Does stimulus pulse width influence contraction fatigability during neuromuscular electrical stimulation?

by

Meng Chen

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Faculty of Kinesiology, Sport, and Recreation

University of Alberta

ABSTRACT

Neuromuscular electrical stimulation (NMES) is used to generate contractions of muscles for rehabilitation after injury or disease to restore functional movements or reduce secondary complications of disuse. However, due to the unnaturally high discharge rates and random recruitment order of motor units (MUs) during conventional NMES ($NMES_{CON}$), contraction fatigability, which is the significant decline in torque over time, occurs rapidly, limiting the duration and intensity of NMES-based programs. It has been reported that sequential NMES ($NMES_{SEQ}$) can produce less contraction fatigability than $NMES_{CON}$ by reducing MU discharge rates. It has also been suggested that delivering NMES using relatively long pulse widths reduces contraction fatigability by recruiting MUs in their natural order via central pathways through the spinal cord. Therefore, the present study explored the effect of pulse width on contraction fatigability of the quadriceps muscles during both $NMES_{SEQ}$ and $NMES_{CON}$. Twelve participants (6 males and 6 females, age: 30.8 ± 11.7) with no known neurological or musculoskeletal injury volunteered for the project and took part in 6 sessions. In the different sessions, $NMES_{SEQ}$ was delivered using a 0.2, 0.5, 1, or 2 ms pulse width and $NMES_{CON}$ was delivered using a 0.2 or 1 ms pulse width. Each session was separated by a minimum of 48 hours. During each experimental session, three different NMES trains were delivered before the fatigue protocol as an assessment of contribution of central pathways to contractions. Then a fatigue protocol was delivered which consisted of 100 contractions (1 s on/1 s off) generated by NMES delivered at 40 Hz. Current was adjusted to generate contractions of 20 % maximum voluntary isometric contraction (MVIC) at the beginning of each fatigue protocol. The contribution of central pathways was quantified as the increase in torque over the course of each type of NMES train.

Contraction fatigability was quantified as the percent decline in torque from the beginning to the end of the fatigue protocols. Pulse width did not affect how torque changed during NMES trains ($p > 0.05$) and we found little evidence of a central contribution to contractions. Contraction fatigability was not different between pulse widths ($p > 0.05$). However, $NMES_{SEQ}$ produced less contraction fatigability (26 ± 13 %) than $NMES_{CON}$ (43 ± 9 %) ($p < 0.001$). Our results suggest that both NMES delivered over the quadriceps muscles, regardless of pulse width or NMES type, generated contractions predominantly by peripheral pathways, and thus pulse width did not influence contraction fatigability. However, $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$. These findings reaffirm that using $NMES_{SEQ}$ would increase the benefits of NMES-rehabilitation programs by reducing contraction fatigability.

PREFACE

This thesis is an original work by Meng Chen. The research project described here has received approval from the University of Alberta Research Ethics Board, project name “Influence of stimulus pulse duration on contraction fatigue of the knee extensor muscles” (No. Pro00081543).

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Dr. David Collins for opening a new and interesting research area for me, supporting me throughout my masters and making my master journey so enjoyable. Without his guidance and patience, I would not have been able to make this thesis possible.

I would like to thank my supervisor committee, Dr. Kelvin Jones and Dr. Vivian Mushahwar, for their valuable time and advice on this thesis, as well as for their kind encouragement.

I would also like to thank all the members of the Human Neurophysiology Laboratory, Alex, Megan, Joao, Brendan, Monika, and David for encouraging me, sharing their experiences to me, and bringing joys to my life.

I would like to thank my friends, Shiyu, Yuzi, and Zhuoxiu for accompanying me to get through all the difficulties and for coloring my life.

Finally, I would like to thank my family for always backing me up. It is the luckiest thing for me to be a member of this lovely family.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

NMES delivered at 40 Hz. Current was adjusted to generated contractions that produced 20 % MVIC. Discomfort was rated by participants after the 5^{th}, 50^{th}, 95^{th} contraction using a visual analog scale (VAS). Participants performed an additional MVIC after the fatigue phase.

Figure 2-3. Torque recorded from a single participant during NMES trains used to assess the "central contribution" during $NMES_{SEQ}$ and $NMES_{CON}$. The left side of each panel displays the torque produced by the three NMES trains when $NMES_{SEQ}$ and $NMES_{CON}$ were delivered using different pulse widths. Dashed boxes indicate the regions used to compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque between these time points for each type of NMES train.

Figure 2-4. Torque recorded from a single participant during each fatigue protocol. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and Panel B displays the percent decline in torque from the first 10 contractions to the last 10 contractions. These data were recorded from the same participant as the data shown in Figure 2-3.

Figure 2-5. Current, charge, and discomfort when $NMES_{SEQ}$ was delivered using 4 pulse widths for the group of 12 participants. Panel A and B display current and charge delivered to generate the contractions for the fatigue protocols, respectively. Panel C displays the average VAS scores during the fatigue protocols. ### denotes $P < 0.001$ and * denotes $P < 0.05$. Dots represent data from single participants.

Figure 2-6. Torque averaged across the group during NMES trains used to assess the effect of pulse width on the "central contribution" during $NMES_{SEQ}$. The left side of each panel displays the torque produced by the three NMES trains averaged across participants (n=12) when $NMES_{SEQ}$ was delivered using four pulse widths. Dashed boxes indicate the regions used to

compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque from the beginning to the end of each type of NMES train. Dots represent data from single participants.

Figure 2-7. Torque recorded during the fatigue protocols delivered using $NMES_{SEQ}$ for the group of 12 participants. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and averaged across participants. Panel B displays the percent decline in torque from the beginning (first 10 contractions) to the end (last 10 contractions) of the fatigue protocols. Dots represent data from single participants.

Figure 2-8. Current, charge, and discomfort during $NMES_{SEQ}$ and $NMES_{CON}$ for the group of 12 participants. Panel A and B display current and charge delivered during the fatigue protocols, respectively. Panel C displays the average VAS scores during the fatigue protocols. * denotes $P < 0.05$ and ### denotes $P < 0.001$. Dots represent data from single participants.

Figure 2-9. Torque averaged across the group during NMES trains used to assess the "central contribution" during $NMES_{SEQ}$ and $NMES_{CON}$. The left side of each panel displays the torque produced by the three NMES trains averaged across participants when $NMES_{SEQ}$ and $NMES_{CON}$ were delivered using two pulse widths. Dashed boxes indicate the regions used to compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque from the beginning to the end of each type of NMES train. * denotes $P < 0.05$ and ### denotes $P < 0.001$. Dots represent data from single participants.

Figure 2-10. Torque recorded during the fatigue protocols delivered using $NMES_{SEQ}$ and $NMES_{CON}$ for the group of 12 participants. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and averaged across participants. Panel B displays the percent decline in torque from the beginning (first 10 contractions) to the end (last

10 contractions) of the fatigue protocols. ### denotes $P < 0.001$. Dots represent data from single participants.

CHAPTER 3

Figure 3-1. Torque generated by the triceps surae muscle recorded from one participant before and during the tibial nerve was blocked. The constant frequency used was 100 Hz (Collins et al. 2001).

LIST OF ABBREVIATIONS

NMES	neuromuscular electrical stimulation
SCI	spinal cord injury
MUs	motor units
$NMES_{SEQ}$	sequential neuromuscular electrical stimulation
$NMES_{CON}$	conventional neuromuscular electrical stimulation
FES	functional electrical stimulation
ACh	acetylcholine
MVIC	maximum voluntary isometric contraction
T_{SD}	strength-duration time constant
ATP	adenosine triphosphate
NTF	neuromuscular transmission failure
iNMES	interleaved NMES
nNMES	nerve stimulation
mNMES	muscle stimulation
VAS	visual analog scale
ANOVA	analysis of variance
EMG	electromyography

CHAPTER 1: GENERAL INTRODUCTION

1.1 Preface

Neuromuscular electrical stimulation (NMES) is used to generate contractions for training in non-injured individuals (Crognale, Crowe, Devito, Minogue, & Caulfield, 2009) and for rehabilitation after injury or disease (Hamid & Hayek, 2008; Sheffler & Chae, 2007). For individuals with spinal cord injury (SCI), NMES is used to restore functional movements and reduce secondary complications. However, these benefits are limited by contraction fatigability that can be measured as a significant decline of torque generated by the muscle over time (Binder-Macleod & Snyder-Mackler, 1993). It is generally thought that two factors influence contraction fatigability during NMES: one is high discharge rates of motor units (MUs), the other is random recruitment of MUs. My thesis work tested an approach that addresses both these issues. Sequential NMES ($NMES_{SEQ}$), first used on human muscles by Pournezam et al. in 1988, was designed to reduce MU discharge rates. It is delivered by rotating electric pulses between several (typically four) electrodes placed on the skin over a muscle belly. In this way, when $NMES_{SEQ}$ is delivered at 40 Hz to the quadriceps muscles, MUs recruited under each stimulation site will discharge at 10 Hz (Downey, Bellman, Kawai, Gregory, & Dixon, 2015; Nguyen, Masani, Micera, Morari, & Popovic, 2011; Popovic & Malesevic, 2009; Sayenko, Popovic, & Masani, 2013). To recruit MUs in their natural order, it has been shown that longer pulse widths can generate contractions via reflex pathways through the spinal cord more than shorter pulse widths (Lagerquist & Collins, 2008, 2010). Recently Jeon and Griffin (2018) reported that longer pulse widths (1 ms) produced less contraction fatigability than shorter pulses (0.2 ms) during conventional, one-channel, NMES. Thus, Project 1 of my thesis work was to

determine whether wider pulse widths also reduce fatigability during $NMES_{SEQ}$.

After data were collected from four participants, there was no apparent difference in contraction fatigability (i.e. the decline in torque over time) between pulse widths during $NMES_{SEQ}$. Thus, a second project was designed to compare the effect of pulse width on contraction fatigability during $NMES_{CON}$ using the same protocol as was used for the $NMES_{SEQ}$ experiments to more thoroughly examine the relationship between pulse width and contraction fatigability.

What follows in the first chapter of this thesis is a review of the literature, including the introduction of NMES, as well as the differences between voluntary contractions and electrically-evoked contractions, the parameters of NMES that influence how torque is generated, the mechanisms that contribute to contraction fatigability, and potential ways to reduce contraction fatigability. Chapter 2 describes the experimental work that this thesis is based on in "paper format", and Chapter 3 provides a general discussion including a summary, overview of the clinical implications, limitations, and future directions.

1.2 Introduction of neuromuscular electrical stimulation (NMES)

1.2.1 Brief history and application of electrical stimulation

The use of electrical stimulation can be traced to 2750 BC when Aristotle showed that the "torpedo fish", a type of fish that can produce electricity, can produce numbness (Bussel, 2015). However, the first treatment using electricity did not emerge until 36 AD, when Emperor Tiberius used torpedo fish to treat his gout pain (Bussel, 2015), and it was not until the middle of the eighteenth century, when electrical stimulation began to be applied over paralyzed muscles to

evoke contractions (Hainaut & Duchateau, 1992). Later Galvani observed muscle twitches when he stimulated the sciatic nerves of dissected frogs and discovered “animal electricity”, the intrinsic electricity conducted by nerves and muscles that contributes to muscle contractions in animal bodies (Hainaut & Duchateau, 1992; Hoff, 1936). However, the common application of electrical stimulation for rehabilitation purposes did not appear until the twentieth century, when less loss of muscle weight was reported using electrical stimulation (Hainaut & Duchateau, 1992).

In recent years, NMES has been used to generate contractions for training in non-injured individuals (Crognale et al., 2009) and rehabilitation after injuries or diseases (Hamid & Hayek, 2008; Sheffler & Chae, 2007). NMES involves the application of electrical stimulation to produce muscle contractions through stimulating axons distributed throughout a muscle belly or nerve trunk (Lake, 1992). It is delivered as a combination of pulse frequency, pulse width, and current amplitude through electrodes placed on the skin over target muscles. Studies have shown NMES can increase muscle strength and improve gait and balance performance (Bax, Staes, & Verhagen, 2005; Langeard, Bigot, Chastan, & Gauthier, 2017; R., 2006). When incorporated into rehabilitation programs, NMES has numerous benefits, especially for individuals experiencing paralysis such as a SCI, stroke, and cerebral palsy (Carmick, 1993; Hamid & Hayek, 2008; Ho et al., 2014; Takeda, Tanino, & Miyasaka, 2017). It can assist individuals in walking and standing (Bijak et al., 2005), improve muscle strength (Sabut, Sikdar, Kumar, & Mahadevappa, 2011), increase blood flow to lower limbs (Phillips, Burkett, Munro, Davis, & Pomeroy, 1995), reduce spasticity (Stein, Fritsch, Robinson, Sbruzzi, & Plentz, 2015), and lower heart rate at submaximal work intensity (Deley, Denuziller, & Babault, 2015; Gibbons, Stock, Andrews, Gall,

& Shave, 2016). Functional electrical stimulation (FES) is the name typically used for NMES when it is used to restore functional movement (Ho et al., 2014; Peckham & Knutson, 2005). FES devices such as the WalkAide system (Everaert et al., 2013; Kim, Eng, & Whittaker, 2004) and HandMaster (Alon, Levitt, & McCarthy, 2007; Snoek, MJ, in 't Groen, Stoffers, & Zilvold, 2000) are available in the market for individuals to reduce "foot drop" during gait and improve grasp and pinch function (Hamid & Hayek, 2008; Takeda et al., 2017), respectively.

1.2.2 Electrically-evoked contractions using NMES

During $NMES_{CON}$, pulses of electrical current are delivered through a single pair of surface electrodes, an anode and a cathode, placed over a muscle belly or a nerve trunk. Typically, the applied current triggers action potentials in axons underneath the electrodes, which in turn generates muscle contractions by activating the skeletal muscle fibers they innervate (Barss et al., 2018; Bickel, Gregory, & Dean, 2011). The process of evoking a muscle contraction can be separated into three phases: initiation of action potentials, transmission at the neuromuscular junction, and excitation-contraction coupling. Details will be discussed in the following sections.

1.2.2.1 The initiation of action potentials

Action potentials are electrical responses evoked by neurons (Kandel, Jessell, Schwartz, Siegelbaum, & Hudspeth, 2013). During NMES, the first step of the initiation of an action potential is the depolarization of axons under the cathode. Generally, negative ions (anions) are repelled by the cathode and move toward the anode, whereas positive ions (cations) are repelled by the anode and move toward the cathode. In this way, anions repelled by the cathode accumulate outside of the axonal membrane, while cations attracted by the cathode accumulate

inside of the axonal membrane, resulting in the decrease of membrane potentials and subsequent depolarization of axons (Peckham, Ackermann, & Moss, 2013). If this depolarization is sufficient to raise the membrane potential to approximately -50 mV, voltage-gated sodium channels are opened. The influx of sodium ions further depolarizes the membrane to approximately +40 mV to +50 mV, close to the equilibrium potential of sodium channels (McCormick, 2014). But before the membrane potential reaches to its peak, voltage-gated sodium channels slowly inactivate, and voltage-gated potassium channels open (Hille, 1992). The efflux of potassium ions returns the membrane potential to a negative value. Due to the slow inactivation of the potassium channels, the efflux of potassium ions overshoots the resting membrane potential and hyperpolarizes membrane. This in turn requires sodium-potassium pumps, which pump three sodium ions out of the membrane and two potassium ions into membrane during a single cycle, to restore the membrane potential to its resting state (Hendry, 2016; McCormick, 2014). An action potential consists of these changes in membrane potentials and is triggered only if the membrane potential reaches its threshold, which is around -50 mV. Once an action potential is evoked, it follows the "all or none" law, which means the amplitude of action potentials that propagate on axons or muscle fibers is not influenced by the strength of the stimulus (Adrian, 1914). The conduction velocity is faster when action potentials propagate along myelinated axons, compared to unmyelinated axons. Along myelinated axons, the conduction velocity of action potentials generally ranges from 70 to 120 meters per second, while the conduction velocity varies between 0.5 and 10 meters per second along unmyelinated axons (Purves et al., 2000). This difference is determined by the axon structures. In peripheral nerves, myelinated axons are surrounded by myelin sheaths that are formed by Schwann cells (Buffington & Rasband, 2013). These myelin sheaths contribute to the fast conduction speed by

increasing resistance and decreasing capacitance of membranes (Salzer & Zalc, 2016). Moreover, there are gaps between myelin sheaths called nodes of Ranvier, which have a high density of voltage-gated sodium channels compared to other regions of the axon. Propagation of action potentials can be achieved by transferring action potentials from node to node instead of traveling along the entire length of axons, allowing a faster conduction velocity, referred to as saltatory conduction (Buffington & Rasband, 2013; Kiernan & Lin, 2012; Salzer & Zalc, 2016). In comparison, action potentials have to travel along the entire length of unmyelinated axons, resulting in a relatively low conduction speed.

1.2.2.2 Neuromuscular transmission and excitation-contraction coupling

When action potentials travel along motor axons to the terminals, they activate voltage-gated calcium channels, resulting in the subsequent influx of calcium ions. This leads to the release of the neurotransmitter – acetylcholine (ACh), into the neuromuscular junction. The neuromuscular junction is a chemical synapse that connects a terminal of an alpha-motoneuron and a muscle fiber (Martyn, Fagerlund, & Eriksson, 2009; Smith & Plowman, 2007). ACh diffuses across the synaptic cleft, then binds to ACh receptors on the motor endplate, opening voltage-gated channels, resulting in the influx of sodium and calcium ions that depolarize the muscle membrane and further contribute to excitation-contraction coupling (Kuo & Ehrlich, 2015; Martyn et al., 2009).

Excitation-contraction coupling was first described by Sandow (1952) as the series of events occurring from the generation of the action potential in the skeletal muscle fibers to the initiation of muscle tension. When action potentials travel across the sarcolemma and depolarize the

transverse tubules, dihydropyridine receptors, the L-type voltage-gated calcium channels located on transverse tubules, are activated (Allen, Westerblad, Lee, & Lännergren, 1992; Calderón, Bolaños, & Caputo, 2014). The activation of dihydropyridine receptors leads to the release of calcium ions from sarcoplasmic reticulum via ryanodine receptors. Calcium ions then bind to troponin C, resulting in a change in position of the troponin complex on actin filaments, which in turn exposes myosin-binding sites, allowing myosin and actin to form cross bridges that trigger muscle contractions (Allen et al., 1992; Calderón et al., 2014; Kuo & Ehrlich, 2015).

1.2.3 Voluntary vs. electrically-evoked contractions

The neural pathways involved in voluntary contractions are different from those involved in electrically-evoked contractions. The initial areas of neural pathways during voluntary contractions involve the cortex, particularly pre-motor cortex and primary motor cortex, which are responsible for motor planning and execution of movement, respectively (Enoka, 2008; Purves et al., 2000). Descending signals travel via upper motor neurons to cell bodies of lower motor neurons in the spinal cord, depolarizing lower motor neurons and sending action potentials to skeletal muscle fibers (Purves et al., 2000). During NMES, however, contractions are typically initiated by the depolarization of motor axons underneath the stimulating electrodes. This pathway is referred to as the peripheral pathway, as shown in Figure 1-1 (Barss et al., 2018). NMES can also depolarize sensory axons that travel to the spinal cord, resulting in depolarization of the motoneurons they synapse with and a subsequent contractions (Barss et al., 2018; Bergquist, Clair, Lagerquist, et al., 2011). This neural pathway via the spinal cord, thus, is referred to as the central pathway (Figure 1-1), and transmission along central pathways toward

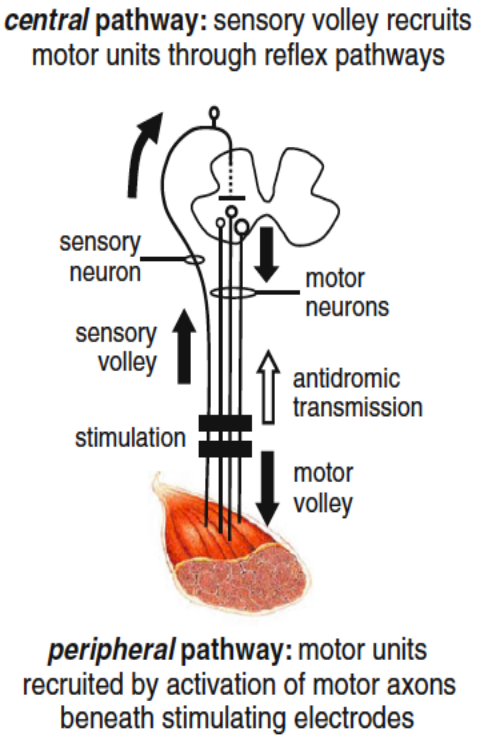

Figure 1-1. Schematic description of peripheral and central pathway (Bergquist et al. 2011).

the spinal cord is referred to as orthodromic transmission. In contrast, action potentials traveling the opposite direction is referred to as antidromic transmission.

Voluntary contractions and electrically-evoked contractions also differ in terms of how MUs are recruited and discharge. During voluntary contractions, MUs discharge asynchronously from one and other at relatively low rates (5 – 20 Hz). During NMES, however, MUs discharge synchronously with each other at a fixed latency from each stimulus pulse (Barss et al., 2018). To generate functional contractions, MU discharge rates need to be high enough to produce fused contractions of sufficient amplitude for a given task. The asynchronous discharge pattern of MUs during voluntary contractions lowers the discharge rates required to produce fused contractions. However, due to repetitive activation of motor axons with each stimulus pulse, the synchronous discharge of MUs during NMES means that higher discharge rates are needed to produce fused contractions (Barss et al., 2018; Dumitru et al., 2014). Moreover, during voluntary contractions, MUs are recruited from smallest to largest, according to Henneman size principle (Henneman, Somjen, & Carpenter, 1965). This order is altered during contractions evoked by $NMES_{CON}$ and MUs are recruited randomly with respect to the type (Binder-Macleod, Halden, & Jungles, 1995; Jubeau, Gondin, Martin, Sartorio, & Maffiuletti, 2007). Due to the random recruitment order,

more large-MUs are recruited during $NMES_{CON}$ than during voluntary contractions. Typically, large MUs have relatively large motoneurons that innervate muscle fibers that fatigue quickly. In contrast, small MUs have small motoneurons that innervate fatigue-resistant muscle fibers. Thus, more fatigable MUs are recruited during $NMES_{CON}$ (Bickel et al., 2011; Sheffler & Chae, 2007). These differences in MU discharge rates and recruitment order between voluntary contractions and contractions evoked by $NMES_{CON}$ result in one of the greatest impediments to the benefits of NMES – contraction fatigability, which can be measured as a significant decline in torque generated by the muscle over time (Binder-Macleod & Snyder-Mackler, 1993).

In summary, a series of events have to occur to produce muscle contractions during both voluntary contractions and contractions evoked by NMES. However, different neural pathways involved in these two types of contractions result in different MU discharge patterns and recruitment orders. Contractions evoked by $NMES_{CON}$ require high MU discharge rates and do not follow natural MU recruitment order. As a result, rapid fatigability occurs, which limits the effectiveness of NMES-based programs. My thesis work was designed to test an approach that addresses both these issues by decreasing MU discharge rates and recruiting MUs in their natural order to reduce contraction fatigability.

1.3 Parameters of NMES

Typically, NMES is delivered as a train or sequence of individual stimulus pulses. NMES trains combine several parameters (see Figure 1-2), including current amplitude (measured in mA), pulse frequency that represents the number of pulses delivered per unit time, and pulse width that represents the duration of each stimulus pulse. These parameters play an important role in

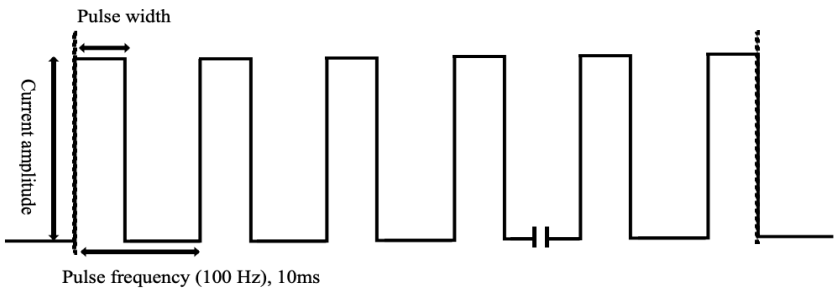

Figure 1-2. Schematic description of NMES parameters.

generating torque during NMES. Many studies have been conducted to explore the optimal combination of these parameters to produce more torque with less contraction fatigability and discomfort. In this section, the effect of these parameters on torque generation will be discussed.

1.3.1 Current amplitude

Increasing current amplitude generally increases contraction amplitude. This is because at a given pulse width and pulse frequency, higher current amplitude increases electrical charge, which in turn depolarizes more motor axons (Barss et al., 2018; Bergquist et al., 2011). Higher current amplitude also depolarizes more sensory axons. However, the orthodromic transmission that travels from sensory axons via the spinal cord collides with the antidromic transmission in motor axons at high current amplitude, which in turn limits the contribution of central pathways to contractions at high stimulation intensity (Bergquist, Clair, Lagerquist, et al., 2011; Glaviano & Saliba, 2016; Pierrot-Deseilligny & Mazevet, 2000). Thus, to evoke contractions through central pathways, current amplitude needs to be set at a low level to minimize collision between orthodromic and antidromic transmission. Unfortunately, low current amplitude cannot evoke contractions required for functional movement. Furthermore, high current amplitude activates nociceptors and triggers pain pathway, producing unpleasant feelings during NMES and limiting

the application of NMES in intact humans (Delitto, Strube, Shulman, & Minor, 1992; Lake, 1992).

1.3.2 Pulse Frequency

Increasing pulse frequency generally increases contraction amplitude. This is because at a given current amplitude and pulse width, increasing pulse frequency increases MU discharge rates. Pulse frequency determines the frequency of axon activation during NMES (Bergquist et al., 2011). Within a certain range (up to 60 Hz), the increase in pulse frequency increases the amplitude of contractions. The relationship between torque production and pulse frequency during NMES was explored by Gregory et al. (2007). They delivered NMES trains over a range of frequencies (10-100 Hz) to quadriceps muscles of 10 able-bodied individuals. The current intensity was that matched to 50% maximum voluntary isometric contractions (MVICs) when a 70 Hz/600 μs pulse train was delivered. Results suggested that torque increased as pulse frequency increased, however improvements plateaued after 60 Hz. Generally, during $NMES_{CON}$, pulse frequency is set at a range from 20 to 40 Hz to produce fused contractions and less contraction fatigability as higher frequencies result in high MU discharge rates that require more metabolic demand (Barss et al., 2018; Bigland-Ritchie, Zijdewind, & Thomas, 2000; Gregory, Dixon, & Bickel, 2007). Further details will be discussed in sections 1.4.1.

1.3.3 Pulse width

Increasing pulse width generally increases contraction amplitude. This is because at a given current amplitude and pulse frequency, wider pulses deliver more electric charge, which activates more MUs (Gregory et al., 2007). Many studies compared torque generation during

NMES with different pulse widths (Gorgey, Mahoney, Kendall, & Dudley, 2006; Gregory et al., 2007). Gorgey et al. (2006) measured torque produced by quadriceps muscles during NMES when two pulse widths (150 μs and 450 μs) were delivered at 100 Hz at the same current amplitude. They reported that wider pulses (450 μs) evoked larger contractions than the narrow pulses (150 μs). Later, the torque-duration curve was explored by Gregory, Dixon, and Bickel (2007). All pulse frequencies ranging from 10 to 100 Hz were combined with each pulse width (100 – 600 μs), the averaged torque was used to plot the torque-duration curve. It was reported that as pulse width increased, averaged torque produced by quadriceps muscles during a range of pulse frequencies also increased.

Pulse width can also influence the relative contribution of central and peripheral pathways during NMES by altering the relative recruitment of sensory and motor axons (Barss et al., 2018; Bergquist et al., 2011). In general, narrow pulses depolarize motor axons predominantly, while relatively more sensory axons are activated by wider pulses. This is due to the different rheobase and strength-duration time constant (T_{SD}) of sensory and motor axons. Rheobase is the threshold current required to evoke action potentials when the pulse width is infinitely long (Bostock, Cikurel, & Burke, 1998; Burke, Kiernan, & Bostock, 2001). T_{SD} is the ratio between the minimum charge threshold and the rheobase (Bostock et al., 1998), in other words, T_{SD} is inferred from the relationship between threshold current and pulse width, representing an apparent membrane time constant (Kiernan & Lin, 2012). It equates to chronaxie that represents the stimulus duration when the threshold current is twice rheobase. Both T_{SD} and rheobase reflect the properties of persistent sodium current (Burke et al., 2001; Kiss, 2008). A long T_{SD} and low rheobase are produced when there is an increased fraction of persistent sodium current (Burke et

al., 2001). Accordingly, Sensory axons have a longer T_{SD} than motor axons due to the more prominent persistent sodium conductance (Bostock et al., 1998; Mogyoros, Kiernan, & Burke, 1996). Thus, delivering wider pulses during NMES activates more sensory axons due to their longer T_{SD}, which enhances the involvement of central pathways. Lagerquist and Collins (2008, 2010) assessed the amplitude of H-reflexes, that represent recruitment of MUs through central pathways by delivering single pulses to the tibial nerve with different pulse widths. They reported that H-reflexes elicited by 50 μs pulses were > 45% smaller than those produced by 200, 500 and 1000 μs pulses. During NMES trains delivered at 20 Hz over the nerve, the amplitude of H-reflexes significantly increased after 100-Hz trains were delivered using relatively wider pulses (200, 500 and 1000 μs) rather than the narrower pulse (50 μs). These results indicate that wide pulses recruit more sensory axons than narrow pulses during both single pulse stimulation and NMES trains. In addition to measuring H-reflexes, another approach to assess the "central contribution" to contractions is as the increase in torque over the course of a single NMES train. Collins et al. (2001, 2002) observed an increase in torque (up to 40 % MVIC) generated by triceps surae muscle when high frequency (100 Hz) and low intensity (current was set to generate contractions ≤ 5% MVIC) trains of NMES were delivered. However, torque remained constant or declined when the nerve between the central pathway and the NMES electrodes was blocked to inhibit the generation and transmission of sensory volley. This indicates the increase in torque was due to the contribution of central pathways. The effect of pulse width on "central contribution" was also compared (Collins, Burke, & Gandevia, 2001, 2002; Lagerquist & Collins, 2010). Typically, torque continues to increase during a train of NMES when delivered using relatively wide pulse widths (1 ms) and high frequencies (>80 Hz) to generate a larger sensory volley. The increase in torque is smaller and less frequent when

shorter pulse durations and lower frequencies are used due to a smaller sensory volley being evoked.

In summary, increasing current amplitude, pulse frequency (up to 60 Hz) or pulse width can increase torque produced by muscles over a certain range (until all motor axons are recruited). My thesis work compared how much the "central contribution" contributes to the torque generated by quadriceps muscles during NMES trains using different pulse widths when the pulse frequency was kept constant and current amplitude was adjusted to match the target torque, as previous studies haven't examined the effect of pulse width on "central contribution" when stimulating quadriceps muscles.

1.4 Mechanisms of contraction fatigability

NMES has numerous benefits, however, these benefits are limited by contraction fatigability. Mechanisms involved in contraction fatigability will be discussed below.

1.4.1 Breakdown of excitation-contraction coupling

In skeletal muscle, excitation-contraction coupling relies on direct coupling among key proteins, the sarcoplasmic reticulum and voltage-gated L-type calcium channels. A failure in excitation-contraction coupling is one of the factors influencing muscle fatigue (Allen, Westerblad, Lee, & Lännergren, 1992; Keeton & Binder-Macleod, 2006; Place, Yamada, Bruton, & Westerblad, 2010; Westerblad & Allen, 1991). A decrease in the concentration of calcium ions and/or decreased calcium sensitivity can result in the failure of excitation-contraction coupling. The intracellular concentration of calcium ions was measured during high-frequency (100 Hz)

stimulation protocols in single mouse muscle fibers by Westerblad and Allen (1991), and a marked decline in both tetanic tension and calcium ions was observed at the end of the fatigue protocols. When caffeine, which has been shown to facilitate the release of calcium from sarcoplasmic reticulum (Weber & Herz, 1968), was injected into the muscle fibers, both tetanic calcium ions and tetanic tension increased at the end of fatigue protocols, whereas only tetanic calcium ions increased at the stable tension production phase where fatigability had not occurred. The results highlight the contribution of decreased calcium release in fatigability. A reduction in the release of calcium ions could be due to a raised concentration of Mg^{2+} and adenosine triphosphate (ATP) metabolites (Allen et al., 1992). Moreover, calcium sensitivity was also compared before and during fatigue protocols by Westerblad and Allen (1991) in the same study, less sensitivity of myofilaments to calcium ions was reported during fatigue protocols. This indicates that a reduction in myofibrillar calcium sensitivity also contributes to muscle fatigue. This reduction in calcium sensitivity could be due to an accumulation of phosphate and protons that can reduce pH and inhibit enzymes such as phosphorylase and phosphofructokinase during the process of glycolysis (Allen et al., 1992). Furthermore, a decrease in ATP availability can also result in the breakdown of excitation-contraction coupling as ATP provides energy for cross-bridge cycling and ionic pumps (Allen et al., 1992; Lamb, 2002; Ortenblad, Westerblad, & Nielsen, 2013). Recently, Luu (2017) compared the peak twitch torque, which is used to assess excitation-contraction coupling process, before and after the fatigue protocols in able-bodied individuals. A significant decline in peak twitch torque of tibialis anterior muscles was observed after a 60-Hz NMES fatigue protocol, indicating the role of the breakdown of excitation-contraction coupling in contraction fatigability.

1.4.2 Neuromuscular transmission failure (NTF)

NTF can contribute to contraction fatigability and can occur at the neuromuscular junction or along muscle membranes (Jones, Bigland-Ritchie, & Edwards, 1979; Sieck & Prakash, 1995). NTF could result from depletion of neurotransmitters (Sieck & Prakash 1995), and/or less excitation of the end-plate. It was suggested that during continued nerve stimulation, an increase in stimulation frequency decreases the quantum content of end-plate potentials (MacIntosh & Collier, 1976). In other words, the output of ACh will decrease at high stimulation frequencies during constant electrical stimulation. Jones et al. (1979) observed an increase in force after changing long-term high-frequency (80 Hz) stimulation to low-frequency (20 Hz) stimulation in fatigued human adductor pollicis muscles. They explained the rapid loss of force during high-frequency stimulation was partly due to a block of neuromuscular transmission along muscle membrane as concentrations of sodium and potassium ions could be changed (an accumulation of sodium ions inside the membrane and a high concentration of potassium ions outside the membrane) during high-frequency electrical activities and result in the failure of membrane propagation of action potentials. However, other studies have argued that the accumulation of external potassium ions may be insufficient to result in the reduction of sarcolemmal excitability (Metzger & Fitts, 1986; Renaud & Light, 1992), and Jones et al. (1979) did not directly measure the NTF along muscle membrane. Moreover, Luu (2017) did not observe failure of transmission at the neuromuscular junction during fatigue protocols by using repetitive nerve stimulation, a clinical test that assesses NTF at the neuromuscular junction, and maximum M-wave, which is used to assess neuromuscular transmission propagation along the muscle membrane. Therefore, it appears that NTF is not an influencing factor of contraction fatigability.

1.4.3 Axonal excitability

Recently, a decrease in the ability to depolarize axons under the stimulating electrodes (i.e. a decrease in axonal excitability) was reported as an influencing factor in contraction fatigability (Matkowski, Lepers, & Martin, 2015; Papaiordanidou, Stevenot, Mustacchi, Vanoncini, & Martin, 2014). The excitability of an axon is a function of both the density and activity of ion channels (Burke et al., 2001). Activity-dependent changes in the concentration of ions across the membrane during NMES might influence the axonal excitability. Papaiordanidou et al. (2014) suggested that one mechanism involved in the significant decrease in torque at 100 Hz was the decreased excitability of motor axons. This conclusion was based on the finding that torque decreased faster at 100 Hz than it did at 30 Hz, however, a positive relationship between torque decline during the fatigue protocol and twitch torque decrease evoked by single supramaximal stimuli was only observed during 30 Hz NMES. This indicates torque decline during the fatigue protocols during high-frequency stimulation (100 Hz) was not only due to a breakdown in excitation-contraction coupling, but also due to a decrease in axonal excitability. It has been suggested that activity-dependent hyperpolarization, which is thought due to overactivation of sodium-potassium pumps, contributes to the decreased axonal excitability (Bostock & Grafe, 1985; Vagg, Mogyoros, Kiernan, & Burke, 1998). Matkowski et al. (2015) found the decrease in twitch amplitude evoked by single stimuli after tetanic contractions was greater at submaximal stimulation intensity than it was at the supramaximal intensity. They suggested a reduction in activated MUs caused by decreased axonal excitability contributes to this torque decline as axonal hyperpolarization could appear at the stimulation site due to repetitive electrical stimulation. Stimuli at submaximal intensity might not be able to activate those hyperpolarized axons, whereas supramaximal intensity could recruit those axons, thus, resulting in less reduction

in torque. Moreover, Luu (2017) observed an increase in required current to evoke a target motor response during fatigue protocols, suggesting the decreased axonal excitability contributes to contraction fatigability. Luu proposed that rapid contraction fatigability during NMES could be due to "dropping out" of large motor axons as they are more prone to be hyperpolarized than smaller motor axons due to their lower expression of I_H current, the conductance of sodium and potassium ions caused by the activation of inward rectifying channels that are activated in response to the hyperpolarization of the membrane (Lorenz & Jones, 2014).

In summary, NTF does not typically influence contraction fatigability during NMES. Breakdown of excitation-contraction coupling and decreased axonal excitability play important roles in rapid fatigability during repetitive electrical stimulation. My thesis work was focused on reducing the breakdown of excitation-contraction coupling and axonal hyperpolarization resulting from high MU discharge rates and unnatural recruitment order.

1.5 Potential ways to reduce contraction fatigability

Research has shown that recruiting MUs in their natural order by increasing involvement of central pathways and decreasing MU discharge rate by utilizing multiple electrodes can reduce contraction fatigability during NMES (Barss et al., 2018; Downey et al., 2015; Gorgey & Dudley, 2008; Lagerquist & Collins, 2010; Nguyen et al., 2011; Popovic & Malesevic, 2009; Sayenko et al., 2013). In the following sections, these potential methods will be discussed.

1.5.1 Recruiting MUs in their natural order

Many studies have been conducted to recruit MUs in a more natural order during NMES. It was suggested that increased involvement of central pathways during NMES can reduce contraction fatigability as recruitment of MUs via central pathway follows Henneman size principle (Barss et al., 2018; Bergquist, Clair, Lagerquist, et al., 2011; Collins, 2007). This central contribution could be achieved by maximising the activation of sensory axons by delivering NMES over nerve trunks and optimizing NMES parameters (Barss et al., 2018; A. J. Bergquist, Clair, Lagerquist, et al., 2011; A. J. Bergquist, Wiest, & Collins, 2012; Bickel et al., 2011; Glaviano & Saliba, 2016; Lagerquist & Collins, 2010).

1.5.1.1 Stimulating nerve trunks

During $NMES_{CON}$, more sensory axons can be depolarized when stimulus pulses are delivered over a nerve trunk rather than a muscle belly. Bergquist, Clair, and Collins (2011) delivered NMES trains to tibial nerves and triceps surae muscles in 10 participants and measured H-reflexes and M-waves. They observed greater H-reflexes and smaller M-waves during tibial nerve stimulation than triceps surae stimulation, indicating that more sensory axons were activated when tibial nerves were stimulated. As MUs recruited from central pathways follow their natural recruitment order, fewer large-MUs are recruited during nerve stimulation than muscle stimulation. Contraction fatigability was compared when NMES was delivered over tibial nerves and triceps surae muscles separately in individuals experiencing a chronic SCI (Bergquist, Wiest, Okuma, & Collins, 2014). Less contraction fatigability was observed in those with H-reflexes (percent torque decline was ~39%) compared those without H-reflexes (percent torque decline was ~70%), and H-reflex amplitude was significantly higher during nerve stimulation than muscle stimulation throughout the entire fatigue protocol.

1.5.1.2 Using wider pulses

Increasing pulse width is another potential way to reduce contraction fatigability and thus many studies have compared the effect of pulse width on contraction fatigability (Table 1-1). Kesar and Binder-Macleod (2006) observed less contraction fatigability when long-duration pulse (600 μs) combined with a frequency at 11.5 Hz were used to evoke contractions, compared to shorter pulses with higher frequencies (150 μs at 30 Hz; 131 μs at 60 Hz). Moreover, Gregory et al. (2007) delivered NMES trains using 200 μs pulses at 50 Hz and 500 μs pulses at 20 Hz over quadriceps and found less contraction fatigability with the longer pulse width and low frequency (500 μs at 20 Hz). However, these results were based on comparing long-duration pulse and low-frequency NMES versus short-duration pulse and high-frequency NMES, even though, high-frequency NMES is known to produce more contraction fatigability than low-frequency NMES. The independent effect of pulse width on contraction fatigability has only been studied in the last ten years. Gorgey, Black, Elder, and Dudley (2009) altered pulse widths (450 μs and 150 μs) to compare their effects on contraction fatigability during NMES delivered at 100 Hz. Initial torque was normalized to 75 % MVICs. No difference in contraction fatigability between pulse widths was observed. Bickel, Gregory, and Azuero (2012) also reported that there was no effect of pulse width on contraction fatigability during NMES. They compared the torque decline produced by short pulse width (167 ± 29μs) and long pulse width (600 μs) when NMES was delivered to quadriceps muscles using trains at 60 Hz to evoke 25 % MVIC at the beginning of the fatigue protocols. Similar level of torque decline was observed between short pulse width (50 % ± 13 %) and long pulse width (48 % ± 14 %). Papaiordanidou et al. (2014) measured percent torque decline of soleus muscles at the end of 4 fatigue protocols in able-bodied individuals using two pulse widths (0.5 and 1 ms) and two pulse frequencies (30 and 100 Hz). The current amplitude

was adjusted to match 30% MVIC. Results showed pulse frequency, rather than pulse width, influenced contraction fatigability of soleus muscles. Later, contraction fatigability during NMES delivered at 30 Hz was compared by Jeon and Griffin (2018) using a wider range of pulse widths (0.2 and 1 ms). Less contraction fatigability was reported when NMES was delivered using wider pulses (25.6 % decrease in torque) than narrower pulses (36 % decrease in torque). The different results among these studies might be due to the range of difference between pulse widths. A narrow range of pulse widths (0.2 – 0.6 ms or 0.5 – 1 ms) used by Gorgey et al. (2009), Bickel et al. (2012), and Papaiordanidou et al. (2014) may be not enough to observe the relative involvement of central pathways between pulse widths. Moreover, the frequencies delivered during NMES by Gorgey et al. (2009) and Bickel et al. (2012) were 100 Hz and 60 Hz, much higher than the frequency used in Jeon and Griffin's study (30 Hz). Perhaps high-frequency stimulation attenuated the influences of pulse width on contraction fatigability as it was reported that frequency modulation affected contraction fatigability more than pulse modulation (Kesar, Chou, & Binder-Macleod, 2008). Other studies have also shown no effect of pulse width on contraction fatigability, however, they did not match the initial torque at the beginning of the fatigue protocols for different pulse widths. Jailani and Tokhi (2012) stimulated SCI quadriceps muscles using 5 pulse widths (range of pulse widths: 0.2 – 0.4 ms) at 30 Hz, however, the current amplitude was set at a constant value for all fatigue protocols. Gorgey, Poarch, Dolbow, Castillo, and Gater (2014) also kept the current intensity at the same value for different pulse width protocols (range of pulse width: 0.2 – 0.5 ms) when stimulating SCI lower extremity muscle groups. Therefore, these results might not be comparable.

1.5.2 Decrease MU discharge rates

Multi-channel NMES, in which stimulus pulses are alternated or rotated between 2 or more cathodes, was designed to reduce high MU discharge rates. By placing more than one cathode over the muscle belly and rotating electric pulses between electrodes, MUs recruited by each site will discharge at a relatively lower frequency with respect to the net frequency delivered to the muscle.

1.5.2.1 Sequential NMES ($NMES_{SEQ}$)

$NMES_{SEQ}$, is delivered through several electrodes placed on the skin over the muscle belly by rotating electrical pulses between electrodes to reduce contraction fatigability. In this way, for example, when $NMES_{SEQ}$ is delivered at 40 Hz to quadriceps muscles by rotating pulses between four electrodes, MUs recruited under each stimulation site will be activated at 10 Hz. Petrofsky (1979) delivered electrical pulses to sciatic nerves of cats and compared the tension produced by synchronous and sequential stimulation. Stimulation amplitudes used to generate tetanic contractions were lower during sequential stimulation than synchronous stimulation. Moreover, contraction fatigability was less during sequential stimulation than during synchronous stimulation. The first application of $NMES_{SEQ}$ on human muscles was conducted by Pournezam et al. in 1988. They introduced two-channel and three-channel $NMES_{SEQ}$ on quadriceps muscles. Results were compared to those collected during $NMES_{CON}$ (one-channel). It was reported that three-channel $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$ and two-channel $NMES_{SEQ}$ (Pournezam, Andrews, Baxendale, Phillips, & Paul, 1988b), thus, reducing contraction fatigability. Studies also have been conducted in individuals with a SCI. Popovic & Malesevic (2009) compared contraction fatigability produced by quadriceps using $NMES_{CON}$ and

four-channel NMES in 6 individuals with chronic SCI. They observed more than a 150% increase in the time it took for torque to decrease by 30% during $NMES_{SEQ}$ compared to $NMES_{CON}$. Other studies have also shown that $NMES_{SEQ}$ produces less contraction fatigability compared to $NMES_{CON}$ when applied over lower limb muscles including the quadriceps muscles (Bergquist et al., 2017; Downey et al., 2015; Laubacher et al., 2017; Malešević et al., 2010) and triceps surae (Nguyen et al., 2011; Sayenko et al., 2013) as well as muscles of the upper limbs that flex and extend the wrist (Maneski et al., 2013). Recently, the application of channel numbers during $NMES_{SEQ}$ was also explored. Sallis et al. (unpublished) compared percent torque decline during fatigue protocols using $NMES_{CON}$ (1-channel NMES), 2-channel, 4-channel, and 8-channel $NMES_{SEQ}$ in 15 able-bodied participants (Figure 1-3). They found that the decrease in torque was greater during $NMES_{CON}$ and 2-channel $NMES_{SEQ}$ than 4-channel and 8-channel $NMES_{SEQ}$, however, there were no differences in contraction fatigability between 4-channel and 8-channel $NMES_{SEQ}$ or between $NMES_{CON}$ and 2-channel $NMES_{SEQ}$. Moreover, 4-channel

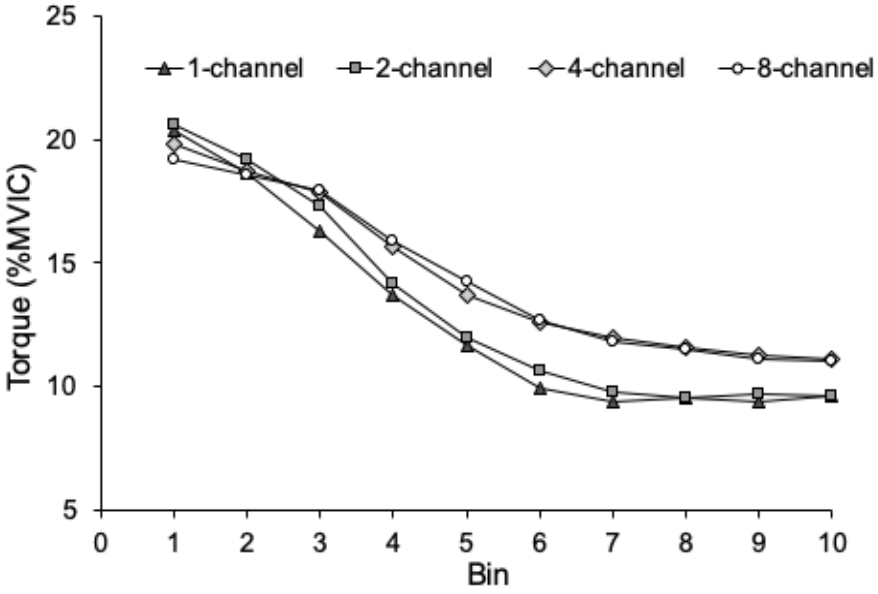

Figure 1-3. Torque recorded during the fatigue protocols delivered using $NMES_{SEQ}$ and $NMES_{CON}$ for the group of 15 participants. Data are represented as % MVIC. Significant difference is represented by the # symbol (Sallis et at., unpublished).

$NMES_{SEQ}$ resulted in less discomfort than 8-channel $NMES_{SEQ}$. Therefore, in my thesis work, 4-channel $NMES_{SEQ}$ was used to reduce contraction fatigability.

1.5.2.2 Interleaved NMES

Interleaved NMES (iNMES) was also designed to reduce contraction fatigability. During iNMES, stimuli are altered or interleaved between electrodes over a nerve trunk (nNMES) and a muscle belly (mNMES). iNMES recruits MUs in their natural order by stimulating the nerve trunk while also decreasing MU discharge rates by using two-channel stimulation (nerve and muscle). Comparison of contraction fatigability between iNMES and $NMES_{CON}$ was also explored. Lou, Bergquist, Aldayel, Czitron, and Collins (2017) compared contraction fatigability produced by tibialis anterior muscles among three types of NMES (iNMES, nNMES, and mNMES) in 10 able-bodied participants. At the end of the fatigue protocol, the torque generated by iNMES was >50% larger than mNMES and >30% larger than nNMES when initial torque was set at the same contraction level. This highlights the potential use of iNMES to reduce of contraction fatigability during rehabilitation programs.

1.5.2.3 Hybrid NMES

Hybrid NMES combines nNMES and $NMES_{SEQ}$. During hybrid NMES, electrical pulses are rotated between the nerve and the muscle. Different from iNMES, hybrid NMES includes multi-channel stimulation at muscle site. It was thought hybrid NMES would reduce contraction fatigability more compared to $NMES_{SEQ}$ or nNMES alone (Claveria-González, 2018). However, deceases in torque during fatigue protocols were similar among hybrid NMES (31±17%), nNMES (17±34%), and $NMES_{SEQ}$ (30±12%) in 14 able-bodied participants (Barss et al., 2018).

Thus, hybrid NMES does not reduce fatigability more than $NMES_{SEQ}$ and nNMES, but it can produce less contraction fatigability compared to $NMES_{CON}$, although comparison of contraction fatigability between hybrid NMES and $NMES_{CON}$ has not been conducted.

In summary, to reduce contraction fatigability, approaches have been developed to reduce MU discharge rates by using $NMES_{SEQ}$, recruit MUs in their natural order by stimulating nerves or using longer pulse widths, or both decrease MU discharge rates and recruit MUs in their natural order by using iNMES or hybrid NMES. My thesis work was aimed to reduce contraction fatigability by recruiting MUs in their natural order using wider pulses and decreasing MU discharge rates using 4-channel $NMES_{SEQ}$. As the effect of pulse width on contraction fatigability is still being debated, the main objective of this thesis work was to compare the effect of pulse width on contraction fatigability during NMES.

1.6 Thesis overview

The main purpose of this thesis work was to explore the effect of pulse width on contraction fatigability of the quadriceps muscles during NMES. We also compared the contraction fatigability produced during $NMES_{SEQ}$ and $NMES_{CON}$. In this experiment, NMES was delivered at 40 Hz using four pulse widths (0.2, 0.5, 1 and 2 ms) for $NMES_{SEQ}$ (see Figure 1-4. Project 1), and two pulse widths (0.2 and 1 ms) for $NMES_{CON}$ (see Figure 1-4. Project 2). We hypothesized that longer pulse widths would produce less contraction fatigability than shorter pulse widths, and the contraction fatigability generated during $NMES_{SEQ}$ would be less than during $NMES_{CON}$.

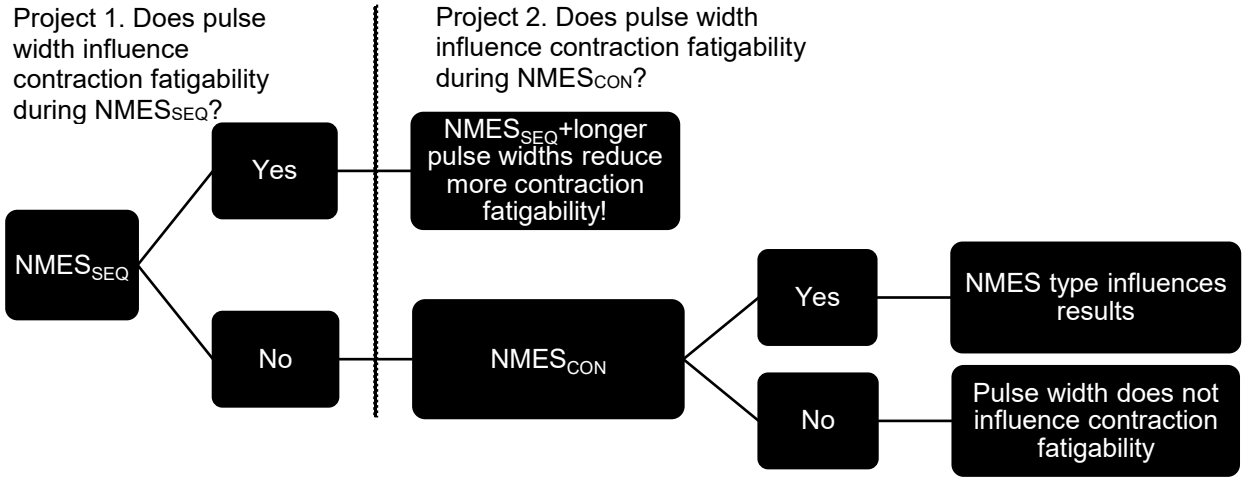

Figure 1-4. Overview of rationales for Project 1-2. The effect of pulse width was initially tested during $NMES_{SEQ}$ using 4 pulse widths (0.2, 0.5, 1 and 2 ms; Project 1). However, after 4 participants were collected, it appeared there was no effect of pulse width on contraction fatigability. Project 2 was then introduced to compare the effect of pulse width on contraction fatigability during $NMES_{CON}$ using 2 pulse widths (0.2 and 1 ms).

Table 1-1. Summary of research has been done to compare effect of pulse width on contraction fatigability during NMES.

Studies	Difference between pulse widths	Pulse width & frequency	Amplitude	Muscle & Fatigue protocol	Fatigue
Kesar & Binder-Macleod (2006)	YES	11.5±1.2Hz/600μs 30Hz/150±21μs 60Hz/131±24μs	20%MVIC	Quadriceps 176 trains 0.3 on/0.7 off	Torque drop 31.3±9.4% (lowest frequency) 51.3±7.5% (highest frequency)
Gregory, Dixon, & Bickel (2007)	YES	20Hz/500μs 50Hz/200μs	Same voltage used to evoke 50%MVIC during a 600μs/70Hz train	Quadriceps 60 trains 1s on/1s off	Torque drop 45.0±4.6% (P500) 62.0±3.6% (P200)
Gorgey, Black, Elder, and Dudley (2009)	NO	100Hz/450μs 100Hz/150μs	75%MVIC	Quadriceps 30 trains 3s on/3s off	Torque drop ～70%
Bickel, Gregory, and Azuero (2012)	NO	167±29μs/60Hz 600μs/60Hz	25%MVIC	Quadriceps 60 trains 1s on/1s off	Torque drop ~50%
Jailani & Tokhi (2012)	NO	200μs, 250μs, 300μs, 350μs, 400μs. 30Hz.	40mA	SCI quadriceps 75 trains 3s on/7s off	Torque drop 62.98 (P200), 74.66 (P250), 56.71 (P300), 51.59 (P350), 55.50% (P400)
Gorgey, Poarch, Dolbow, Castillo, and Gater (2014)	NO	200, 350, 500μs. 33.3Hz	140mA, 140mA and 100mA	SCI quadriceps, hamstring, gluteus maximus Resistance 1 Nm, fatigue threshold 18 RPM	Time to fatigue 11 ± 8, 10 ± 8, and 10 ± 8 min during P200, P350, and P500
Papaiordanidou, Stevenot, Mustacchi, Vanoncini, and Martin (2014)	NO	30Hz/500μs 30Hz/1ms 100Hz/500μs 100Hz/1ms	30%MVIC	Soleus 60 trains 4s on/6s off	Torque drop ～20% for 30 Hz protocols, percent torque drop off for 100 Hz protocols was not reported
Jeon & Griffin (2018)	YES	200μs/30Hz 1000μs/30Hz	25%MVIC	Quadriceps 80 trains, 0.3s on/0.7s off, 10 trains a cycle, 5s rest between two cycles	Torque drop 36.0±8.8% (P200) 25.6±9.4% (P1000)

*High-frequency width-pulse verses low-frequency narrow-pulse studies were not included as they are not comparable.

CHAPTER 2. DOES STIMULUS PULSE WIDTH INFLUENCE CONTRACTION FATIGABILITY DURING NEUROMUSCULAR ELECTRICAL STIMULATION?

2.1 Introduction

Neuromuscular electrical stimulation (NMES) involves the application of pulses of electrical stimulation to produce muscle contractions by stimulating axons distributed throughout a muscle belly or within a nerve trunk (Lake, 1992). NMES is used to generate contractions for training in non-injured individuals (Crognale et al., 2009) and for rehabilitation after injury or disease (Hamid & Hayek, 2008; Sheffler & Chae, 2007). When incorporated into rehabilitation programs, NMES can restore functional movement and reduce secondary complications of disuse. However, the benefits of NMES are limited by contraction fatigability, which is the significant decline in torque generated by a muscle over time (Binder-Macleod & Snyder-Mackler, 1993).

One factor that contributes to contraction fatigability during NMES is the unnaturally high discharge rates of motor units (MUs) (Barss et al., 2018; Dumitru et al., 2014; Nguyen et al., 2011). During conventional NMES ($NMES_{CON}$), which is typically delivered through a single cathode, all MUs discharge synchronously with each other, due to repetitive activation of motor axons with each stimulus pulse. The synchronous discharge of MUs means that high discharge rates are needed to produce large contractions, and this contributes to contraction fatigability (Barss et al., 2018; Dumitru et al., 2014). One way to lower MU discharge rates and reduce contraction fatigability is sequential NMES ($NMES_{SEQ}$). $NMES_{SEQ}$ is delivered by rotating stimulus pulses between several electrodes (typically four) over a muscle belly. Several studies

have shown that $NMES_{SEQ}$ produces less contraction fatigability compared to $NMES_{CON}$ when applied over lower limb muscles including the quadriceps muscles (Bergquist et al., 2017; Downey et al., 2015; Laubacher et al., 2017; Malešević et al., 2010; Popovic & Malesevic, 2009) and triceps surae (Nguyen et al., 2011; Sayenko et al., 2013), as well as muscles of the upper limbs that flex and extend the wrist (Maneski et al., 2013). In the original study, Pournezam et al (1988) compared contraction fatigability between $NMES_{CON}$, two-channel, and three-channel $NMES_{SEQ}$ in individuals with a SCI and found three-channel $NMES_{SEQ}$ generated contractions that took longer (7 min) to decrease by 50 % than $NMES_{CON}$ (~ 1 min) and two-channel $NMES_{SEQ}$ (5 min). All of the six other studies that have compared contraction fatigability between $NMES_{CON}$ and 4-channel $NMES_{SEQ}$ have also reported that $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$ (Bergquist et al., 2016; Downey et al., 2015; Malešević et al., 2010; Maneski et al., 2013; Popovic & Malesevic, 2009; Sayenko et al., 2013).

Another factor that contributes to contraction fatigability during $NMES_{CON}$ is the unnatural recruitment order of MUs (Sheffler & Chae, 2007). During $NMES_{CON}$, in contrast to during voluntary contractions, MUs are recruited randomly with respect to type. Therefore, relatively more fatigable MUs are recruited during $NMES_{CON}$ than during voluntary contractions of similar amplitude (Bickel, Gregory, & Dean, 2011; Sheffler & Chae, 2007). One way to recruit MUs in their natural order to reduce contraction fatigability is to deliver NMES using relatively long pulse widths. Longer pulse widths favor the activation of sensory axons over motor axons (Bostock, Cikurel, & Burke, 1998; Burke, Kiernan, & Bostock, 2001), and thus can generate contractions with a significant contribution from reflex pathways through the spinal cord (see "central pathway" in Figure 1-1). In contrast, when shorter pulse widths are used, as is typical

during $NMES_{CON}$, contractions are generated predominantly by the stimulation of motor axons (Lagerquist & Collins, 2008, 2010; see "peripheral pathway" in Figure 1-1). Reflex inputs (except cutaneous inputs) to the spinal cord recruit MUs according to Henneman's size principle, with the most fatigue-resistant recruited first (Henneman, Somjen, & Carpenter, 1965). Several studies have compared contraction fatigability when NMES is delivered using different pulse widths and the results have been variable. Two studies found less contraction fatigability when wider pulses were used compared to narrower pulses (Gregory et al., 2007; Kesar & Binder-Macleod, 2006). However, those studies compared long-duration pulse and low-frequency NMES (500 μs/20 Hz; 600 μs/11.5 Hz) versus short-duration pulse and high-frequency NMES (200 μs/50 Hz; 131 μs/60 Hz), and thus, the lower contraction fatigability is likely due at least in part to the lower frequencies rather than the wider pulses. Two other studies suggested that there was no effect of pulse width on contraction fatigability, however, in those studies pulse width was increased without changing current, and thus contractions produced by the wider pulses generated up to 2-fold more torque at the beginning of the fatigue protocols than when the narrower pulses were used (Gorgey et al., 2014; Jailani & Tokhi, 2012). Therefore, the lack of an effect of pulse width may be related to the greater contraction amplitude when wider pulses were used. Four studies have explored the effect of pulse width on contraction fatigability while keeping other variables such as frequency and initial contraction amplitude constant. Three of those studies found no effect of pulse width on contraction fatigability (Gorgey et al., 2009; Bickel et al., 2012; Papaiordanidou et al., 2014), however, the lack of an effect of pulse width may be due to the small range of pulse widths (0.2 – 0.6 ms or 0.5 – 1 ms) tested. More recently, Jeon and Griffin (2018) compared contraction fatigability over a wider range of pulse widths (0.2 – 1 ms) when NMES was delivered at 30 Hz to produce an initial contraction of 25 % MVIC.

They found significantly less fatigability when NMES was delivered using the wider pulses (26 % decrease in torque) than the narrower pulses (36 % decrease in torque).

My thesis research was initially designed to compare the effect of pulse width on contraction fatigability during 4-channel $NMES_{SEQ}$ of quadriceps muscles. This was based on the rationale that Jeon and Griffin (2018) showed that wider pulses (1 ms vs. 0.2 ms) reduced contraction fatigability during $NMES_{CON}$. Thus, we anticipated that the beneficial effects of $NMES_{SEQ}$ on contraction fatigability would be even greater when wider pulses were used. However, after data were collected from four participants and there was no clear difference in fatigability over a 10-fold range in pulse widths (0.2 – 2 ms), we decided to also include a comparison of the effect of pulse width on contraction fatigability during $NMES_{CON}$ in the present study to provide a more complete description of the effect of pulse width on contraction fatigability. Therefore, the main objective of this thesis work was to explore the effect of pulse width on contraction fatigability of the quadriceps muscles. We anticipated that longer pulse widths would generate contractions with a greater central contribution and thus produce less contraction fatigability than shorter pulse widths. To assess central contribution, we compared the effect of pulse widths on the torque generated by the three different NMES trains. Based on previous studies (Collins et al., 2001, 2002), we expected that torque would increase when there was a central contribution and thus we hypothesized that torque would increase more when longer pulse widths were used than shorter pulse widths due to the increased central contribution when using the longer pulse widths. To assess contraction fatigability, we calculated the percent decline in torque during fatigue protocols consisting of 100 contractions. We hypothesized that longer pulse widths would result in less decline in torque than shorter pulse widths over the course of the fatigue

protocols. Secondary objectives included comparing contraction fatigability between NMES types (i.e. $NMES_{SEQ}$ and $NMES_{CON}$) and comparing discomfort between pulse widths and NMES types, given that discomfort is also a barrier to the benefits of FES programs for some individuals. The results of these experiments contribute to our understanding of how best to deliver NMES to reduce contraction fatigability and discomfort when NMES is used to generate contractions of the quadriceps muscles.

2.2 Methods

2.2.1 Participants

All experiments were conducted in the Human Neurophysiology Laboratory at the University of Alberta (4-219 Van Vliet complex). Twelve participants (6 males and 6 females, age: 30.8 ± 11.7) with no known neurological or musculoskeletal injury volunteered for this project and provided informed consent. Each individual participated in 6 sessions. Each session lasted ~1 hour and sessions were separated by a minimum of 48 hours. A different combination of pulse widths and NMES types were delivered in the different sessions. Pulse widths tested for $NMES_{SEQ}$ were 0.2 ms, 0.5 ms, 1 ms, and 2 ms. For $NMES_{CON}$, 0.2 ms and 1 ms pulse widths were tested. The first four participants started with 4 $NMES_{SEQ}$ sessions following by 2 $NMES_{CON}$ sessions. The order of $NMES_{SEQ}$ sessions was not randomized for these 4 participants, whereas the order of $NMES_{CON}$ sessions was randomized. For the subsequent 8 participants, the order of 6 experimental sessions was randomized. All procedures were approved by the University of Alberta's Health Research Ethics Board.

2.2.2 General methodology

Torque. Participants were seated in the chair of a Biodex System III dynamometer (Biodex Medical Systems, Inc.,) to measure the isometric torque of knee extension. The right knee and hip were positioned at ~110° and ~85°, respectively, and the center of rotation of the right knee was aligned with the axis of rotation of the dynamometer.

Sequential NMES ($NMES_{SEQ}$). For $NMES_{SEQ}$, stimuli were rotated sequentially between four cathodes over the quadriceps muscles (Figure 2-1 A) using a custom-built stimulation distributor connected to a constant-current stimulator (DS7AH, Digitimer). The stimulating electrodes (5x10 cm; Axelgaard, Fallbrook, US) were placed on the skin over the quadriceps muscles and targeted the motor points of the vastus lateralis (electrodes #1 and #4), vastus medialis (electrode #2), and rectus femoris (electrode #3). Reference electrodes (anodes) were placed proximal to electrode #1 and #3 (electrode #6), and distal to electrode #2 and #4 (electrode #5). Electrode#1 and #3

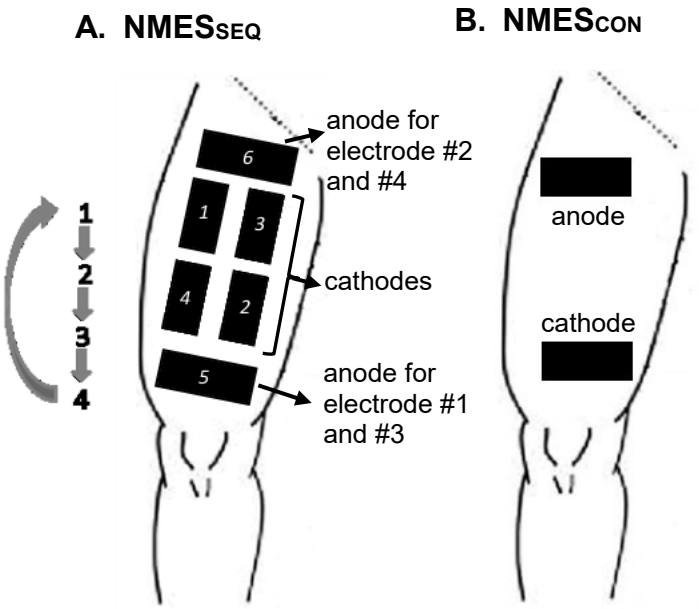

Figure 2-1. Electrode placements. Electrode placements for sequential ($NMES_{SEQ}$; Panel A) and conventional NMES ($NMES_{CON}$; Panel B). For $NMES_{SEQ}$, pulses were rotated between cathodes at a net frequency of 40 Hz, resulting in a frequency at each cathode of 10 Hz. For $NMES_{CON}$, the cathode was "fixed" and stimulus pulses were delivered at 40 Hz.

shared a common anode (electrode #5), and electrode #2 and #4 shared a common anode (electrode #6).

Conventional NMES ($NMES_{CON}$). For $NMES_{CON}$, stimuli were delivered between a single "fixed" cathode and a single anode (5x10 cm; Axelgaard, Fallbrook, US; see Figure 2-1 B) using a constant-current stimulator (DS7AH, Digitimer). The cathode was placed over the distal portion of rectus femoris and vastus medialis muscles. The anode was placed over the proximal portion of the rectus femoris and vastus lateralis muscles (Jeon & Griffin, 2018).

2.2.3 Experimental procedures

Each session consisted of three phases: pre-fatigue, fatigue, and post-fatigue as described below. A schematic representation of the experimental protocol is shown in Figure 2-2.

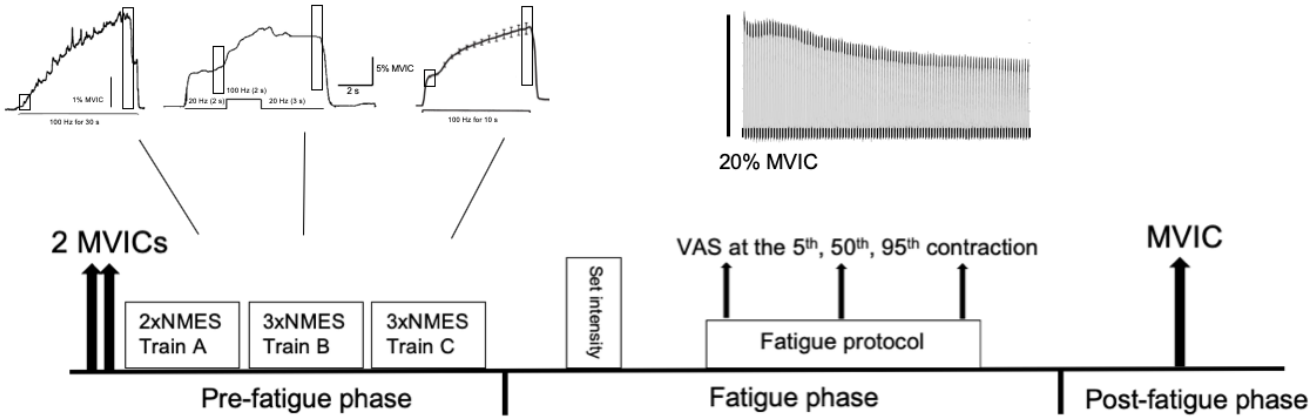

Figure 2-2. Schematic representation of the experimental protocol. Each session consisted of three phases: pre-fatigue phase, fatigue phase, and post-fatigue phase. During the pre-fatigue phase, two MVICs were performed, followed by three different NMES trains that were delivered to assess the central contribution. The fatigue phase consisted of 100 contractions generated by NMES delivered at 40 Hz. Current was adjusted to generate contractions that produced 20 % MVIC. Discomfort was rated by participants after the 5th, 50th, 95th contraction using a visual analog scale (VAS). Participants performed an additional MVIC after the fatigue phase.

Pre-fatigue phase

Maximum voluntary isometric contractions (MVICs). At the beginning of each session, participants performed two MVICs lasting 3-5 s each that were separated by at least 1 minute. Participants were strapped in the Biodex and were asked to put their hands on the handle bars. During MVIC, participants received verbal encouragement to contract as forcefully as possible and were given visual feedback of their torque production on a computer monitor.

NMES trains. Three different trains of NMES were delivered to assess the involvement of central pathways to the evoked contraction, according to Collins et al. (2001, 2002) and Lagerquist and Collins (2010).

NMES train A. Two trains were delivered at motor threshold intensity at 100 Hz. Each train was delivered for 30 s and separated by 5 s. To set stimulation intensity during $NMES_{CON}$, single pulses were delivered as the current was gradually increased to the lowest intensity at which a visible muscle twitch was observed. To set stimulation intensity for $NMES_{SEQ}$ sessions, stimulus pulses were delivered through one channel (electrodes #1 and #5) and current was increased as described above. After the current intensity was identified, stimulus pulses were delivered to four channels to generate contractions.

NMES train B. NMES train B was delivered in a "step-like" pattern at 20 Hz for 2 s, 100 Hz for 2 s, and then 20 Hz for 3 s. Three trains were delivered and successive trains were separated by 5 s. To set stimulation intensity for $NMES_{CON}$, 1 s trains were delivered at 20 Hz and the current was adjusted to generate a contraction of 5 % MVIC. To set stimulation intensity for $NMES_{SEQ}$, 1 s trains were delivered at 20 Hz to four channels.

NMES train C. Train C was also delivered to generate an initial contraction of 5 % MVIC.

However, the frequency was held constant at 100 Hz. Each of the 3 trains were delivered for 10 s and were separated by 5 s. To set stimulation intensity for $NMES_{CON}$, 1 s trains were delivered at 100 Hz, and the current was adjusted to generate 5 % MVIC. To set stimulation intensity for $NMES_{SEQ}$, 1 s trains were delivered at 100 Hz to four channels.

Fatigue phase.

During the fatigue phase, a fatigue protocol was applied, during which NMES was delivered at 40 Hz to produce 100 contractions (1 s on/1 s off). To set the stimulation intensity for $NMES_{CON}$, current was adjusted until the torque generated by a 1 s train at 40 Hz produced 20 percent MVIC. To set the stimulation intensity for $NMES_{SEQ}$, 1s train was delivered at 40 Hz to four channels and current was adjusted until the torque generated by 4 channels produced 20 percent MVIC. 1-2 mins after this stimulation intensity was identified, NMES was delivered to generate 100 contractions.

Discomfort. Participants were asked to rate their discomfort after the 5^{th}, 50^{th}, and 95^{th} contraction of each fatigue protocol using a visual analog scale (VAS). The VAS consisted of a 100 mm long straight line on a piece of paper, with 0 corresponding to "no discomfort" and 100 corresponding to "the worst possible pain." Participants were asked to verbally indicate their discomfort levels using whole numbers between 0 and 100.

Post-fatigue phase

Participants performed one MVIC immediately (<1 min) after each fatigue protocol ended.

Data analyses

Data were sampled at 5000 Hz using LabView (National Instruments) software and stored on a computer for later analysis that was conducted using custom-written Matlab software. Torque produced during the MVICs was quantified over a 0.3 s window centered on the peak torque. Torque produced during the three different NMES trains used to assess the contribution of central pathways was calculated by averaging the torque over two 1 s windows. For NMES train A and C, these windows included the first 1 s torque and the last 1 s of each contraction. For NMES train B, these windows included the second 1 s torque (i.e. the last 1 s torque produced by the first 20 Hz train) and the last 1 s torque produced by the last 20 Hz train. Changes in torque during NMES train A and C were assessed and calculated according to the equation below:

$$\text{Torque changes (\% MVIC)} = \text{Torque at the last 1 s} - \text{Torque at the first 1 s}$$

Changes in torque during NMES train B were assessed and calculated according to the equation below:

$$\text{Torque changes (\% MVIC)} = \text{Torque at the last 1 s} - \text{Torque at the 2nd 1 s}$$

Torque generated during each contraction of each fatigue protocol was quantified by averaging the torque over a 0.3 s window centered on the peak torque. Torque generated during fatigue protocols was binned by averaging data over every 10 successive contractions, therefore, 10 bins were obtained. Contraction fatigability was calculated as the difference between the mean torque generated during the first 10 contractions (bin 1) and the last 10 contractions (bin 10) and then

divided by the mean torque generated during first 10 contractions (bin 1), according to the equation below:

$$\%\ \text{decline in torque} = \frac{Bin\ 1 - Bin\ 10}{Bin\ 1} \times 100$$

To facilitate a comparison of our results with those of Jeon and Griffin (2018), we also calculated contraction fatigability using the same procedure as they did. Thus, contraction fatigability was calculated as the difference between torque produced during the first contraction and the last contraction, then divided by the torque produced during the first contraction, according to the equation used by Jeon and Griffin (2018). The equation is shown below:

$$\%\ \text{decline in torque} = \frac{Torque\ of\ first\ contraction - Torque\ of\ last\ contraction}{Torque\ of\ first\ contraction} \times 100$$

VAS scores were averaged over the course of each fatigue protocol at 3 time points: the beginning, the middle and the end, as there was no main effect of time ($F_{(1.038, 11.419)} = 0.927$, $p = 0.359$) during $NMES_{SEQ}$ and during $NMES_{CON}$ ($F_{(1.048, 11.532)} = 0.040$, $p = 0.856$).

Statistics

Statistical analyses were performed using SPSS software (SPSS, Chicago, Illinois). Unless specifically mentioned, all data were normally distributed as determined using Shapiro-Wilk tests. If Mauchly's test was violated, Greenhouse-Geisser corrections would be used. Post hoc analyses with Bonferroni corrections were used if there was a significant interaction. A p-value

less than 0.05 was considered statistically significant. Data are presented as mean ± standard deviation. Data were analyzed in 2 parts. For part 1, we tested the effect of pulse width on outcome measures during $NMES_{SEQ}$ across a wide range of pulse widths (0.2 – 2 ms). For part 2, we explored the effect of pulse width on outcome measures during $NMES_{SEQ}$ and $NMES_{CON}$ using 2 pulse widths (0.2 and 1 ms).

Influence of pulse width: $NMES_{SEQ}$ (0.2, 0.5, 1.0 and 2.0 ms)

To assess the effect of pulse width on current, charge, and VAS scores during the fatigue protocols delivered using $NMES_{SEQ}$, separate 1-way repeated-measures analyses of variance (ANOVA) were conducted for each outcome measure. To assess the effect of pulse width on the contribution of central pathways to the contractions, torque produced during the three different NMES trains (A, B, and C) was analyzed in two ways. Firstly, to determine whether torque changed from the beginning to the end of NMES trains, we used a 2 (time) x 4 (pulse width) ANOVA. Secondly, to assess the effect of pulse width on changes in torque (expressed as a % MVIC) during each of different NMES trains, we used a 1-way repeated-measures ANOVA to compare changes in torque from the beginning to the end of NMES trains between pulse widths. To assess the effect of pulse width on contraction fatigability, a 1-way repeated-measures ANOVA was conducted to compare the percent decline in torque between 4 pulse widths. Percent change in MVICs recorded before and after the fatigue protocols was compared between pulse widths using a 1-way repeated-measures ANOVA.

Influence of pulse width: $NMES_{SEQ}$ vs. $NMES_{CON}$ (0.2 and 1.0 ms)

To assess the effect of NMES type ($NMES_{SEQ}$ vs. $NMES_{CON}$) on current, charge, and VAS

scores, separate 2 (NMES type) x 2 (pulse width) ANOVAs were conducted for each outcome measure. To assess the effect of NMES type on the contribution of central pathways to the contractions, torque produced during three different NMES trains (A, B, and C) was also analyzed in two ways. Firstly, to determine whether torque changed from the beginning to the end of $NMES_{CON}$ trains that were used to measure central contribution, we used 2 (time) x 2 (pulse width) ANOVAs. This analysis was not conducted for the $NMES_{SEQ}$ data as that was done for the data in part 1. Secondly, to assess the effect of NMES type on changes in torque during each of different NMES trains, we used a 2 (NMES type) x 2 (pulse width) ANOVA to compare changes in torque (expressed as a % MVIC) from the beginning to the end of NMES trains between 4 NMES sessions. To assess the effect of NMES type on contraction fatigability, a 2 (NMES type) x 2 (pulse width) ANOVA was conducted to compare the percent decline in torque between 4 NMES sessions. Percent change in MVICs recorded before and after the fatigue protocols was compared between 4 NMES sessions using a 2 (NMES type) x 2 (pulse width) ANOVA.

2.3 Results

Single participant data:

Data collected from a single participant are presented in Figure 2-3, which shows torque (left side) and the percent change in torque (right side) during the three different NMES trains that were used to assess the "central contribution". In this participant, during NMES train A, torque tended to decline more during $NMES_{CON}$ (see dotted lines in Figure 2-3) than during $NMES_{SEQ}$ (see sold lines in Figure 2-3). Torque produced during NMES train B tended to increase or stay constant when both $NMES_{SEQ}$ and $NMES_{CON}$ were delivered, interestingly, however, much more

torque was generated during the 100 Hz stimulation during $NMES_{SEQ}$ than $NMES_{CON}$. During NMES train C, torque decreased during $NMES_{CON}$, whereas torque tended to increase or stay constant during $NMES_{SEQ}$. There was no clear effect of pulse width on how torque changed during any of the three NMES trains.

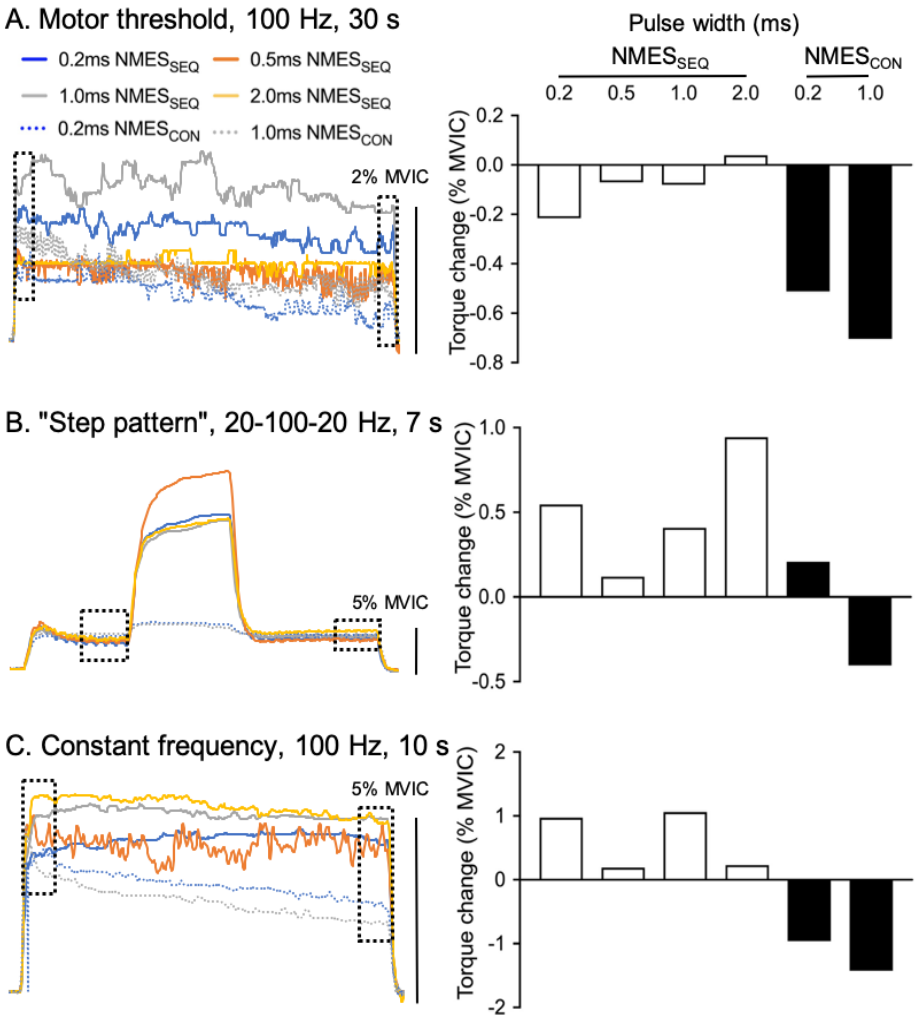

Figure 2-3. Torque recorded from a single participant during NMES trains used to assess the "central contribution" during $NMES_{SEQ}$ and $NMES_{CON}$. The left side of each panel displays the torque produced by the three NMES trains when $NMES_{SEQ}$ and $NMES_{CON}$ were delivered using different pulse widths. Dashed boxes indicate the regions used to compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque between these time points for each type of NMES train.

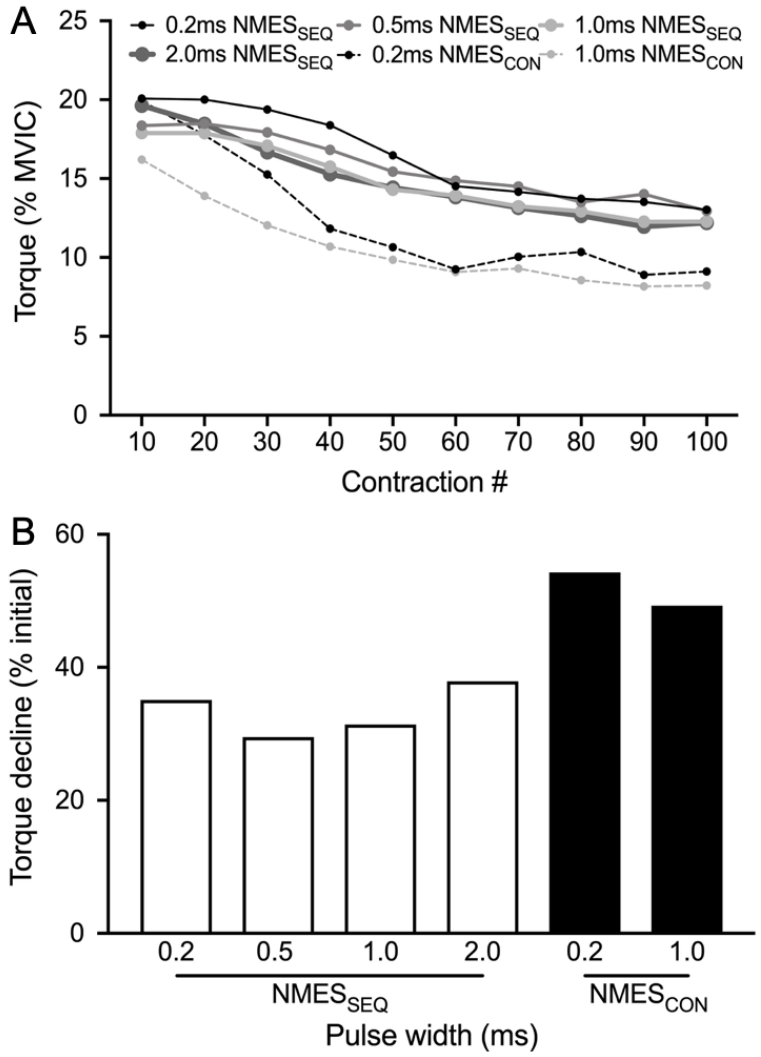

Figure 2-4. Torque recorded from a single participant during each fatigue protocol. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and Panel B displays the percent decline in torque from the first 10 contractions to the last 10 contractions. These data were recorded from the same participant as the data shown in Figure 2-3.

Figure 2-4 displays the torque recorded during the fatigue protocols for the participant whose data are shown in Figure 2-3. In this participant, there was a clear effect of NMES type on how torque declined during the fatigue protocols as torque declined more during $NMES_{CON}$ than $NMES_{SEQ}$. However, there did not appear to be an effect of pulse width on contraction fatigability. During $NMES_{CON}$, torque declined by 52 %, in contrast, torque declined by 33 % during $NMES_{SEQ}$. Discomfort rated by this participant was lower during $NMES_{CON}$ (45 ± 9) than $NMES_{SEQ}$ (65 ± 5) during the fatigue protocols, whereas pulse width did not appear to influence discomfort (data not shown).

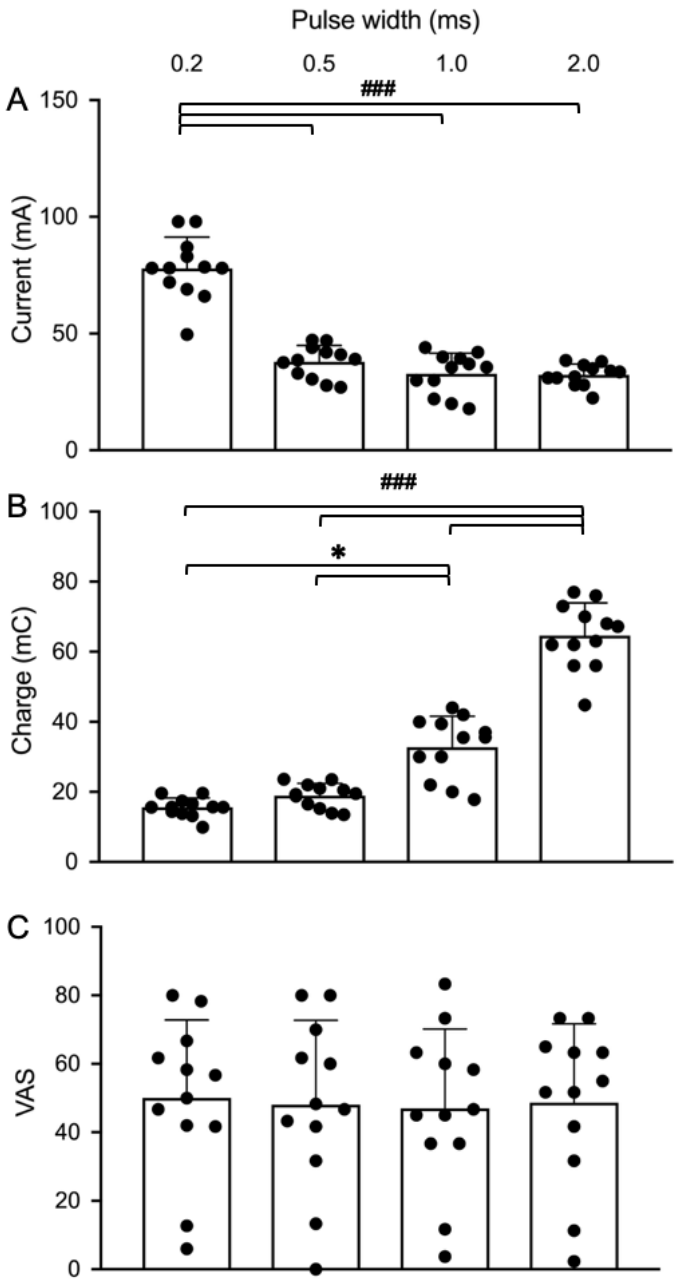

Figure 2-5. Current, charge, and discomfort when $NMES_{SEQ}$ was delivered using 4 pulse widths for the group of 12 participants. Panel A and B display current and charge delivered to generate the contractions for the fatigue protocols, respectively. Panel C displays the average VAS scores during the fatigue protocols. ### denotes $P < 0.001$ and * denotes $P < 0.05$. Dots represent data from single participants.

Group data:

Influence of pulse width: $NMES_{SEQ}$ (0.2, 0.5, 1.0 and 2.0 ms)

Current, charge and discomfort.

Figure 2-5 shows current (Panel A), charge (Panel B), and VAS scores (i.e. discomfort, Panel C) recorded during $NMES_{SEQ}$, for the group of 12 participants. There was a main effect of pulse width on current ($F_{(1.461, 16.069)} = 93.318$, $p < 0.001$). Post hoc tests identified that to generate 20 % MVIC at the beginning of the fatigue protocols, more current was required when 0.2 ms pulse widths were used than 0.5 ms, 1 ms and 2 ms. For charge, there was also a main effect of pulse width ($F_{(1.669, 18.360)} = 206.109$, $p < 0.001$). More charge was delivered to generate contractions of 20 % MVIC when using a 2 ms pulse width than the other 3 pulse widths. Similarly,

NMES delivered using a 1 ms pulse width required more charge than 0.2 ms and 0.5 ms pulse widths. There was no difference in charge between 0.2 ms and 0.5 ms pulse widths ($p = 0.263$). There was no significant main effect of pulse width on VAS scores ($F_{(3, 33)} = 0.207$, $p = 0.891$).

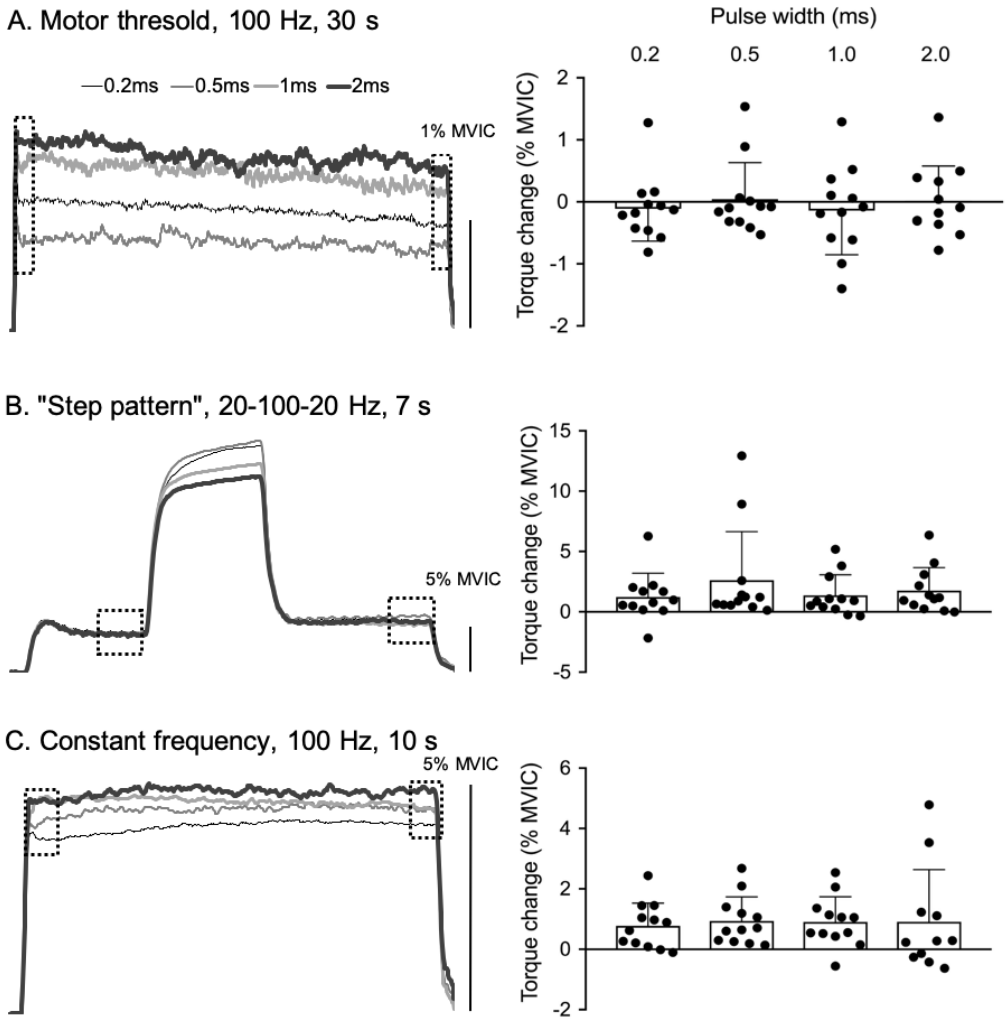

Figure 2-6. Torque averaged across the group during NMES trains used to assess the effect of pulse width on the "central contribution" during $NMES_{SEQ}$. The left side of each panel displays the torque produced by the three NMES trains averaged across participants (n=12) when $NMES_{SEQ}$ was delivered using four pulse widths. Dashed boxes indicate the regions used to compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque from the beginning to the end of each NMES train. Dots represent data from single participants.

Torque changes during three different NMES trains. Torque averaged across the 12 participants during NMES trains used to assess "central contribution" is shown in the left panels of Figure 2-

6, and the change in torque, as a % MVIC, is shown on the right side. These data were analyzed in 2 different ways. Firstly, after 2 (time) x 4 (pulse width) ANOVAs were conducted to determine whether torque changed from the beginning to the end of NMES trains (data not shown). For NMES train A, there was no significant main effect of time ($F_{(1, 11)} = 0.201$, $p = 0.662$) or pulse width ($F_{(1.455, 16.006)} = 2.311$, $p = 0.141$), and there was no interaction between time and pulse width ($F_{(1.975, 21.721)} = 0.242$, $p = 0.785$). In contrast, there was a main effect of time during NMES train B ($F_{(1, 11)} = 13.304$, $p < 0.01$) and C ($F_{(1, 11)} = 25.791$, $p < 0.001$), however, there was no main effect of pulse width (NMES train B: $F_{(2.189, 21.891)} = 0.161$, $p = 0.870$; NMES train C: $F_{(3, 33)} = 1.263$, $p = 0.303$) and there was no interaction between time and pulse width during those two types of NMES trains (NMES train B: $F_{(1.523, 15.235)} = 0.690$, $p = 0.479$; NMES train C: $F_{(3, 33)} = 0.466$, $p = 0.708$). On average, torque increased by 1 ± 1 % MVIC and 0.7 ± 0.5 % MVIC during NMES train B and C, respectively. For the second type of analysis, a 1-way repeated-measures AVOVAs was used to compare the effect of pulse width on changes in torque during each of different NMES trains (see right side of Figure 2-6). Based on these analyses, altering pulse width did not influence how torque changed during any of the three NMES trains tested – there was no main effect of pulse width during any NMES trains (NMES train A: $F_{(3, 33)} = 0.376$, $p = 0.682$; NMES train B: $F_{(3, 33)} = 1.0$, $p = 0.360$; NMES train C: $F_{(3, 33)} = 0.075$, $p = 0.973$).

Torque decline during fatigue protocols. Torque averaged across the 12 participants recorded during the fatigue protocols when $NMES_{SEQ}$ was delivered using 4 pulse widths is shown in Figure 2-7. Torque generated at the beginning of the fatigue protocols was not different between pulse widths ($F_{(3, 33)} = 1.611$, $p = 0.205$) as there was no main effect of pulse width on torque

produced over the first 10 contractions (Bin 1). On average, mean contraction amplitude over the first 10 contractions was 20 ± 2 % MVIC across the 4 sessions. Pulse width did not influence contraction fatigability as there was no main effect of pulse width on the percent torque decline (see Panel B in Figure 2-7; $F_{(3, 33)} = 0.993$, $p = 0.408$). On average, torque declined by 27 ± 13 % during $NMES_{SEQ}$, regardless of the pulse width used. There was also no main effect of pulse width on contraction fatigability ($F_{(3, 33)} = 1.151$, $p = 0.343$) when contraction fatigability was calculated by comparing the percent decline in torque from the first contraction to the last contraction, as was done by Jeon and Griffin (2018).

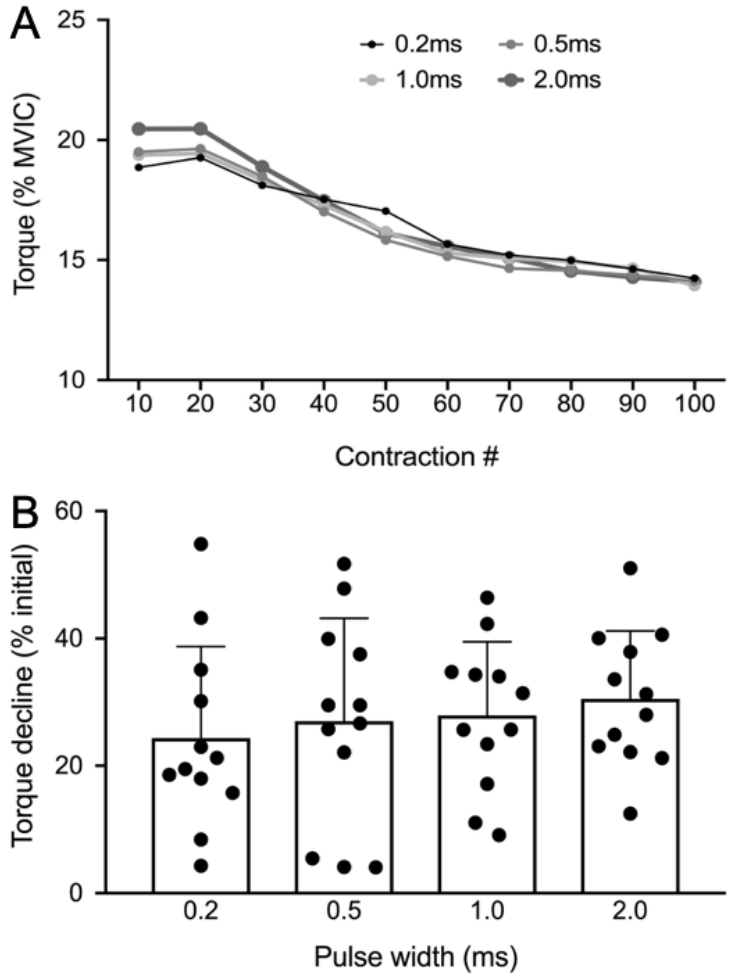

Figure 2-7. Torque recorded during the fatigue protocols delivered using $NMES_{SEQ}$ for the group of 12 participants. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and averaged across participants. Panel B displays the percent decline in torque from the beginning (first 10 contractions) to the end (last 10 contractions) of the fatigue protocols. Dots represent data from single participants.

Percent changes in MVICs. There was no significant effect of pulse width on the percent change in MVICs recorded before and after the fatigue protocols (data not shown, $F_{(3, 33)} = 0.365$, $p = 0.778$). On average, the torque

generated during MVICs was 7 ± 10 % less after the fatigue protocols than before the fatigue protocols.

Influence of pulse width: $NMES_{SEQ}$ vs. $NMES_{CON}$ (0.2 and 1.0 ms)

Current, charge, and discomfort. Data collected from 12 participants when $NMES_{SEQ}$ and $NMES_{CON}$ were delivered using 2 pulse widths are shown in Figure 2-8. Panels A-C display current, charge, and VAS scores, respectively. For current, there was an interaction between NMES type and pulse width ($F_{(1, 11)} = 30.845$, $p < 0.001$). Post hoc tests indicated that more current was required when using 0.2 ms pulse widths than 1 ms pulse widths during $NMES_{SEQ}$ ($F_{(1, 11)} = 232.012$, $p < 0.001$). During $NMES_{CON}$, current was also greater when 0.2 ms pulse widths was used than 1 ms pulse widths ($F_{(1, 11)} = 130.513$, $p < 0.001$). When comparing current between NMES types, more current was required for $NMES_{SEQ}$ than $NMES_{CON}$ when using 0.2 ms pulse widths ($F_{(1, 11)} = 22.548$, $p < 0.05$). However, there was no difference in current between NMES types when 1 ms pulse width was used ($F_{(1, 11)} = 0.033$, $p = 0.859$). For charge, there was a main effect of pulse width ($F_{(1, 11)} = 124.038$, $p < 0.001$). To produce initial contractions of 20 % MVIC, delivering NMES using a 1 ms pulse width required more charge than when using 0.2 ms pulse widths. However, there was no main effect of NMES type ($F_{(1, 11)} = 1.720$, $p = 0.216$) and no interaction between pulse width and NMES type ($F_{(1, 11)} = 1.893$, $p = 0.196$). When comparing VAS scores, there was no main effect of pulse width ($F_{(1, 1)} = 0.592$, $p = 0.458$) or NMES type ($F_{(1, 11)} = 0.027$, $p = 0.872$) and no interaction between pulse width and NMES type ($F_{(1, 11)} = 0.132$, $p = 0.723$). The average VAS scores during the fatigue protocols across NMES sessions was 48 ± 21.

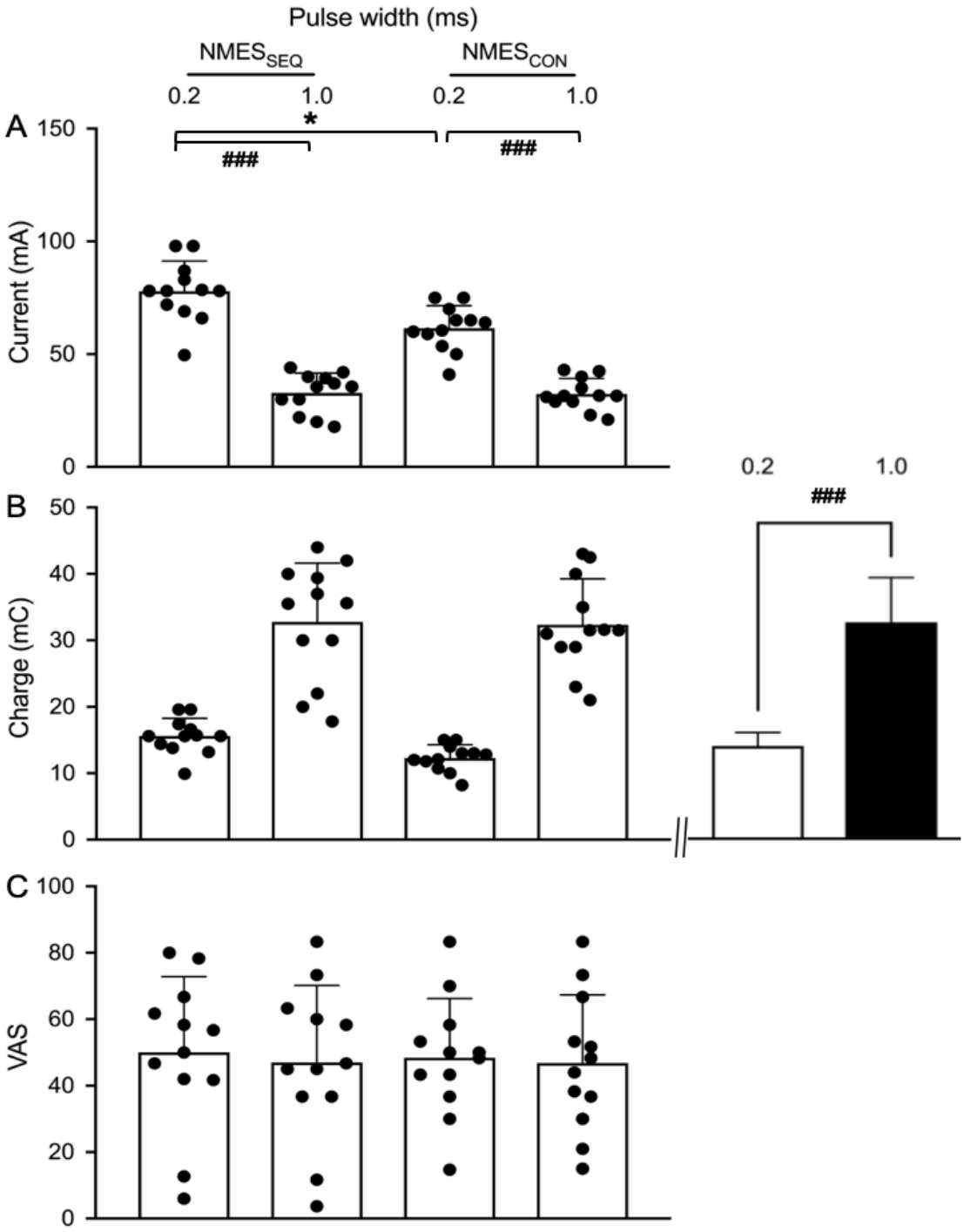

Figure 2-8. Current, charge, and discomfort during NMES$_{SEQ}$ and NMES$_{CON}$ for the group of 12 participants. Panel A and B display current and charge delivered during the fatigue protocols, respectively. Panel C displays the average VAS scores during the fatigue protocols. * denotes $P < 0.05$ and ### denotes $P < 0.001$. Dots represent data from single participants.

Torque changes during three different NMES trains. Torque averaged across the 12 participants during NMES trains used to assess the "central contribution" during $NMES_{SEQ}$ and $NMES_{CON}$ is shown in Figure 2-9, which displays torque (left side) and change (% MVIC) in torque (right side) during the three different NMES trains. Using 2 (time) x 2 (pulse width) ANOVAs to determine whether torque changed from the beginning to the end of the $NMES_{CON}$ trains, there was a main effect on time during both NMES train A ($F_{(1, 11)} = 40.806$, $p < 0.001$) and C ($F_{(1, 11)} = 9.831$, $p < 0.01$), however, there was no main effect of pulse width (NMES train A: $F_{(1, 11)} = 0.034$, $p = 0.857$; NMES train C: $F_{(1, 11)} = 2.368$, $p = 0.152$) and no interaction between time and pulse width (NMES train A: $F_{(1, 11)} = 4.369$, $p = 0.061$; NMES train C: $F_{(1, 11)} = 0.013$, $p = 0.912$). During NMES train B, torque stayed constant as there was no main effect of time ($F_{(1, 11)} = 3.002$, $p = 0.111$) or pulse width ($F_{(1, 11)} = 0.911$, $p = 0.360$) and no interaction between time and pulse width ($F_{(1, 11)} = 1.547$, $p = 0.239$; data not shown). On average, during $NMES_{CON}$, torque declined by -0.6 ± 0.3 % MVIC and -0.6 ± 0.7 % MVIC during NMES train A and C, respectively.

When using 2 (NMES type) x 2 (pulse width) ANOVAs to assess the effect of NMES type on the % MVIC change in torque during of different NMES trains, there was no main effect of pulse width on torque changes for all three NMES trains (NMES train A: $F_{(1, 11)} = 2.061$, $p = 0.179$; NMES train B: $F_{(1, 11)} = 0.307$, $p = 0.591$; NMES train C: $F_{(1, 11)} = 0.203$, $p = 0.661$). There was, however, a main effect of NMES type on how torque changed during NMES train A ($F_{(1, 11)} = 7.449$, $p < 0.05$) and C ($F_{(1, 11)} = 44.508$, $p < 0.001$). For NMES train A, torque decreased less during $NMES_{SEQ}$ (-0.1 ± 0.6 % MVIC) than $NMES_{CON}$ (-0.6 ± 0.5 % MVIC) and for NMES train C, torque increased during $NMES_{SEQ}$ (0.8 ± 0.8 % MVIC), whereas decreased during

$NMES_{CON}$ (-0.6 ± 0.8% MVIC). There was no main effect of NMES type ($F_{(1, 11)} = 1.752$, $p = 0.212$) on the percent change in MVIC torque during NMES train B, and there was no interaction between NMES type and pulse width during any of three NMES trains (NMES train A: $F_{(1, 11)} = 1.722$, $p = 0.216$; NMES train B: $F_{(1, 11)} = 1.691$, $p = 0.220$; NMES train C: $F_{(1, 11)} = 0.046$, $p = 0.834$).

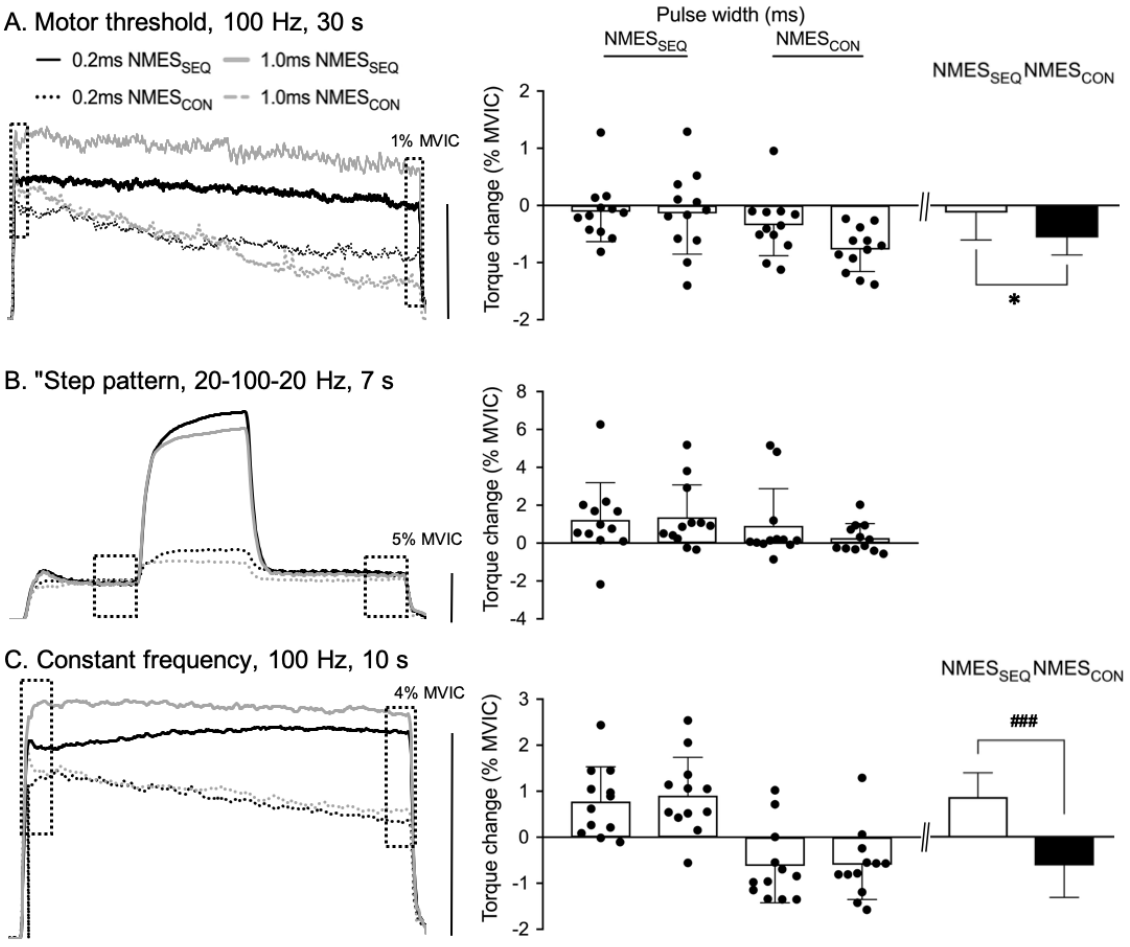

Figure 2-9. Torque averaged across the group during NMES trains used to assess the "central contribution" during $NMES_{SEQ}$ and $NMES_{CON}$. The left side of each panel displays the torque produced by the three NMES trains averaged across participants when $NMES_{SEQ}$ and $NMES_{CON}$ were delivered using two pulse widths. Dashed boxes indicate the regions used to compare torque produced at the beginning and end of each contraction. The right side of each panel displays changes in torque from the beginning to the end of each type of NMES train. * denotes $P < 0.05$ and ### denotes $P < 0.001$. Dots represent data from single participants.

Torque decline during fatigue protocols. Torque averaged across the 12 participants recorded during the $NMES_{SEQ}$ and $NMES_{CON}$ fatigue protocols is shown in Figure 2-10. Torque produced at the beginning of the fatigue protocols was not different between sessions ($p > 0.05$), as there was no main effect of session on torque produced over the first 10 contractions (Bin 1). On average, mean contraction amplitude over the first 10 contractions was 19 ± 2 % MVIC across 4

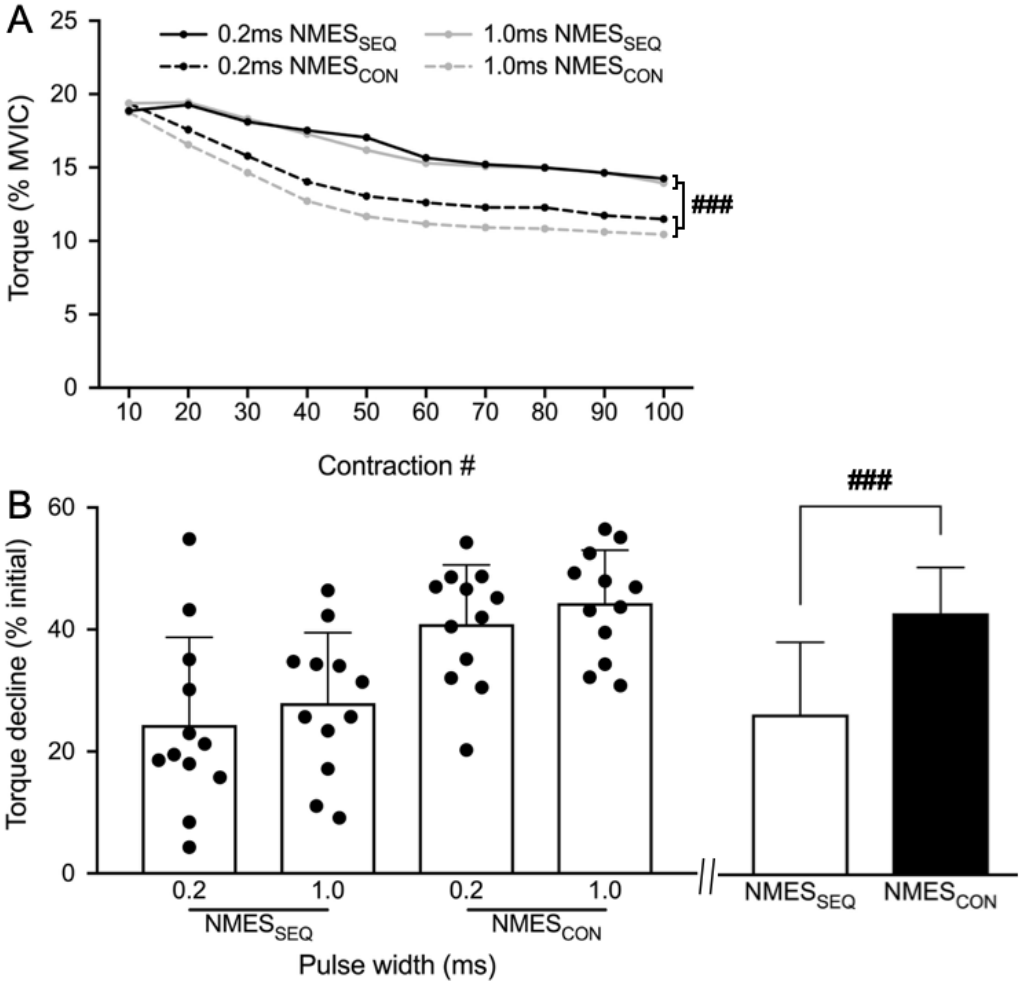

Figure 2-10. Torque recorded during the fatigue protocols delivered using $NMES_{SEQ}$ and $NMES_{CON}$ for the group of 12 participants. Panel A displays the torque binned over every 10 successive contractions of the fatigue protocols and averaged across participants. Panel B displays the percent decline in torque from the beginning (first 10 contractions) to the end (last 10 contractions) of the fatigue protocols. ### denotes $P < 0.001$. Dots represent data from single participants.

sessions. There was no main effect of pulse width on contraction fatigability ($F_{(1, 11)} = 2.173$, $p = 0.168$). There was, however, a main effect of NMES type ($F_{(1, 11)} = 35.260$, $p < 0.001$): torque declined more during $NMES_{CON}$ (43 ± 9 %) than $NMES_{SEQ}$ (26 ± 13 %). There was no interaction between NMES type and pulse width ($F_{(1, 11)} = 0.002$, $p = 0.969$). When contraction fatigability was also calculated by comparing the percent torque decline from the first contraction to the last contraction using the equation of Jeon and Griffin (2018), there was also a main effect of NMES type ($F_{(1, 11)} = 34.050$, $p < 0.001$), but no significant main effect of pulse width ($F_{(1, 11)} = 3.406$, $p = 0.092$) on contraction fatigability and there was no interaction between NMES type and pulse width ($F_{(1, 11)} = 0.119$, $p = 0.736$).

Percent changes in MVICs. There was no main effect of pulse width ($F_{(1, 11)} = 0.525$, $p = 0.484$) or NMES type ($F_{(1, 11)} = 0.862$, $p = 0.373$) on the percent changes in MVICs recorded before and after the fatigue protocols (data not shown). There was also no interaction between pulse width and NMES type ($F_{(1, 11)} = 0.746$, $p = 0.406$). The average changes in MVICs were 8 ± 8 % across NMES sessions.

2.4 Discussion

The main objective of this thesis research was to explore the effect of pulse width on contraction fatigability of the quadriceps muscles. Secondary objectives were to compare contraction fatigability between NMES types (i.e. $NMES_{SEQ}$ and $NMES_{CON}$) and discomfort between pulse widths and NMES types. We hypothesized that longer pulse widths would generate contractions with a greater central contribution and thus produce less contraction fatigability than shorter pulse widths. However, we found that pulse width did not influence either the central

contribution to the evoked contractions or contraction fatigability. Consistent with our hypothesis that $NMES_{CON}$ would produce more contraction fatigability than $NMES_{SEQ}$, $NMES_{CON}$ resulted in a percent of torque decline 1.5 times greater than $NMES_{SEQ}$. Meanwhile, discomfort was not influenced by either pulse width or NMES type.

In the present study, we used three different NMES trains to assess whether there was a central contribution to the evoked contractions. This central contribution was identified as an increase in torque over the course of those NMES trains. Torque only increased during NMES train B and C when $NMES_{SEQ}$ was delivered, and there was no relationship between pulse width and our measure of the central contribution. In addition, the observed central contribution was small (< 2 % MVIC) compared to what had been reported by Collins et al. (2001, 2002), who found that central contribution was up to 40 % MVIC in both triceps surae and tibialis anterior muscles. Thus, in the present study we found little or no evidence for a central contribution to the evoked contractions and thus, we conclude that contractions were generated predominantly by motor axons. Thus would explain why increased pulse width did not influence how torque changed over the course of those NMES trains or during the fatigue protocols. Bergquist et al. (2012) also found there was no contribution of central pathways to evoked contractions during both constant-frequency and step-frequency trains when the quadriceps muscles were stimulated using 1 ms pulse width. Therefore, the present results suggest that stimulation over the quadriceps muscles generated contractions predominantly by motor axons, regardless of pulse width or NMES type.

However, Bergquist, Clair, and Collins (2011) conducted similar experiments on the triceps surae muscles using 1 ms pulse widths. In those experiments, both torque and H-reflexes were

significantly augmented following 100-Hz NMES during step-frequency trains that were similar to NMES train B in the present study. The different results may be due to the different stimulation sites. NMES delivered over the nerve trunk can generate torque more via central pathways, compared to NMES delivered over the muscle belly, evidenced by the larger H-reflexes when stimulating the tibial and femoral nerves than stimulating the triceps surae and quadriceps muscles (Bergquist, Clair, & Collins, 2011; Bergquist et al., 2012). Furthermore, reflex pathways for the quadriceps muscles may be weaker than those for the triceps surae muscle. For example, when stimulating the femoral nerves, the H-max to M-max ratio was 0.2 ± 0.1 mV (Bergquist et al., 2012), whereas the H-max to M-max ratio was three times greater (0.6 ± 0.1 mV) when tibial nerves were stimulated (Bergquist, Clair, & Collins, 2011) using the same pulse width (1 ms) and waveform (monopolar). Therefore, the small role or the absence of "central contribution" during NMES trains applied over the quadriceps muscles may be due to the weaker reflex pathways to those muscles.

Contrary to our hypothesis that longer pulse widths would produce less contraction fatigability than shorter pulse widths, pulse width did not influence contraction fatigability. In the present study, we tested a wide range of pulse widths (0.2 – 2 ms) during $NMES_{SEQ}$ and we also compared contraction fatigability between $NMES_{SEQ}$ and $NMES_{CON}$ using 0.2 and 1 ms pulse widths, based on the rationale that Jeon & Griffin (2018) showed 1 ms pulse widths produced less contraction fatigability than 0.2 ms pulse widths during $NMES_{CON}$. We believe that the lack of an effect of pulse width on contraction fatigability was due to the fact that contractions were generated predominantly by motor axons. Therefore, during the fatigue protocols, all pulse widths recruited MUs in a similar order, which resulted in a similar contraction fatigability.

Gorgey et al. (2009), Bickel et al. (2012) and Papaiordanidou et al. (2014) also reported that pulse width did not influence contraction fatigability of the quadriceps and soleus muscles during NMES, even though they used a small range of pulse widths (0.2 – 0.6 ms or 0.5 – 1ms). Our results are consistent with these studies, since altering pulse width either from 0.2 to 0.5 ms or from 0.5 to 1 ms did not change the contraction fatigability produced during $NMES_{SEQ}$.

However, our results differ from those of Jeon and Griffin (2018) who showed that 1 ms pulse widths produced less contraction fatigability than 0.2 ms pulse widths. It is difficult to explain the different results shown by these two studies, although differences in the fatigue protocols may be responsible. For example, Jeon and Griffin (2018) delivered $NMES_{CON}$ at 30 Hz to generate 80 contractions (0.3 s on/0.7 s off), whereas we delivered $NMES_{CON}$ at 40 Hz to generate 100 contractions (1 s on/1 s off). Alternatively, the "central contribution" is highly variable between individuals (Wegrzyk et al., 2015), which may be related to the different intrinsic properties of the quadriceps muscles (Lieber & Kelly, 1991). Lieber and Kelly observed large variation in the efficiency of NMES between individuals, regardless of current intensity and electrode size, and this those who responded well to the stimulation were referred to as responders, those who did not were referred to as non-responders. Therefore, the study of Jeon and Griffin (2018) may have included more participants who are responders than the present study.

Consistent with our hypothesis, more contraction fatigability was produced by $NMES_{CON}$ than $NMES_{SEQ}$. This finding supports a growing body of evidence showing that $NMES_{SEQ}$ results in less contraction fatigability than $NMES_{CON}$ (Bergquist et al., 2017; Downey et al., 2015;

Laubacher et al., 2017; Malešević et al., 2010; Maneski et al., 2013; Nguyen et al., 2011; Popovic & Malesevic, 2009; and Sayenko et al., 2013). In the present study, $NMES_{CON}$ generated 1.5 times more contraction fatigability than $NMES_{SEQ}$. In addition, for NMES train A and C, torque decreased during $NMES_{CON}$ rather than during $NMES_{SEQ}$, which likely due to the lower MU discharge rates during $NMES_{SEQ}$ than $NMES_{CON.}$ These present results highlight the importance of utilizing $NMES_{SEQ}$ in clinical NMES programs. In terms of discomfort, there were no differences between pulse widths or NMES types. Gregory et al. (2007) reported that discomfort was not influenced by pulse width, however, they did not explore the independent effect of pulse width on discomfort – they compared the discomfort produced by wide-pulse and low-frequency NMES versus narrow-pulse and high-frequency NMES. Interestingly, a different result was reported by Liebano et al. (2013). When they kept pulse width constant but increased pulse charge, which is the product of pulse width and current amplitude, no changes in discomfort occurred, however, increasing pulse width increased the discomfort level. This finding suggests that pulse width influences discomfort more than current amplitude. Recently, in contrast, Jeon and Griffin (2018) suggested that current amplitude affected discomfort more than pulse width, as higher current amplitude can active more small-diameter afferents, such as A-delta fibers and some unmyelinated C-fibers, and trigger pain pathways. In the present study, pulse width did not influence discomfort, even though the current was greater when 0.2 ms pulse width was used than the other wider pulse widths. Overall, VAS is a subjective scale, and the values rated by the same participant between sessions may have varied a lot.

In conclusion, in the present study, pulse width did not influence contraction fatigability of the quadriceps muscles. We propose that this was because contractions were generated

predominantly by the stimulation of motor axons, as there was little or no evidence for a central contribution to the evoked contractions during the three types of NMES trains. However, $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$ and we suggest that this was as a result of decreased MU discharge rates. Discomfort was not influenced by either pulse width or NMES type. Thus, the present study shows that to reduce contraction fatigability when stimulating over the quadriceps muscles, increasing pulse width to recruit motor units in the natural order is not effective, however, delivering NMES through multiple electrodes to reduce MU discharge rates, as during $NMES_{SEQ}$ is effective. Thus, we suggest the use of $NMES_{SEQ}$ for NMES-based rehabilitation programs.

CHAPTER 3. GENERAL DISCUSSION

The main objective of this thesis work was to explore the effect of pulse width on contraction fatigability of the quadriceps muscles. Contraction fatigability is a major limiting factor to the benefits of FES programs and we hypothesized that one way to reduce contraction fatigability was to use longer pulse widths. A secondary objective was to compare contraction fatigability between $NMES_{SEQ}$ and $NMES_{CON}$, because $NMES_{SEQ}$ has been suggested to produce less contraction fatigability than $NMES_{CON}$. The other secondary objective was to compare discomfort between pulse widths and NMES types, given that discomfort is also a barrier to the benefits of FES programs for some individuals. In this chapter, a summary of the findings is presented in Section 3.1, followed by description of clinical implications of this thesis work in Section 3.2. Section 3.3 and 3.4 include limitations and future directions, respectively, and Section 3.5 provides a summary of this thesis work.

3.1 Overview

The primary outcome of this thesis work was the finding that pulse width did not influence contraction fatigability of the quadriceps muscles during NMES. As a parameter of NMES, pulse width influences how MUs are recruited and thus how torque is generated. Generally, an increase in pulse width at a given current amplitude delivers more charge and depolarizes more axons, which in turn recruits more MUs and generates more torque (Bergquist, Clair, Lagerquist, et al., 2011; Gregory et al., 2007). Pulse width, however, also plays a role in the relative recruitment of sensory and motor axons during NMES (Barss et al., 2018; Bergquist, Clair, Lagerquist, et al., 2011; Bostock et al., 1998; Burke et al., 2001). As mentioned in Chapter 1 (see Section 1.3.3), sensory axons have longer strength-duration time constants (T_{SD}) and lower rheobase than motor

axons (Mogyoros, Kiernan, & Burke, 1996), thus, delivering longer pulse widths favors the activation of sensory axons over motor axons and generates contractions with a larger contribution from "central pathways" through the spinal cord. As MUs recruited through central pathways follow their natural recruitment order, with fatigue-resistant MUs recruited first (Henneman, Somjen, & Carpenter, 1965), we expected that longer pulse widths would recruit more MUs through central pathways and thus reduce contraction fatigability that arises due to the unnatural MU recruitment order. However, we did not find any evidence to support the idea that longer pulse widths (1 ms and 2 ms) generated contractions with a greater central contribution than shorter pulse widths (0.2 ms and 0.5 ms). In fact, we found little if any evidence for a central contribution to the evoked contractions in the present study. The central contribution was assessed by comparing the changes in torque between pulse widths during three different NMES trains. This analysis was based on previous studies showing that torque increased up to 40 % MVIC during trains at low intensity and high frequency (>80 Hz, see Figure 3-1), however, this increase in torque did not occur when the nerve was blocked between central pathways and NMES electrodes (Collins et al., 2001, 2002). This result therefore indicates that the increase in torque was due to the contribution of central pathways. The lack of central contribution to

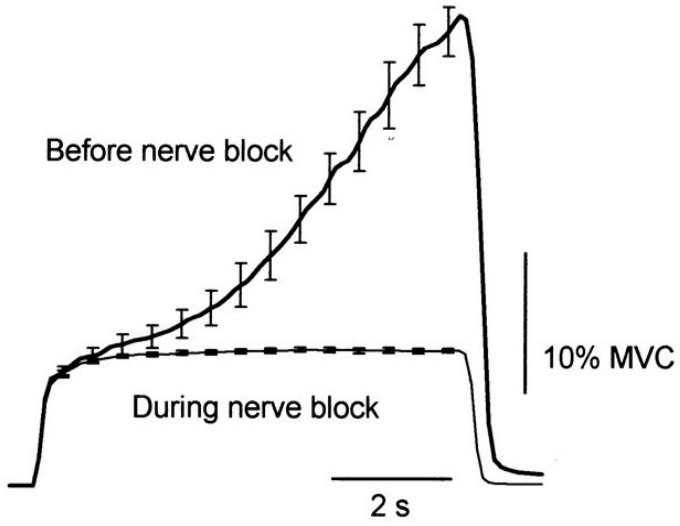

Figure 3-1. Torque generated by the triceps surae muscle recorded from one participant before and during the tibial nerve was blocked. The constant frequency used was 100 Hz (Collins et al. 2001).

contractions in the present study can explain why longer pulse widths did not produce less contraction fatigability than shorter pulse widths – since predominantly peripheral pathways contributed to the recruitment of MUs during the fatigue protocols, regardless of pulse widths. Therefore, MUs were recruited in a similar way with all pulse widths, resulting in a similar decline in torque by the end of the fatigue protocols.

A secondary outcome of this thesis work was the finding that $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$. Less contraction fatigability produced by $NMES_{SEQ}$ was expected because $NMES_{SEQ}$ was designed to reduce contraction fatigability by rotating stimulus pulses between several electrodes placed over the skin of muscle belly to reduce MU discharge rates compared to $NMES_{CON}$ (Bergquist et al., 2017; Downey et al., 2015; Laubacher et al., 2017; Malešević et al., 2010; Nguyen et al., 2011; Popovic & Malesevic, 2009; Sayenko et al., 2013). Thus, the present findings provide further evidence of this result and also show that this difference between $NMES_{SEQ}$ and $NMES_{CON}$ is independent of pulse width.

Another secondary outcome was the finding that discomfort was not influenced by either the pulse width or NMES type. Liebano et al. (2013) had previously reported that pulse width influenced discomfort more than current amplitude, whereas Jeon and Griffin (2018) suggested current amplitude plays a more important role in discomfort than pulse width. This thesis work did not find any differences in discomfort between high current-amplitude and short pulse-width NMES versus low current-amplitude and long pulse-width NMES. Therefore, it was not clear whether current amplitude or pulse width played a more important role in discomfort in the present study. Furthermore, to our knowledge, the effect of $NMES_{SEQ}$ versus $NMES_{CON}$ on

discomfort has not been compared, and thus this finding is not supported by any previous studies. Given that discomfort was assessed using the self-rated scale (VAS), the variation of VAS scores may vary a lot for a single participant during different sessions and contribute to those different results.

3.2 Clinical implications

In rehabilitation programs, NMES is used to restore functional movement or reduce secondary complications of disuse (Hamid & Hayek, 2008). However, during $NMES_{CON}$, the high discharge rates and unnatural recruitment order of MUs produce contraction fatigability rapidly, limiting the duration and intensity of NMES programs. Our results suggest that it may not be possible to reduce contraction fatigability by recruiting MUs in their natural order using wide pulse widths during stimulation over the quadriceps muscles. However, our results contribute to a growing body of evidence suggesting that $NMES_{SEQ}$ should replace $NMES_{CON}$ during rehabilitation programs that use NMES as a treatment. $NMES_{SEQ}$ produces less contraction fatigability than $NMES_{CON}$, and therefore, should allow participants to maintain a target contraction level for longer than when using $NMES_{CON}$. Given that pulse width did not affect contraction fatigability or discomfort, we recommend using 0.5 ms pulse width for NMES-based programs that need a high contraction amplitude. This is because 0.5 ms pulse width can generate more torque than 0.2 ms pulse width and increasing pulse width to 1 or 2 ms does not produce more torque.

3.3 Limitations

We did not record the electromyography (EMG) activity from the muscles that were stimulated in the present study, and this was one of the limitations of this thesis work. EMG is a more direct

way to measure the central and peripheral recruitment during NMES than the torque measurements currently used and would provide more conclusive evidence of the relative central and peripheral contributions to contractions. However, as we currently used 6 stimulating electrodes during $NMES_{SEQ}$, there was little room to place EMG electrodes on the legs of some participants. Moreover, EMG signals would be contaminated by large stimulation artifacts, particularly when they are placed close to the stimulating electrodes as would be the case for $NMES_{SEQ}$. Furthermore, it would not be possible to measure H-reflexes in the EMG signals as their latency (40 – 60 ms) is longer than the stimulation period (25 ms) when NMES was delivered at 40 Hz.

In addition, the sample size (n=12) in the present study was small, and thus our sample may not have been included people who are "responders", individuals who have a large contribution via central pathways to evoked contractions when stimulating the quadriceps muscles. Furthermore, the participants of the present study were individuals without any neurological impairments, however, NMES is most commonly used in individuals with neurological or musculoskeletal impairments. Typically, after periods of disuse, there is an increase in the proportion of type II muscle fibers that fatigue rapidly (Biering-Sorensen, Kristensen, Kjaer, & Biering-Sorensen, 2009; Burnham et al., 1997) and axons below the lesion have decrease in excitability (Lin et al., 2007) after a SCI, compared to able-bodied individuals. The fatigue-prone properties of type II muscle fibers can result in contraction fatigability through the breakdown of excitation-contraction coupling and the deceased axonal excitability contributes to the contraction fatigability especially during high-frequency NMES. Thus, these physiological differences may

augment or attenuate the differences in contraction fatigability between $NMES_{SEQ}$ and $NMES_{CON}$.

3.4 Future directions

Future studies should compare the contraction fatigability over a wide range of pulse widths when delivering stimulus pulses over the femoral nerve, where the central contribution to the evoked contractions is stronger than when stimulating the quadriceps muscles (Bergquist et al., 2012). Thus, unlike contractions generated predominantly through peripheral pathways when stimulating the quadriceps muscles, central pathways would likely play a significant role in generating contractions when stimulating the femoral nerve. In this case, the proportion of contractions generated through central pathways may be more strongly influenced by pulse width and longer pulse widths may produce less contraction fatigability than shorter pulse widths.

Future studies could also explore the effect of pulse width on contraction fatigability of other muscles such as the triceps surae or tibialis anterior muscles, which can generate contractions with a large central contribution during both NMES over the muscle belly or nerve trunk (Collins et al., 2001, 2002). Thus, the effect of pulse width on contraction fatigability may also be more prevalent in those muscles than in the quadriceps. This effect of pulse width could be explored for both $NMES_{CON}$ and $NMES_{SEQ}$.

Furthermore, future studies could involve a large sample size to explore the effect of pulse width on contraction fatigability in individuals who have a SCI, to include those who have contribution of central pathways during the quadriceps muscles stimulation. In this case, longer pulse widths

may produce less contraction fatigability than shorter pulse widths in those individuals by generating a greater central contribution.

For clinical use, future research could involve "training" studies that compare the effect of $NMES_{SEQ}$ versus $NMES_{CON}$ on physiological indices, such as heart rate, muscle strength, peak oxygen uptake and MU type, in individuals with a SCI after long-term FES exercise programs. This idea is based on the finding that $NMES_{SEQ}$ produced less contraction fatigability than $NMES_{CON}$, and thus contractions can be maintained at a target amplitude for longer during $NMES_{SEQ}$ than during $NMES_{CON}$ and the benefits to individuals with a SCI would be greater. This is important because it can provide important evidence to further the development and translation of this technology into FES-based programs.

3.5 Summary

This thesis work shows that $NMES_{SEQ}$, rather than longer pulse widths, produces less contraction fatigability when stimulating the quadriceps muscles. This result suggests the use of $NMES_{SEQ}$ for NMES-based rehabilitation programs. This thesis work did not assess the central contribution to evoked contractions in a direct way (EMG), and thus cannot provide more conclusive evidence of relative central and peripheral contributions to contractions. However, it is more important to explore the benefits of $NMES_{SEQ}$ to individuals with a SCI after a longer-term FES training as this may further the development and translation of this technology into FES-based programs.

REFERENCE

Adrian, E. D. (1914). The all-or-none principle in nerve. *J Physiol, 47*(6), 460-474.

Allen, D. G., Westerblad, H., Lee, J. A., & Lännergren, J. J. S. M. (1992). Role of Excitation-Contraction Coupling in Muscle Fatigue. *13*(2), 116-126. doi:10.2165/00007256-199213020-00007

Alon, G., Levitt, A. F., & McCarthy, P. A. (2007). Functional electrical stimulation enhancement of upper extremity functional recovery during stroke rehabilitation: a pilot study. *Neurorehabil Neural Repair, 21*(3), 207-215. doi:10.1177/1545968306297871

Barss, T. S., Ainsley, E. N., Claveria-Gonzalez, F. C., Luu, M. J., Miller, D. J., Wiest, M. J., & Collins, D. F. (2018). Utilizing Physiological Principles of Motor Unit Recruitment to Reduce Fatigability of Electrically-Evoked Contractions: A Narrative Review. *Arch Phys Med Rehabil, 99*(4), 779-791. doi:10.1016/j.apmr.2017.08.478

Bax, L., Staes, F., & Verhagen, A. (2005). Does neuromuscular electrical stimulation strengthen the quadriceps femoris? A systematic review of randomised controlled trials. *Sports Med, 35*(3), 191-212. doi:10.2165/00007256-200535030-00002

Bergquist, A. J., Babbar, V., Ali, S., Popovic, M. R., & Masani, K. (2017). Fatigue reduction during aggregated and distributed sequential stimulation. *56*(2), 271-281. doi:10.1002/mus.25465

Bergquist, A. J., Clair, J. M., & Collins, D. F. (2011). Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae. *J Appl Physiol (1985), 110*(3), 627-637. doi:10.1152/japplphysiol.01103.2010

Bergquist, A. J., Clair, J. M., Lagerquist, O., Mang, C. S., Okuma, Y., & Collins, D. F. (2011). Neuromuscular electrical stimulation: implications of the electrically evoked sensory volley. *Eur J Appl Physiol, 111*(10), 2409-2426. doi:10.1007/s00421-011-2087-9

Bergquist, A. J., Wiest, M. J., & Collins, D. F. (2012). Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: quadriceps femoris. *J Appl Physiol (1985), 113*(1), 78-89. doi:10.1152/japplphysiol.00074.2011

Bergquist, A. J., Wiest, M. J., Okuma, Y., & Collins, D. F. (2014). H-reflexes reduce fatigue of evoked contractions after spinal cord injury. *50*(2), 224-234. doi:10.1002/mus.24144

Bickel, C. S., Gregory, C. M., & Azuero, A. (2012). Matching initial torque with different stimulation parameters influences skeletal muscle fatigue. *J Rehabil Res Dev, 49*(2), 323-331.

Bickel, C. S., Gregory, C. M., & Dean, J. C. (2011). Motor unit recruitment during neuromuscular electrical stimulation: a critical appraisal. *Eur J Appl Physiol, 111*(10), 2399-2407. doi:10.1007/s00421-011-2128-4

Biering-Sorensen, B., Kristensen, I. B., Kjaer, M., & Biering-Sorensen, F. (2009). Muscle after spinal cord injury. *Muscle Nerve, 40*(4), 499-519. doi:10.1002/mus.21391

Bigland-Ritchie, B., Zijdewind, I., & Thomas, C. K. (2000). Muscle fatigue induced by stimulation with and without doublets. *Muscle Nerve, 23*(9), 1348-1355.

Bijak, M., Rakos, M., Hofer, C., Mayr, W., Strohhofer, M., Raschka, D., & Kern, H. (2005). Stimulation parameter optimization for FES supported standing up and walking in SCI patients. *Artif Organs, 29*(3), 220-223. doi:10.1111/j.1525-1594.2005.29039.x

Binder-Macleod, S. A., Halden, E. E., & Jungles, K. A. (1995). Effects of stimulation intensity on the physiological responses of human motor units. *Med Sci Sports Exerc, 27*(4), 556-565.

Binder-Macleod, S. A., & Snyder-Mackler, L. (1993). Muscle fatigue: clinical implications for fatigue assessment and neuromuscular electrical stimulation. *Phys Ther, 73*(12), 902-910.

Bostock, H., Cikurel, K., & Burke, D. (1998). Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve, 21*(2), 137-158.

Bostock, H., & Grafe, P. (1985). Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. *J Physiol, 365*, 239-257.

Buffington, S. A., & Rasband, M. N. (2013). Chapter 35 - Structure and Function of Myelinated Axons. In J. L. R. Rubenstein & P. Rakic (Eds.), *Patterning and Cell Type Specification in the Developing CNS and PNS* (pp. 707-722). Oxford: Academic Press.

Burke, D., Kiernan, M. C., & Bostock, H. (2001). Excitability of human axons. *Clin Neurophysiol, 112*(9), 1575-1585.

Burnham, R., Martin, T., Stein, R., Bell, G., MacLean, I., & Steadward, R. (1997). Skeletal muscle fibre type transformation following spinal cord injury. *Spinal Cord, 35*(2), 86-91.

Bussel, B. (2015). History of electrical stimulation in rehabilitation medicine. *Ann Phys Rehabil Med, 58*(4), 198-200. doi:10.1016/j.rehab.2015.04.008

Calderón, J. C., Bolaños, P., & Caputo, C. (2014). The excitation-contraction coupling mechanism in skeletal muscle. *Biophys Rev, 6*(1), 133-160. doi:10.1007/s12551-013-0135-x

Carmick, J. (1993). Clinical use of neuromuscular electrical stimulation for children with cerebral palsy, Part 1: Lower extremity. *Phys Ther, 73*(8), 505-513; discussion 523-507.

Claveria-González, F. C. (2018). Comparison of three types of neuromuscular electrical stimulation on fatigability in the quadricpes muscles (Unpublished master's thesis). University of Alberta, Edmonton, Canada.

Collins, D. F. (2007). Central contributions to contractions evoked by tetanic neuromuscular electrical stimulation. *Exerc Sport Sci Rev, 35*(3), 102-109. doi:10.1097/jes.0b013e3180a0321b

Collins, D. F., Burke, D., & Gandevia, S. C. (2001). Large involuntary forces consistent with plateau-like behavior of human motoneurons. *J Neurosci, 21*(11), 4059-4065.

Collins, D. F., Burke, D., & Gandevia, S. C. (2002). Sustained contractions produced by plateau-like behaviour in human motoneurones. *J Physiol, 538*(Pt 1), 289-301.

Crognale, D., Crowe, L., Devito, G., Minogue, C., & Caulfield, B. (2009). Neuro-muscular electrical stimulation training enhances maximal aerobic capacity in healthy physically active adults. *Conf Proc IEEE Eng Med Biol Soc, 2009*, 2137-2140. doi:10.1109/iembs.2009.5333972

Deley, G., Denuziller, J., & Babault, N. (2015). Functional electrical stimulation: cardiorespiratory adaptations and applications for training in paraplegia. *Sports Med, 45*(1), 71-82. doi:10.1007/s40279-014-0250-2

Delitto, A., Strube, M. J., Shulman, A. D., & Minor, S. D. (1992). A study of discomfort with electrical stimulation. *Phys Ther, 72*(6), 410-421; discussion on 421-414.

Downey, R. J., Bellman, M. J., Kawai, H., Gregory, C. M., & Dixon, W. E. (2015). Comparing the Induced Muscle Fatigue Between Asynchronous and Synchronous Electrical Stimulation in Able-Bodied and Spinal Cord Injured Populations. *IEEE Trans Neural Syst Rehabil Eng, 23*(6), 964-972. doi:10.1109/TNSRE.2014.2364735

Enoka, R. M. (2008). *Neuromechanics of Human Movement*: Human Kinetics.
Everaert, D., B Stein, R., Abrams, G., Dromerick, A., Francisco, G., Hafner, B., . . . V Kufta, C. (2013). *Effect of a Foot-Drop Stimulator and Ankle-Foot Orthosis on Walking Performance After Stroke: A Multicenter Randomized Controlled Trial* (Vol. 27).
Gibbons, R. S., Stock, C. G., Andrews, B. J., Gall, A., & Shave, R. E. (2016). The effect of FES-rowing training on cardiac structure and function: pilot studies in people with spinal cord injury. *Spinal Cord, 54*, 822. doi:10.1038/sc.2015.228
Glaviano, N. R., & Saliba, S. (2016). Can the Use of Neuromuscular Electrical Stimulation Be Improved to Optimize Quadriceps Strengthening? *Sports Health, 8*(1), 79-85. doi:10.1177/1941738115618174
Gorgey, A. S., Black, C. D., Elder, C. P., & Dudley, G. A. (2009). Effects of electrical stimulation parameters on fatigue in skeletal muscle. *J Orthop Sports Phys Ther, 39*(9), 684-692. doi:10.2519/jospt.2009.3045
Gorgey, A. S., & Dudley, G. A. (2008). The role of pulse duration and stimulation duration in maximizing the normalized torque during neuromuscular electrical stimulation. *J Orthop Sports Phys Ther, 38*(8), 508-516. doi:10.2519/jospt.2008.2734
Gorgey, A. S., Mahoney, E., Kendall, T., & Dudley, G. A. J. E. J. o. A. P. (2006). Effects of neuromuscular electrical stimulation parameters on specific tension. *97*(6), 737-744. doi:10.1007/s00421-006-0232-7
Gorgey, A. S., Poarch, H. J., Dolbow, D. D., Castillo, T., & Gater, D. R. (2014). Effect of adjusting pulse durations of functional electrical stimulation cycling on energy expenditure and fatigue after spinal cord injury. *J Rehabil Res Dev, 51*(9), 1455-1468. doi:10.1682/jrrd.2014.02.0054
Gregory, C. M., Dixon, W., & Bickel, C. S. (2007). Impact of varying pulse frequency and duration on muscle torque production and fatigue. *Muscle Nerve, 35*(4), 504-509. doi:10.1002/mus.20710
Hainaut, K., & Duchateau, J. J. S. M. (1992). Neuromuscular Electrical Stimulation and Voluntary Exercise. *14*(2), 100-113. doi:10.2165/00007256-199214020-00003
Hamid, S., & Hayek, R. (2008). Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: an overview. *Eur Spine J, 17*(9), 1256-1269. doi:10.1007/s00586-008-0729-3
Hendry, S. (2016). Post-Synaptic Potentials and Action Potentials: Membrane Potentials. In D. W. Pfaff & N. D. Volkow (Eds.), *Neuroscience in the 21st Century: From Basic to Clinical* (pp. 113-136). New York, NY: Springer New York.
Henneman, E., Somjen, G., & Carpenter, D. O. (1965). FUNCTIONAL SIGNIFICANCE OF CELL SIZE IN SPINAL MOTONEURONS. *J Neurophysiol, 28*, 560-580. doi:10.1152/jn.1965.28.3.560
Hille, B. (1992). *Ionic Channels of Excitable Membranes*: Oxford University Press, Incorporated.
Hoff, H. E. (1936). Galvani and the pre-Galvanian electrophysiologists. *Annals of Science, 1*(2), 157-172. doi:10.1080/00033793600200131
Ho, C. H., Triolo, R. J., Elias, A. L., Kilgore, K. L., DiMarco, A. F., Bogie, K., . . . Mushahwar, V. K. (2014). Functional electrical stimulation and spinal cord injury. *Phys Med Rehabil Clin N Am, 25*(3), 631-654, ix. doi:10.1016/j.pmr.2014.05.001

Jailani, R., & Tokhi, M. O. (2012, 23-25 March 2012). *The effect of functional electrical stimulation (FES) on paraplegic muscle fatigue.* Paper presented at the 2012 IEEE 8th International Colloquium on Signal Processing and its Applications.

Jeon, W., & Griffin, L. (2018). Effects of pulse duration on muscle fatigue during electrical stimulation inducing moderate-level contraction. *Muscle Nerve, 57*(4), 642-649. doi:10.1002/mus.25951

Jones, D. A., Bigland-Ritchie, B., & Edwards, R. H. T. (1979). Excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions. *Experimental Neurology, 64*(2), 401-413. doi:https://doi.org/10.1016/0014-4886(79)90279-6

Jubeau, M., Gondin, J., Martin, A., Sartorio, A., & Maffiuletti, N. A. (2007). Random motor unit activation by electrostimulation. *Int J Sports Med, 28*(11), 901-904. doi:10.1055/s-2007-965075

Kandel, E. R., Jessell, T. M., Schwartz, J. H., Siegelbaum, S. A., & Hudspeth, A. J. (2013). *Principles of Neural Science, Fifth Edition*: McGraw-Hill Education.

Keeton, R. B., & Binder-Macleod, S. A. (2006). Low-Frequency Fatigue. *Phys Ther, 86*(8), 1146-1150. doi:10.1093/ptj/86.8.1146 %J Physical Therapy

Kesar, T., & Binder-Macleod, S. (2006). Effect of frequency and pulse duration on human muscle fatigue during repetitive electrical stimulation. *91*(6), 967-976. doi:doi:10.1113/expphysiol.2006.033886

Kesar, T., Chou, L. W., & Binder-Macleod, S. A. (2008). Effects of stimulation frequency versus pulse duration modulation on muscle fatigue. *J Electromyogr Kinesiol, 18*(4), 662-671. doi:10.1016/j.jelekin.2007.01.001

Kiernan, M. C., & Lin, C. S. Y. (2012). Chapter 15 Nerve Excitability A Clinical Translation. In *Aminoff's Electrodiagnosis in Clinical Neurology* (pp. 345-365).

Kim, C. M., Eng, J. J., & Whittaker, M. W. (2004). Effects of a simple functional electric system and/or a hinged ankle-foot orthosis on walking in persons with incomplete spinal cord injury. *Archives of Physical Medicine and Rehabilitation, 85*(10), 1718-1723. doi:https://doi.org/10.1016/j.apmr.2004.02.015

Kiss, T. (2008). Persistent Na-channels: origin and function. A review. *Acta Biol Hung, 59 Suppl*, 1-12. doi:10.1556/ABiol.59.2008.Suppl.1

Kuo, I. Y., & Ehrlich, B. E. (2015). Signaling in muscle contraction. *Cold Spring Harbor perspectives in biology, 7*(2), a006023-a006023. doi:10.1101/cshperspect.a006023

Lagerquist, O., & Collins, D. F. (2008). Stimulus pulse-width influences H-reflex recruitment but not Hmax/Mmax ratio. *37*(4), 483-489. doi:10.1002/mus.20957

Lagerquist, O., & Collins, D. F. (2010). Influence of stimulus pulse width on M-waves, H-reflexes, and torque during tetanic low-intensity neuromuscular stimulation. *Muscle Nerve, 42*(6), 886-893. doi:10.1002/mus.21762

Lake, D. A. J. S. M. (1992). Neuromuscular Electrical Stimulation. *13*(5), 320-336. doi:10.2165/00007256-199213050-00003

Lamb, G. D. (2002). Excitation-contraction coupling and fatigue mechanisms in skeletal muscle: studies with mechanically skinned fibres. *J Muscle Res Cell Motil, 23*(1), 81-91.

Langeard, A., Bigot, L., Chastan, N., & Gauthier, A. (2017). Does neuromuscular electrical stimulation training of the lower limb have functional effects on the elderly?: A systematic review. *Exp Gerontol, 91*, 88-98. doi:10.1016/j.exger.2017.02.070

Laubacher, M., Aksöz, A. E., Riener, R., Binder-Macleod, S., & Hunt, K. J. J. E. J. o. A. P. (2017). Power output and fatigue properties using spatially distributed sequential stimulation in a dynamic knee extension task. *117*(9), 1787-1798. doi:10.1007/s00421-017-3675-0

Liebano, R. E., Rodrigues, T. A., Murazawa, M. T., & Ward, A. R. (2013). The influence of stimulus phase duration on discomfort and electrically induced torque of quadriceps femoris. *Brazilian Journal of Physical Therapy, 17*, 479-486.

Lieber, R. L., & Kelly, M. J. (1991). Factors influencing quadriceps femoris muscle torque using transcutaneous neuromuscular electrical stimulation. *Phys Ther, 71*(10), 715-721; discussion 722-713. doi:10.1093/ptj/71.10.715

Lin, C. S.-Y., Macefield, V. G., Elam, M., Gunnar Wallin, B., Engel, S., & Kiernan, M. C. (2007). Axonal changes in spinal cord injured patients distal to the site of injury. *Brain, 130*(4), 985-994. doi:10.1093/brain/awl339 %J Brain

Lorenz, C., & Jones, K. E. (2014). IH activity is increased in populations of slow versus fast motor axons of the rat. *8*(766). doi:10.3389/fnhum.2014.00766

Lou, J. W., Bergquist, A. J., Aldayel, A., Czitron, J., & Collins, D. F. (2017). Interleaved neuromuscular electrical stimulation reduces muscle fatigue. *Muscle Nerve, 55*(2), 179-189. doi:10.1002/mus.25224

Luu, M. J. (2017). Decrease in the excitability of motor axons contribute substantially to contraction fatigability during neuromuscular electrical stimulation (Unpublished master's thesis). University of Alberta, Edmonton, Canada.

MacIntosh, F. C., & Collier, B. (1976). Neurochemistry of Cholinergic Terminals. In E. Zaimis & J. Maclagan (Eds.), *Neuromuscular Junction* (pp. 99-228). Berlin, Heidelberg: Springer Berlin Heidelberg.

Malešević, N. M., Popović, L. Z., Schwirtlich, L., & Popović, D. B. (2010). Distributed low-frequency functional electrical stimulation delays muscle fatigue compared to conventional stimulation. *42*(4), 556-562. doi:10.1002/mus.21736

Maneski, L. Z. P., Malešević, N. M., Savić, A. M., Keller, T., & Popović, D. B. (2013). Surface-distributed low-frequency asynchronous stimulation delays fatigue of stimulated muscles. *48*(6), 930-937. doi:10.1002/mus.23840

Martyn, J. A. J., Fagerlund, M. J., & Eriksson, L. I. (2009). Basic principles of neuromuscular transmission. *64*(s1), 1-9. doi:doi:10.1111/j.1365-2044.2008.05865.x

Matkowski, B., Lepers, R., & Martin, A. (2015). Torque decrease during submaximal evoked contractions of the quadriceps muscle is linked not only to muscle fatigue. *118*(9), 1136-1144. doi:10.1152/japplphysiol.00553.2014

McCormick, D. A. (2014). Chapter 12 - Membrane Potential and Action Potential. In J. H. Byrne, R. Heidelberger, & M. N. Waxham (Eds.), *From Molecules to Networks (Third Edition)* (pp. 351-376). Boston: Academic Press.

Metzger, J. M., & Fitts, R. H. (1986). Fatigue from high- and low-frequency muscle stimulation: role of sarcolemma action potentials. *Exp Neurol, 93*(2), 320-333.

Mogyoros, I., Kiernan, M. C., & Burke, D. (1996). Strength-duration properties of human peripheral nerve. *Brain, 119 (Pt 2)*, 439-447.

Nguyen, R., Masani, K., Micera, S., Morari, M., & Popovic, M. R. (2011). Spatially distributed sequential stimulation reduces fatigue in paralyzed triceps surae muscles: a case study. *Artif Organs, 35*(12), 1174-1180. doi:10.1111/j.1525-1594.2010.01195.x

Ortenblad, N., Westerblad, H., & Nielsen, J. (2013). Muscle glycogen stores and fatigue. *J Physiol, 591*(18), 4405-4413. doi:10.1113/jphysiol.2013.251629
Papaiordanidou, M., Stevenot, J.-D., Mustacchi, V., Vanoncini, M., & Martin, A. (2014). Electrically induced torque decrease reflects more than muscle fatigue. *50*(4), 604-607. doi:10.1002/mus.24276
Peckham, P. H., Ackermann, D. M., & Moss, C. W. (2013). Chapter II.5.12 - The Role of Biomaterials in Stimulating Bioelectrodes. In B. D. Ratner, A. S. Hoffman, F. J. Schoen, & J. E. Lemons (Eds.), *Biomaterials Science (Third Edition)* (pp. 981-996): Academic Press.
Peckham, P. H., & Knutson, J. S. (2005). Functional electrical stimulation for neuromuscular applications. *Annu Rev Biomed Eng, 7*, 327-360. doi:10.1146/annurev.bioeng.6.040803.140103
Petrofsky, J. S. (1979). Sequential motor unit stimulation through peripheral motor nerves in the cat. *Medical and Biological Engineering and Computing, 17*(1), 87-93. doi:10.1007/BF02440958
Phillips, W., Burkett, L. N., Munro, R., Davis, M., & Pomeroy, K. (1995). Relative changes in blood flow with functional electrical stimulation during exercise of the paralyzed lower limbs. *Spinal Cord, 33*(2), 90-93. doi:10.1038/sc.1995.21
Pierrot-Deseilligny, E., & Mazevet, D. (2000). The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. *Neurophysiologie Clinique/Clinical Neurophysiology, 30*(2), 67-80. doi:https://doi.org/10.1016/S0987-7053(00)00062-9
Place, N., Yamada, T., Bruton, J. D., & Westerblad, H. J. E. J. o. A. P. (2010). Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres. *110*(1), 1-15. doi:10.1007/s00421-010-1480-0
Popovic, L. Z., & Malesevic, N. M. (2009). Muscle fatigue of quadriceps in paraplegics: comparison between single vs. multi-pad electrode surface stimulation. *Conf Proc IEEE Eng Med Biol Soc, 2009*, 6785-6788. doi:10.1109/iembs.2009.5333983
Pournezam, M., Andrews, B. J., Baxendale, R. H., Phillips, G. F., & Paul, J. P. (1988a). Reduction of muscle fatigue in man by cyclical stimulation. *J Biomed Eng, 10*(2), 196-200.
Pournezam, M., Andrews, B. J., Baxendale, R. H., Phillips, G. F., & Paul, J. P. (1988b). Reduction of muscle fatigue in man by cyclical stimulation. *J Biomed Eng, 10*(2), 196-200. doi:https://doi.org/10.1016/0141-5425(88)90100-8
Purves, D., Fitzpatrick, D., Katz, L. C., Lamantia, A. S., McNamara, J. O., Williams, S. M., & Augustine, G. J. (2000). *Neuroscience*: Sinauer Associates.
R., H. W. (2006). Effect of training with neuromuscular electrical stimulation on elbow flexion strength. *J Sports Sci Med, 5*(2), 276-281.
Renaud, J. M., & Light, P. (1992). Effects of K+ on the twitch and tetanic contraction in the sartorius muscle of the frog, Rana pipiens. Implication for fatigue in vivo. *Can J Physiol Pharmacol, 70*(9), 1236-1246.
Sabut, S. K., Sikdar, C., Kumar, R., & Mahadevappa, M. (2011). Improvement of gait & muscle strength with functional electrical stimulation in sub-acute & chronic stroke patients. *Conf Proc IEEE Eng Med Biol Soc, 2011*, 2085-2088. doi:10.1109/iembs.2011.6090387
Salzer, J. L., & Zalc, B. (2016). Myelination. *Current Biology, 26*(20), R971-R975. doi:https://doi.org/10.1016/j.cub.2016.07.074

Sandow, A. (1952). Excitation-contraction coupling in muscular response. *The Yale journal of biology and medicine, 25*(3), 176-201.

Sayenko, D. G., Popovic, M. R., & Masani, K. (2013). Spatially distributed sequential stimulation reduces muscle fatigue during neuromuscular electrical stimulation. *Conf Proc IEEE Eng Med Biol Soc, 2013*, 3614-3617. doi:10.1109/embc.2013.6610325

Sheffler, L. R., & Chae, J. (2007). Neuromuscular electrical stimulation in neurorehabilitation. *Muscle Nerve, 35*(5), 562-590. doi:10.1002/mus.20758

Sieck, G. C., & Prakash, Y. S. (1995). Fatigue at the Neuromuscular Junction. In S. C. Gandevia, R. M. Enoka, A. J. McComas, D. G. Stuart, C. K. Thomas, & P. A. Pierce (Eds.), *Fatigue: Neural and Muscular Mechanisms* (pp. 83-100). Boston, MA: Springer US.

Smith, D. L., & Plowman, S. A. (2007). Chapter 2 - Understanding Muscle Contraction**Based on Plowman SA, Smith DL: Exercise physiology for health, fitness, and performance, ed 2, San Francisco, 2003, Benjamin Cummings. In R. Donatelli (Ed.), *Sports-Specific Rehabilitation* (pp. 15-38). Saint Louis: Churchill Livingstone.

Snoek, G. J., MJ, I. J., in 't Groen, F. A., Stoffers, T. S., & Zilvold, G. (2000). Use of the NESS handmaster to restore handfunction in tetraplegia: clinical experiences in ten patients. *Spinal Cord, 38*(4), 244-249.

Stein, C., Fritsch, C. G., Robinson, C., Sbruzzi, G., & Plentz, R. D. (2015). Effects of Electrical Stimulation in Spastic Muscles After Stroke: Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Stroke, 46*(8), 2197-2205. doi:10.1161/strokeaha.115.009633

Takeda, K., Tanino, G., & Miyasaka, H. (2017). Review of devices used in neuromuscular electrical stimulation for stroke rehabilitation. *Med Devices (Auckl), 10*, 207-213. doi:10.2147/mder.S123464

Vagg, R., Mogyoros, I., Kiernan, M. C., & Burke, D. (1998). Activity-dependent hyperpolarization of human motor axons produced by natural activity. *J Physiol, 507 (Pt 3)*(Pt 3), 919-925. doi:10.1111/j.1469-7793.1998.919bs.x

Weber, A., & Herz, R. (1968). The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. *J Gen Physiol, 52*(5), 750-759.

Wegrzyk, J., Fouré, A., Le Fur, Y., Maffiuletti, N. A., Vilmen, C., Guye, M., . . . Gondin, J. (2015). Responders to Wide-Pulse, High-Frequency Neuromuscular Electrical Stimulation Show Reduced Metabolic Demand: A 31P-MRS Study in Humans. *PLOS ONE, 10*(11), e0143972. doi:10.1371/journal.pone.0143972

Westerblad, H., & Allen, D. G. (1991). Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J Gen Physiol, 98*(3), 615-635.