trains of NMES was normalized by the torque evoked during plantarflexion MVIC performed in both ankle positions. Torque produced by NMES when the ankle was at A90 was normalized by the MVIC recorded at A90 and torque produced by NMES when the ankle was at A120 was normalized by the MVIC recorded at A120. Torque was averaged over each of two time periods (T_1: 2 - 3 s into the NMES; T_2: 7 - 8 s into the NMES) during a single NMES train. Subsequently the average of each time period was calculated across the three trains of stimulation. Extra torque was calculated as the percent of change from T_1 to T_2 [(torque evoked during T_2 / torque evoked during T_1) × 100] (Frigon et al., 2011) and also as a significant increase from T_1 to T_2 (Bergquist et al., 2011a).

The M-waves and H-reflexes were recorded during the trains of NMES. These EMG data were normalized by the largest M-wave (M_{max}) recorded during a recruitment curve protocol (50 nNMES pulses delivered 8 to 10 seconds apart with progressive increases on stimulation amplitude) (Bergquist et al., 2011a; Bergquist et al., 2012b). The same windows that were used to analyse torque were also used to analyze EMG data. Contamination of the M-waves by the stimulation artifact was removed using a two-step software-based signal processing procedure (O'Keeffe et al., 2001). Contamination of the H-reflexes by the preceding M-wave was removed using a four-step software-based signal processing procedure (Lang et al., 1967). Both protocols were described in a previous study (Bergquist et al., 2012b).

All statistical analyses were performed on group data using Statistica software (StatSoft, Tulsa, OK). The normal distribution of the data was confirmed using the Shapiro-Wilk test. Repeated measure analyses of variance (rmANOVA) tests were used to compare Torque and EMG (M-waves and H-reflexes) data taking in consideration the factors *Time* (2 levels: T_1 and T_2), *Muscle length* (A90 and A120) and *NMES type* (mNMES and nNMES). Tukey post-hoc

tests were used when appropriate. EMG data from mNMES and nNMES, or M-waves and H-reflexes, were not compared since the 2 NMES types contribute differently to the EMG signal due to the recruitment of MU distributed in different locations of the muscle (Okuma et al., 2013; Vanderthommen et al., 2000). The level of significance was set at 0.05. All data are reported as mean ± standard deviation (SD).

A.3 Results

A.3.1 Extra Torque

There was no difference in the torque produced during the first 2-3 s of stimulation (T_1) between ankle positions or NMES types [$p<0.05$]. Extra torque was compared in two ways: as the significant difference from T_1 to T_2 and as percent of increase from T_1 to T_2. These results are displayed on A-1.

A.3.1.1 T_1 vs. T_2

Using this comparison, significant extra torque was only generated when the TS was lengthened. There was an interaction between *Time* and *Muscle length* [$F_{(1,12)}=5.64$, $p=.035$, observed power=0.58]; torque was larger at T_2 compared to T_1 only when the ankle was at 90 degrees [$p=0.001$]. No other main effects or interactions were identified [$p>0.05$] (Figure A-1A).

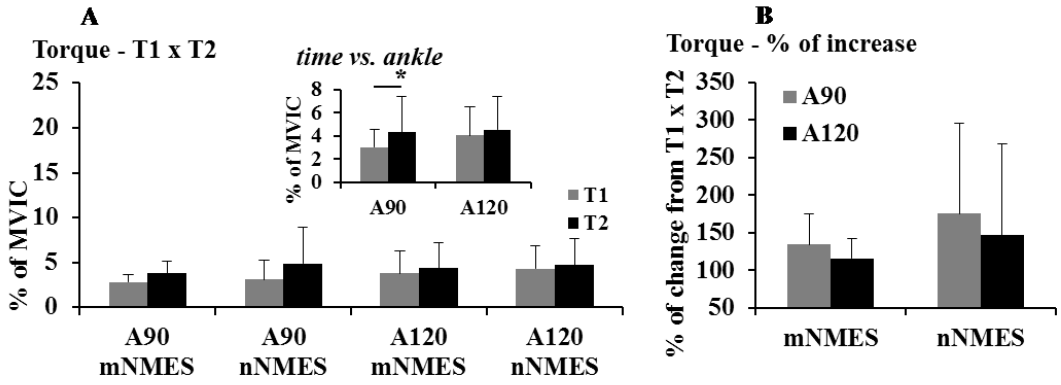

Figure A-1. Group torque data representing the percent of MVIC torque in T_1 and T_2 **(A)** and percent of change in torque **(B)**. ***A.*** Torque normalized by the MVIC and reported for T_1 and T_2. Significant interactions identified by the rmANOVA are displayed within *insets*. ***B.*** Percent of change in torque during mNMES and nNMES while the TS muscle was lengthened (A90) or shortened (A120). * $p<0.05$.

A.3.1.2 Percent of increase from T_1 to T_2

When extra torque was expressed as percent of change from T_1 to T_2 no main effect or interactions were identified [p>0.05]. (Figure A-1B).

A.3.2 M-waves

The amplitude of M-waves was analyzed as the difference from T_1 to T_2. There was an interaction between *Muscle length* and *NMES type* [$F_{(1,12)}$=14.94, p=0.002, observed power=0.94]; M-waves generated by mNMES were larger when TS was shortened compared to lengthened [p<0.001]. There were no other significant main effects or interactions [p>0.05] (Figure A-2).

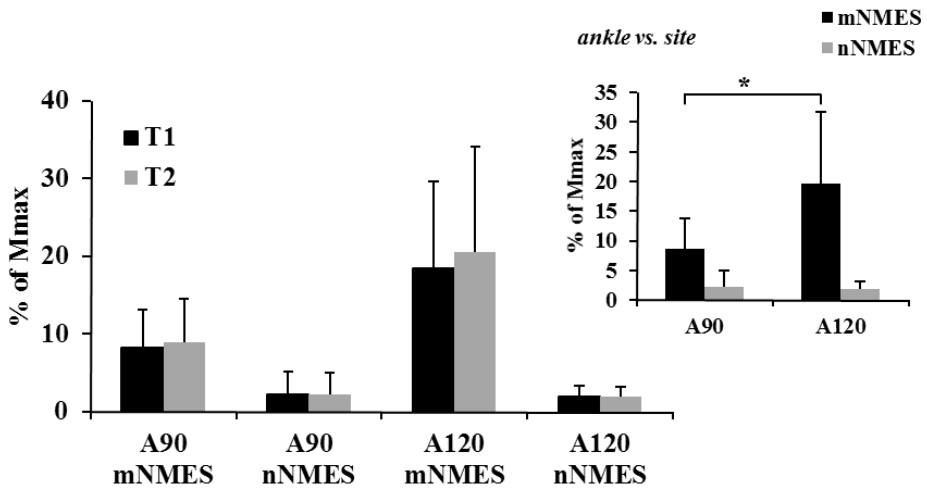

Figure A-2. M-waves recorded from the soleus muscle. M-waves were normalized using the largest M-wave (M_{max}) identified during the recruitment curve. mNMES and nNMES were compared with the TS lengthened (A90) and shortened (A120). Significant interactions identified by the rmANOVA are displayed within *insets*. * p<0.05.

A.3.3 H-reflexes

The amplitude of H-reflexes were compared between T_1 and T_2. An interaction was identified between *Time* and *NMES type* [$F_{(1,12)}$=6.46, p=0.02, observed power=0.64]. The H-reflexes generated during nNMES were larger at T_2 compared to T_1 [p=0.001]. No other significant main effects or interactions were identified [p<0.05] (Figure A-3).

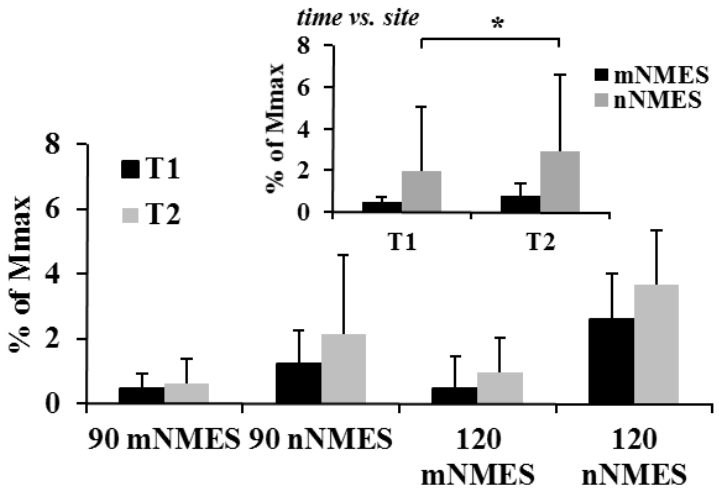

Figure A-3. H-reflexes recorded from the soleus muscle. H-reflexes were normalized using the largest M-wave (M_{max}) identified during the recruitment curve. mNMES and nNMES were compared with the TS lengthened (A90) and shortened (A120). Significant interactions identified by the rmANOVA are displayed within *insets*. * $p<0.05$.

A.4 Discussion

The aim of this study was to compare extra torque generated by mNMES and nNMES when the muscle was positioned at different lengths. Extra torque was only identified when the TS was lengthened and there was no effect of NMES type. Muscle contractions were generated mainly through peripheral pathways (M-waves) during mNMES and through central pathways (H-reflexes) during nNMES.

An important concept that requires clarification is the definition of "*extra torque*". Frigon et al. (2011) defined extra torque as "*forces or torques that are larger than what would be expected from the input or stimuli*". Extra torque in our previous studies resulted in increases in torque from 50-412% in the TS (Bax et al., 2005; Bergquist et al., 2011a; Collins et al., 2001, 2002; Klakowicz et al., 2006). However, Frigon et al. (2011) reported much smaller increases in torque ranging from -5% (TS shortened) to 50% (TS lengthened), where --5% represents a small decrease in the torque generated at T_1 compared to T_2, which does not fit the definition of extra torque. These results suggest that the phenomenon that we described as extra torque is different than the one described by Frigon et al. (2011).

Our first hypothesis was that lengthened muscles would produce larger extra torque due to plateau-like behavior of motor neurons (Hyngstrom et al., 2007). Frigon et al. (2011) rejected this hypothesis showing that larger extra torque was produced when the TS was in a short length attributing this behavior to intrinsic properties of the muscle such as post tetanic potentiation, staircase and doublet potentiation phenomena (Rassier, 2000; Rassier et al., 2002; Sandercock et al., 1997). We could not replicate Frigon et al. (2011) since we did not identify an effect of muscle length on extra torque generation represented as percent of increase. We only identified extra torque when the muscle was lengthened but only when reported as a significant change in torque from T_1 to T_2, confirming our first hypothesis. The reasons for the differences between our present results and those of Frigon et al. (2011) are unclear, since we carefully controlled variables such as electrode position and joint angles.

One of the goals of this study was to contribute to our understanding regarding how central pathways contribute to extra torque. Our second hypothesis was based on previous studies which showed that M-waves were larger during mNMES and H-reflexes were larger during nNMES (Bergquist et al., 2011a). The results from this study confirmed our second hypothesis as larger M-waves (peripheral pathway) were identified during mNMES and larger H-reflexes (central pathway) were identified during nNMES. Also, H-reflexes generated by nNMES were larger at T_2 compared to T_1, suggesting a central upregulation of H-reflexes as a result of the introduction of a burst of high-frequency wide-pulse stimulation. Our third hypothesis stated that extra torque would be larger during nNMES than mNMES due to the larger central contribution to torque generation. We rejected this hypothesis since the amount of torque generated by mNMES and nNMES was the same before and after the bursts of high frequency stimulation, even though the level of central contribution was higher (larger H-reflexes) during nNMES compared to mNMES. Our results suggest that the magnitude of the increase in torque recorded in our study was similar to that shown in the Frigon et al. (2011) study. Therefore, the increase in torque reported here and by Frigon et al. (2011) is a consequence of intrinsic properties of the muscle and not from central pathways.

It is still not clear the reason why we were not able to replicate Frigon et al. (2011) results or produce the amount of extra torque previously reported in the literature. One of the main candidates is the fact that participants respond differently to NMES. Previous studies from our lab suggested that extra torque was seen in 85-100% of the participants when small

sample sizes were tested. This view was challenged by a recent study using a large cohort (n=42) showing that only 60% of the participants were responders (Wegrzyk et al., 2015b). These authors also introduced a robust method to identify responders and non-responders. One of the characteristics of the responder group was that H-reflexes were depressed after the end of the wide-pulse high frequency (100 Hz) stimulation trains, suggesting a larger contribution of spinal mechanisms in the torque generated by the responders group. The extra torque generated by the responders group was around 3-fold, confirming previous results (Bax et al., 2005; Bergquist et al., 2011a; Collins et al., 2001, 2002; Klakowicz et al., 2006). The H-reflexes and torque were unchanged before and after high frequency trains in the non-responder group. Based on these results from Wegrzyk et al. (2015b), we could assume that participants from our study as well as the participants from Frigon's study were non-responders, explaining the prevalence of intrinsic properties of the muscle mediating the small extra torque produced. Future studies should use this method to address the problem of identifying responders and non-responders when aiming to clarify the effect of NMES type and muscle length on extra torque generation.

A.5 Conclusion

In this study we described the differences of the phenomenon described as extra torque by our group and other researches (Frigon et al., 2011). We showed that intrinsic properties of the muscle are part of the extra torque generation that are strongly present during muscle belly stimulation (mNMES). There was an increase in the central contribution when the nerve trunk was stimulated (nNMES) as the increase on H-reflex amplitude demonstrated. However, this increase in central contribution did not result in differences in extra torque during nNMES compared to mNMES. It was inferred that most of the participants were non-responders to extra torque generation explaining the low extra torque recorded, even when H-reflexes were present. Further research is needed to identify subjects that are more responsive to this phenomenon since a larger muscle contraction with the use of less current of stimulation is beneficial for rehabilitation purposes.

A.6 References

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