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*Where* electrical stimulation is delivered affects *how* contractions are generated in the tibialis anterior muscle

by

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## ABSTRACT

This thesis describes experiments designed to investigate how motor units in tibialis anterior (TA) were recruited when electrical stimulation was applied over the TA muscle belly versus the common peroneal nerve trunk. The data from the first study (Chapter 2) showed that contractions were generated predominantly by depolarizing motor axons, regardless of stimulation site. The second study (Chapter 3) showed that single pulses of stimulation delivered over the muscle belly recruited motor units from superficial to deep as stimulation amplitude increased, but single pulses delivered over the nerve trunk recruited motor units evenly throughout the muscle, regardless of stimulus amplitude. Contrary to the results of Chapter 3, the final study (Chapter 4) provided preliminary evidence to suggest that repetitive stimulation recruited motor units from superficial to deep, regardless of stimulation site. In general, these findings support the idea that *where* electrical stimulation is delivered markedly affects *how* contractions are generated.

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## List of Abbreviations

| | |
|---|---|
| **ANOVA** | analysis of variance |
| **CNS** | central nervous system |
| **CP** | common peroneal |
| **EMG** | electromyography |
| **FES** | functional electrical stimulation |
| **H-reflex** | Hoffman reflex |
| **$M_{max}$** | maximum motor wave |
| **$M_{TH}$** | motor wave threshold |
| **$M_{50\%max}$** | motor wave at 50% of maximum motor wave |
| **M-wave** | motor wave |
| **MVIC** | maximum voluntary isometric contraction |
| **NMES** | neuromuscular electrical stimulation |
| **RMS** | root-mean-square |
| **TA** | tibialis anterior |
| **TES** | therapeutic electrical stimulation |

# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 Preface

Damage to the central nervous system (CNS) can disrupt activity in afferent and efferent pathways that control movement. As a result, voluntary control of the muscles innervated by the affected pathways can be reduced or abolished. The tibialis anterior muscle (TA) dorsiflexes the ankle and is commonly affected following CNS trauma. To compensate for reduced voluntary control of TA, neuromuscular electrical stimulation (NMES) can be applied either over the CP nerve trunk (Liberson et al., 1961; Stein et al., 2010) or the TA muscle belly (Merletti et al., 1978; Tsang et al., 1994). The primary goal of the experiments described in this thesis was to investigate how electrical stimulation generates contractions when stimulation was applied over the TA muscle belly or the CP nerve trunk. A secondary goal was to examine how recruited motor units were spatially distributed during voluntary contractions. The results of these studies contribute to our understanding of how electrically-evoked contractions are generated and provide further evidence that *where* the stimulation is delivered markedly affects *how* the contractions are produced.

This thesis consists of one unpublished study (Chapter 2), one project that was submitted for publication (Chapter 3) and one preliminary study (Chapter 4). The experiments described in Chapter 2 comprise the first series of experiments I conducted to compare how electrical stimulation generates contractions of the TA muscle when applied over the muscle belly versus the CP nerve trunk. As such, these experiments were conducted before the experiments described in Chapter 3

and 4. The goal of the experiments described in Chapter 2 was to determine what pathways contributed to contractions when stimulation was delivered at the two sites. The results from this study led to the questions addressed in Chapter 3 and 4. The experiments described in Chapter 3 and 4 were conducted during the same experimental sessions. The goals of those experiments were to investigate how recruited motor units were spatially distributed when single pulse (Chapter 3) or repetitive stimulation (i.e. NMES; Chapter 4) was delivered over the two sites. In the experiments described in Chapter 4, the spatial distribution of motor units recruited during voluntary isometric contractions, the secondary goal of this thesis was also investigated.

This General Introduction comprises three main sections. The first section (1.2) provides a brief background on NMES. The second section (1.3) provides an overview of motor unit recruitment during NMES and voluntary contractions. The last section (1.4) introduces how contractions are generated when NMES is applied at two different stimulation sites.

## 1.2 Neuromuscular Electrical Stimulation (NMES)

NMES involves the application of electrical current to generate muscle contractions. In this section, the history, contemporary use and limitations of NMES are discussed.

### 1.2.1 History of NMES

In 1791, a revolutionary discovery was made by Luigi Galvani, an Italian physician and physicist, and the field of electrophysiology was born. Before Galvani's discovery, a bulge in a muscle during contractions was thought to be

due to an inflation of the muscle by a mysterious fluid ("animal spirits") carried by nerves (Piccolino, 1998). However, Galvani witnessed vigorous muscle twitches evoked by static electricity when he inserted two metals into the body of a dead frog. His discovery provided the first scientific evidence that muscle contractions involve an electrical event. Galvani suggested that current may be stored in muscles. In contrast to Galvani's idea, Alessandro Volta (1800) proposed that muscle contractions could be induced by current flow external to the nerve and muscle. When Volta repeated Galvani's experiment, he was surprised how sensitive frog muscles were to externally applied current and proposed that the electrical event of contractions originated outside of the body. Later, he invented the battery, and displayed that external current could induce muscle contractions in the frog. However, Volta's work did not completely discount Galvani's view of electrical current originating inside of the body and generating contractions. Many researchers, including Du Bois-Reymond (1849), supported Galvani's view. Using a technique known as electromyography (EMG), Du Bois-Reymond was the first to confirm the origin of electrical activity in human muscles. Now we know electrical events are responsible for generating contractions, and contractions can be evoked by both intrinsically and extrinsically applied currents.

As the field of electrophysiology developed, it became clear that muscle contractions are associated with the excitation of motoneurons. Unlike voluntary contractions, electrical stimulation can induce contractions without the involvement of the CNS. Thus, electrical stimulation has often been used to

restore or improve body functions after trauma to the CNS. Electrical stimulation has been used for many different purposes, but its first therapeutic application for movement disorders was for the treatment of foot-drop in hemiplegic patients (Liberson et al., 1961). Liberson et al. (1961) termed this type of stimulation "functional electrotherapy" due to its goal to generate purposeful muscle contractions that help to restore or improve body functions. Later, the term functional electrotherapy was re-termed "functional electrical stimulation (FES)" by Moe and Post (1962). It was noticed that electrical stimulation could improve gait for people with foot-drop. When the stimulation was on, the participants could walk faster and further with a better gait pattern without any adverse side effects (Liberson et al., 1961; Moe and Post, 1962). This improvement in gait was apparent even after the stimulation was turned off resulting in long-lasting improvements in walking performance (Liberson et al., 1961; Moe and Post, 1962; Stein et al., 2010). When NMES is used to produce these long-lasting outcomes, by improving or maintaining muscle quality and movement after the stimulation is turned off, it is often called "therapeutic electrical stimulation (TES)". As long as motoneurons are intact, contractions can be evoked relatively easily by electrical stimulation as a replacement for inputs from the CNS (Popovic et al., 2001; Salmons et al., 2005; Sheffler and Chae, 2007). NMES produces immediate and lasting effects without any serious side effects in most participants. Perhaps the most potentially serious side-effect is autonomic dysreflexia in individuals who have had a spinal cord injury, a potentially life-threatening increase in blood pressure brought on by noxious stimuli, but participants can be

screened for this before their participation in a NMES program and autonomic dysreflexia is easily managed if noticed early enough. Nowadays, NMES is used for people with movement disorders to improve or maintain muscle function, as well as for athletes to enhance muscle performance.

### 1.2.2 Contemporary use of NMES

NMES can be used for many different purposes such as walking (Kralj and Bajd, 1989), grasping (Prochazka et al., 1997), bladder function (Van Kerrebroeck et al., 1996), breathing (Glenn and Phelps, 1985), muscle strengthening (Glanz et al., 1996; Maffiuletti et al., 2006; Shields and Dudley-Javoroski, 2006), motor learning (Chae, 2003; de Kroon et al., 2005), maintenance of bone mineral density (Shields and Dudley-Javoroski, 2006; Dudley-Javoroski and Shields, 2008), joint stability, activities of daily living, exercise (Van Peppen et al., 2004), and reduction of spasticity (Aydin et al., 2005; Ping Ho Chung and Kam Kwan Cheng, 2010). Since NMES can improve function without adverse side effects (Moe and Post, 1962), it is used among people with physical disabilities and those without disabilities (Liberson et al., 1961; Maffiuletti et al., 2006; Maffiuletti, 2010).

### 1.2.3 How NMES produces muscle contractions

It is possible to electrically-activate denervated muscle but the current required is typically an order of magnitude higher than is required to activate muscles with intact motoneurons. This thesis focuses on generating contractions in individuals with intact motoneurons. The purpose of this section is to describe

how electrical stimulation induces muscle contractions from both a biophysical and a physiological (pathways) point of view.

*1.2.3.1 Biophysics*

During NMES, an anode and a cathode are typically placed over surface of the skin and current is delivered to stimulate peripheral nerves beneath the electrodes as shown in Figure 1-1. When the stimulation is on, negatively charged ions (anions) move from the anode to the cathode and positively charged ions (cations) move in the opposite direction. Some ions travel through the extracellular fluid, and some travel through the intracellular fluid of the nerve membrane (Gersh, 1992). Under the anode, cations in the extracellular fluid are repelled and move toward the nerve membrane and cations in the intracellular fluid are repelled away from the nerve membrane. Under the cathode, anions are repelled and move toward the nerve membrane and cations are attracted to the negatively charged cathode. As a result of these ionic movements in the extracellular and intracellular fluids, the nerve membrane under the anode becomes more negative (hyperpolarized) and the nerve membrane under the cathode more positive (depolarized). When the stimulation intensity is low, the net effect of the current flow causes slight depolarization of the nerve membrane; however, the change in transmembrane potential may be so small that the potential will quickly return to the resting membrane potential. When the stimulation intensity is high enough, the change in transmembrane potential is large enough to open voltage-gated sodium and potassium channels and action potentials are generated which propagate along motor and sensory axons in both

normal (orthodromic) and reverse (antidromic) directions. In the next section, the pathways that contribute to electrically-evoked contractions are discussed.

*1.2.3.2 Pathways*

Traditionally it has been thought that NMES evokes muscle contractions by the repetitive depolarization of motor axons beneath the stimulating electrodes and any contribution made by the activation of sensory axons was seldom considered (Jacobs and Nash, 2004; Sheffler and Chae, 2007). However, there is now evidence that, at least under experimental conditions, the activation of both motor and sensory axons can contribute to electrically-evoked contractions (Collins et al., 2001; Collins et al., 2002b; Klakowicz et al., 2006; Bergquist et al., 2011a; Bergquist et al., 2012).

When NMES is applied over either a muscle or a nerve trunk, both motor and sensory axons beneath the stimulating electrodes are depolarized as shown in Figure 1-2. The depolarization of motor axons produces contractions by signals travelling from the stimulation site to the muscle via a "peripheral pathway." The discharge of motor units recruited through this peripheral pathway is synchronized or "time-locked" to each stimulus pulse and can be seen in the EMG signal ~5 ms after a stimulus pulse. This response in the EMG is known as a motor- or M-wave. Motor unit recruitment through M-waves tends to be random in relation to axon diameter (Knaflitz et al., 1990; Binder-Macleod et al., 1995; Gregory and Bickel., 2005; Maffiuletti, 2010). Electrical stimulation also activates sensory axons from muscle spindles, Golgi tendon organs and cutaneous receptors (Burke et al., 1983). This afferent volley produces contractions by

signals travelling to the CNS and back to the muscle via "central pathways." The discharge of motor units recruited through central pathways can be both synchronized and unsynchronized from the stimulus pulses. The synchronous response is due to transmission along short-latency reflex pathways though the spinal cord, resulting in a response in the EMG signal ~30 ms after a stimulus pulse, called the Hoffmann reflex (H-reflex; Hoffmann,1918; Hoffmann, 1922; Zehr, 2002; Misiaszek, 2003). Unlike the M-wave and H-reflex, asynchronous activity is temporally dispersed, not time-locked to the stimulus pulses (Lang and Vallbo, 1967; Collins et al., 2001; Bergquist et al., 2011a) and can be sustained even after stimulation is turned-off (Collins et al., 2001; Collins et al., 2002a; Collins et al., 2002b). While the origin of this asynchronous activity is presently not clear, it has been suggested that it may be due to the activation of persistent inward currents in spinal neurons (Gorassini et al., 1998; Nozaki et al., 2003). However, the preliminary data I collected during my MSc work suggested that there may be a contribution from signals travelling through the motor cortex (Okuma et al., 2010).

At the level of the cortex, the afferent volley generated during NMES increases cortical excitability (Mang et al., 2010), inducing plastic changes (Ridding et al., 2000; Khaslavskaia et al., 2002) which can, over time, strengthen corticospinal pathways which is advantageous for rehabilitative purposes (Everaert et al., 2010). At the level of the spinal cord, the electrically-evoked afferent volley recruits motor units synaptically via reflex pathways and their recruitment follows Henneman's size principle (Bawa et al., 1984; see section

1.3.1;). As a result, in theory, motor unit recruitment through central pathways will activate more fatigue resistant muscle fibres than contractions generated through peripheral pathways. After CNS trauma, fatigue resistant muscle fibres are prone to developing disuse atrophy or shifting their properties to become more like fast-fatigable fibres (Rochester et al., 1995; Shields. 2002). Therefore, enhancing the contractions through central pathways may be beneficial for promoting recovery in the CNS and in muscle after CNS trauma.

Compared to the triceps surae and quadriceps muscles, TA has smaller and less frequent H-reflexes (Schieppati, 1987; Zehr, 2002; Klakowicz et al., 2006) and electrically-evoked contractions of TA tend to have a smaller central contribution (Nickolls et al., 2004; Klakowicz et al., 2006). Therefore, since TA is the target muscle that I studied in this thesis, unless specifically stated, the bulk of the discussion in this thesis pertains to motor unit recruitment via depolarization of motor axons.

### 1.2.4 Limitations of NMES

Even though NMES is used to restore function of affected muscles after CNS trauma, the use of NMES is restricted by its limitations. Firstly, the application of NMES on the surface of the skin can cause discomfort (Delitto et al., 1992). Some people with CNS trauma are hypersensitive to the stimulation (Curatolo et al., 2006) and cannot participate in NMES programs. Even for individuals with normal or reduced sensitivity, discomfort can be a barrier to NMES use. Secondly, electrically-evoked contractions fatigue more quickly than voluntary contractions. During NMES, the same motor units are activated

repeatedly and synchronously (Maffiuletti, 2010) and the order of motor unit recruitment is non-physiological (Trimble and Enoka, 1991; Enoka, 2002). Thirdly, the use of NMES is restricted by limited spatial recruitment of motor units (Vanderthommen et al., 2000; Farina et al., 2004; Maffiuletti, 2010; Mesin et al., 2010), which is also one of the factors that promotes fatigue (Maffiuletti, 2010). During sub-maximal voluntary contractions, motor unit discharge can "rotate" whereby the discharge of active motor units is replaced by previously inactive motor units with similar recruitment thresholds (Bawa et al., 2006; Bawa and Murnaghan, 2009). Unlike voluntary contractions, NMES activates the same muscle fibres repeatedly, at least for contractions driven by the activation of motor axons only. Together these factors mean that when the same amount of torque is generated by NMES and voluntary drive, the metabolic cost on individual motor units is higher for the NMES and, as a result, NMES induces more muscle fatigue (Deley et al., 2006; Theurel et al., 2007; Jubeau et al., 2008). Early fatigue limits the duration of exercise and this is possibly the biggest issue limiting the potential benefits for people who use FES-based exercise programs. Lastly, controlling a consistent amount of torque using NMES can be challenging, especially during stimulation over a nerve trunk (Collins et al., 2001; Baldwin et al., 2006). The amount of torque generated through central pathways can be inconsistent from trial-to-trial and muscle-to-muscle. Despite these limitations, NMES has proven to be beneficial for improving and even restoring muscle function after trauma to the CNS. Details of the differences between muscle

contractions evoked by NMES and those evoked by voluntary drive will be discussed in the next few sections.

## 1.3 Recruitment of Motor Units during Voluntary Contractions and NMES

A motor unit is the basic functional unit of motor control and it was defined by Sir Charles Sherrington as a single motoneuron and all the muscle fibres it innervates (Sherrington, 1906; for review see Burke, 2007). Motor units can be divided into three types depending on physiological, biomechanical and immunohistochemical properties. Accordingly, there are type I (slow oxidative), type IIa (fast oxidative-glycolytic) and type IIb (fast glycolytic) motor units. Type I motor units have the smallest a motoneuron, generate the least force and are the most fatigue-resistant. Type IIb units have a largest motoneuron, generates the most force and fatigue the fastest. In the first half of the 20$^{th}$ century, many studies were devoted to identifying the rules that govern the activation of motor units. In this section, motor unit recruitment during voluntary contractions and contractions evoked by electrical stimulation are discussed.

### 1.3.1 Recruitment order

Researchers have long debated how motor units are recruited during muscle contractions (for review see Duchateau and Enoka, 2011). Elwood Henneman and his colleagues studied motor unit recruitment through reflex pathways, and formulated the "size principle" (1957; 1965). According to this principle, the smallest motoneurons have the lowest recruitment threshold and thus start to discharge before larger motoneurons (Henneman, 1957; Henneman et

al., 1965). Milner-Brown et al. (1973) presented the first conclusive evidence that the size principle holds true during voluntary contractions in humans. In their study, the contractile properties of single motor units in the first dorsal interosseous muscle were investigated during voluntary isometric contractions, and they found a correlation between recruitment thresholds and twitch tension of motor units. Specifically, progressively larger motor units were recruited as the intensity of the voluntary contraction increased. They also found that motor units recruited at lower contraction intensities had longer contraction times compared with units recruited at higher contraction intensities. Other researchers have used different indexes of motor unit type such as twitch amplitude, motor axon or muscle fibre conduction velocity and amplitude of evoked EMG wave and have investigated a variety of human muscles including the first dorsal interosseous (Milner-Brown et al., 1973), the flexor carpi radialis (Calancie and Bawa, 1985), the TA (Desmedt and Godaux, 1977a), and the vastus lateralis (see reviews for Binder and Mendell, 1990; Calancie and Bawa, 1985). The general agreement of all these studies has been that as long as the muscle is working as a primary mover, motor unit recruitment during slow isometric contractions is orderly. Additionally, the size principle holds true regardless of the speed of muscle contractions (slow ramp or ballistic contraction; Desmedt and Godaux, 1977a; Desmedt and Godaux, 1977b) and direction of muscle contraction in one degree of freedom (concentric or eccentric contraction; Stotz and Bawa, 2001; Duchateau and Enoka, 2011). However, recruitment order can be modified by cutaneous inputs which facilitate the recruitment of high-threshold units (Duchateau and

Enoka, 2011). Thus, overall, Henneman's size principle is the general principle guiding the recruitment of motor units during voluntary contractions. However, this may not be the case for contractions evoked during NMES.

It was traditionally thought that the order of motor unit recruitment during NMES was reversed compared to that of voluntary contractions due to large diameter axons having a lower resistance to externally-applied current (Sinacore et al., 1990; Enoka, 2002). However, human studies have shown that motor unit recruitment during NMES is random compared to that during voluntary contractions (Kim et al., 1995; Binder-Macleod et al., 1995). This discrepancy can be explained by NMES activating axons through the skin rather than activating motoneuron cell bodies by synaptic drive. Skin impedance, subcutaneous tissue, the orientation of peripheral nerves (Kim et al., 1995; Gregory and Bickel, 2005) and activation of cutaneous afferents (Garnett and Stephens, 1981) may influence motor unit recruitment during NMES (for review see Maffiuletti, 2010). Experimental and simulation data have shown that at the level of the nerve trunk, recruitment of motor axons is random in relation to axon diameter and depends predominantly on the anatomical location of axons relative to the electrode (Doherty and Brown, 1993; Major and Jones, 2005). As a result, stimulation through the skin may recruit motor units randomly in relation to fibre type. For example, if the size principle were reversed and the stimulation preferentially recruited fast-twitch muscle fibres, the time to peak twitch would be expected to be lower at lower stimulation intensities. However, the time to peak twitch was not different at stimulation intensities that evoked 20, 30 and 50% of MVIC

(Binder-Macleod et al., 1995). Using magnetic resonance imaging, Adams et al. (1993) investigated the pattern of motor unit activation during electrically-evoked contractions in the quadriceps muscles. If NMES preferentially recruited large motor units, fast-twitch muscle fibres would be activated at low stimulation intensities and slow-twitch muscle fibres would be recruited as intensity increased. If this were true, the relationship between the cross-sectional area activated by stimulation and the torque generated during stimulation would be nonlinear because fast-twitch muscle fibres produce a larger torque than do slow-twitch muscle fibres. However, Adams et al. (1993) found a strong linear relationship between these variables ($r^2$=0.74) suggesting that motor unit recruitment during NMES of the quadriceps is random.

### 1.3.2 Temporal recruitment

During voluntary contractions, motor units are recruited asynchronously relative to one another (Lind and Petrofsky, 1978; Clamann and Schelhorn, 1988; Jakobsson et al, 1988). Such asynchronous firing of motor units contributes to smooth contractions at lower firing frequencies (Robinson and Snyder-Mackler, 2008; Maffiuletti, 2010), and thus induces less fatigue (Maffiuletti, 2010) due to less metabolic demand (Lind and Petrofsky, 1978; Clamann and Schelhorn, 1988) on individual motor units. This asynchronous activity of motor units is clearly evident in surface EMG during a voluntary contraction.

When electrical stimulation is delivered over a nerve or a muscle belly, the stimulus activates axons beneath the electrodes synchronously with each stimulus pulse. This synchronous activity results in responses that are “time-locked” to

each stimulus pulse and appear in the surface EMG as either M-waves or H-reflexes (see section 1.3.2.2). As with voluntary contractions, motor units can also fire asynchronously during NMES (Collins et al., 2001; Bergquist et al., 2011a; Bergquist et al., 2011b) but to a lesser extent than occurs during voluntary contractions. During NMES this asynchronous activity develops over time, and is more prevalent when NMES is applied over a muscle belly than over a nerve trunk (Bergquist et al., 2011a). In general, however, compared to voluntary contractions NMES predominantly recruits motor axons synchronously and repeatedly (Gregory and Bickel, 2005; Maffiuletti, 2010), which results in fatigue in muscle fibres.

### 1.3.3 Spatial recruitment

Contractions can be induced voluntarily and electrically in the same muscle, but the spatial distribution of recruited motor units is different between these two stimulations. Given that the muscle fibres innervated by a single motoneuron are of the same type and motor units are recruited according to size during voluntary contractions, the spatial distribution of recruited muscle fibres depends on the distribution of muscle fibres types within a muscle (Lexell et al., 1983). In contrast, during stimulation over a muscle belly, the spatial recruitment of muscle fibres is predominantly, but not exclusively (Adams et al., 1993), superficial (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010). Using positron emission tomography, Vanderthommen et al. (2000) measured blood flow in the quadriceps muscles as an indirect measurement of contractile activity. They found that blood flow increased as stimulation intensity increased

and the motor units closest to the stimulation electrodes showed higher blood flow compared with those more distant from the electrodes. Farina et al. (2004) quantified the average rectified value, spectral frequencies (mean and median frequency) and conduction velocity to investigate properties of motor units recruited as M-waves in bicep muscles and also ran computer simulations for the M-wave properties. They found that average conduction velocity increased and spectral frequencies decreased when stimulation amplitude was slowly increased. Based on their simulation data, motor units tended to be recruited from those with high to low conduction velocities and from superficial to deep with increasing stimulation amplitudes. Mesin et al. (2010) used experimental data and computer simulations for TA and found that M-wave amplitude increased and then plateaued while torque continued to increase with further increases in stimulation amplitude. They also found that conduction velocity increased as stimulation amplitude increased. Combining their simulation and experimental data, they concluded that larger motor units are preferentially located in deep portions of TA and motor units close to the surface are recruited before deeper motor units. Place et al. (2010) compared the time to peak torque of twitch responses during stimulation over the quadriceps muscles and the femoral nerve trunk and found that time to peak torque was shorter for stimulation over the muscle. Since more type II muscle fibres are located in the superficial portion the vastus lateralis muscle (Knight and Kamen, 2005), Place et al. suggested that stimulation over the muscle recruited more superficial motor units than did stimulation over the nerve trunk. These three studies came to the same conclusion, that muscle fibres close to

the stimulus electrodes are activated preferentially when stimulation is applied over the muscle belly. Using functional magnetic resonance imaging, however, Adams et al. (1993) showed that in some participants motor units were recruited in deep portions of the quadriceps, even at relatively low stimulus amplitudes, when NMES was applied over the muscle belly. A possible explanation for the Adams et al. (1993) finding is that the motor axons close to the stimulus electrodes happened to branch to deep portions of the muscle (Gregory and Bickel, 2005). Alternatively, some of the motor units may have been recruited through central pathways, in which case slower motor units which are located deeper in the quadriceps may have been recruited first. In general, most studies have described only group data whereas Adams et al. described individual differences in the spatial distribution of recruited motor units. A more comprehensive study that includes analyses of recruitment patterns in individual participants needs to be conducted to better understand the spatial distribution of motor units recruited during NMES.

## 1.4 Stimulation Locations

In the experiments described in this thesis, stimulation was always delivered non-invasively using surface electrodes applied either over a muscle belly or a nerve trunk. In the next sections, some of the different ways to apply NMES are introduced.

### 1.4.1 Stimulation types

Electrical stimulation can be delivered through 3 different types of electrodes: implanted electrodes, percutaneous intramuscular electrodes and

surface electrodes. Implanted electrodes are situated such that they reside close to a nerve underneath the skin. The benefits of using implanted electrodes are the low current required to depolarize the nerve, which minimizes discomfort, and the selective activation of a specific target muscle (Kralj and Bajd, 1989). The drawbacks of using an implanted electrode are its invasiveness and high cost. Fine wires are used for percutaneous intramuscular electrodes. Percutaneous intramuscular electrodes are invasive and have the same benefits and drawbacks as implanted electrodes. However, long-term use of percutaneous intramuscular electrodes is not recommended due to its poor cosmetic aesthetics, the vulnerability of the wires to damage and the risk of infection (Kralj and Bajd, 1989). Surface electrodes adhere to the skin and thus require adequate skin integrity. Compared with implanted and percutaneous intramuscular electrodes, surface electrodes typically require more current to generate a contraction and can induce pain from the activation of cutaneous receptors or axons in the skin. Surface electrodes also generally have poor selectivity for activating target muscles and cause skin irritation from the electrode gel for some people. Overall, although surface electrodes have disadvantages, they are the most common way to apply NMES due to their non-invasive nature, lower cost and ease of application. In the next section, some details regarding the use of surface electrodes for NMES are discussed.

### 1.4.2 NMES over a muscle belly versus a nerve trunk

Using surface electrodes, muscle contractions can be evoked by delivering stimulation over either a muscle belly or a nerve trunk. Intuitively one might think

that the placement of the electrode does not affect how contractions are generated since both stimulation sites end up contracting the target muscle. However, the way in which axons are activated is different between the two stimulation sites. At the muscle belly site, axons beneath the stimulating electrodes are distributed diffusely throughout the muscle. On the contrary, at the nerve trunk site, all of the axons to the muscle are directly beneath the stimulating electrodes, in close proximity to each other and to the stimulating electrodes.

The pathways that contribute to contractions can be different depending on where NMES is delivered. In triceps surae and quadriceps muscles, contractions during stimulation over the muscle belly were generated predominantly through peripheral pathways (M-waves). In contrast, during stimulation over the nerve trunk, contractions were generated primarily through central pathways (H-reflexes and asynchronous activity; (Baldwin et al., 2006; Bergquist et al., 2011a; Bergquist et al., 2012). Since the central contribution to electrically-evoked contractions of TA is typically small (Schieppati, 1987; Zehr, 2002; Nickolls et al., 2004; Klakowicz et al., 2006), where the stimulation is delivered may not have as large an impact on how the contractions are generated for TA as it has for the triceps surae and quadriceps muscles. Accordingly, contractions generated in TA are more likely to be through peripheral pathways regardless of the stimulation site. This idea is supported by the data presented in Chapter 2 of this thesis.

The temporal recruitment of motor units is also different when stimulation is delivered over a muscle belly or a nerve trunk. Bergquist et al (2011a; 2012)

found that more asynchronous activity developed during stimulation over the triceps surae muscle compared stimulation over the tibial nerve trunk when same amount of torque was generated by NMES at the two sites. This can be explained by the way in which axons are activated at the two stimulation sites as described in the first paragraph in this section. During stimulation over a muscle belly, terminal branches of axons are selectively activated (Hultman et al., 1983). Conversely, axons in a nerve bundle are activated during stimulation over a nerve trunk. Since terminal branches in a muscle are diffuse underneath the electrodes and axons in the nerve trunk are in close proximity to each other, stimulation over a muscle belly may recruit motor units relatively asynchronously compared to stimulation over a nerve trunk. This may be one reason that the recruitment of motor units during stimulation over a muscle belly is more temporally dispersed than during stimulation over a nerve trunk.

The spatial distribution of motor units recruited during stimulation over a muscle belly and a nerve trunk may be different and this forms a main focus of the work in this thesis. The general consensus regarding the spatial distribution of muscle fibres recruited during stimulation over a muscle belly is that it is primarily, but not entirely (Adams et al., 1993), superficial (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010; see 1.3.3 Spatial Recruitment). As shown in Figure 1-3, when stimulation is applied over a muscle belly, motor units closest to the stimulating electrodes are preferentially activated (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010). In contrast, when stimulation is applied over a nerve trunk, I predicted that recruited motor

units would be more evenly distributed throughout the muscle. This idea was tested in the experiments described in Chapter 3 and 4.

## 1.5 Thesis Outline

Four questions are addressed in this thesis. 1) What pathways contribute to contractions when stimulation is applied over the TA muscle belly or the CP nerve trunk (Chapter 2). 2) How are electrically recruited motor units spatially distributed within TA when single pulse stimulation is delivered over the two stimulation sites (Chapter 3). 3) How are electrically recruited motor units spatially distributed when repetitive stimulation (i.e. NMES) is delivered over the two stimulation sites. 4) How are recruited motor units distributed during voluntary contractions (Chapter 4). The results of this thesis will help us to understand how electrical stimulation generates muscle contractions and will help determine the extent to which *where* the stimulation is delivered affects *how* contractions are produced.

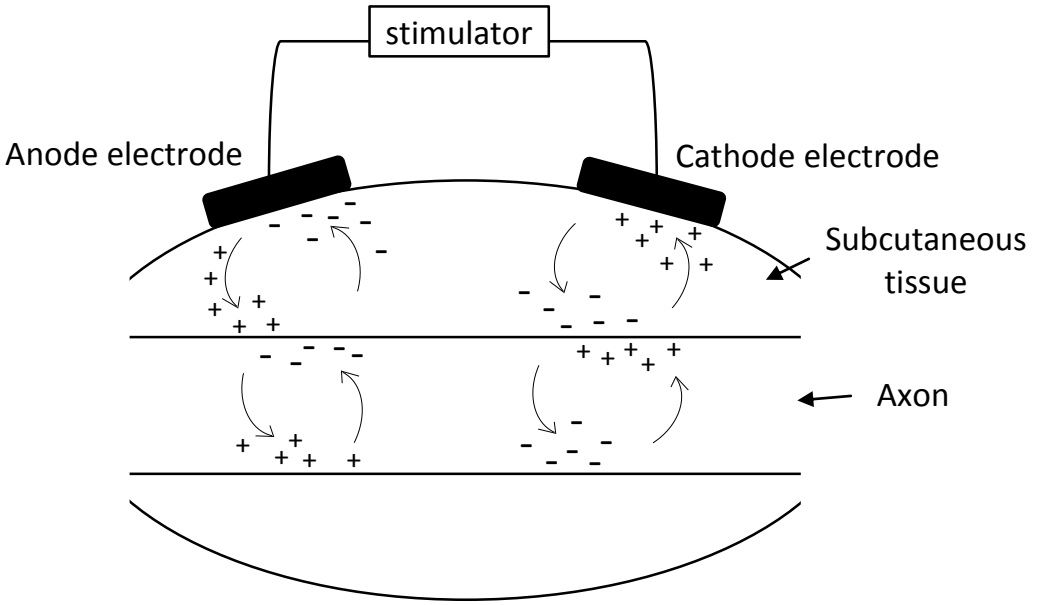
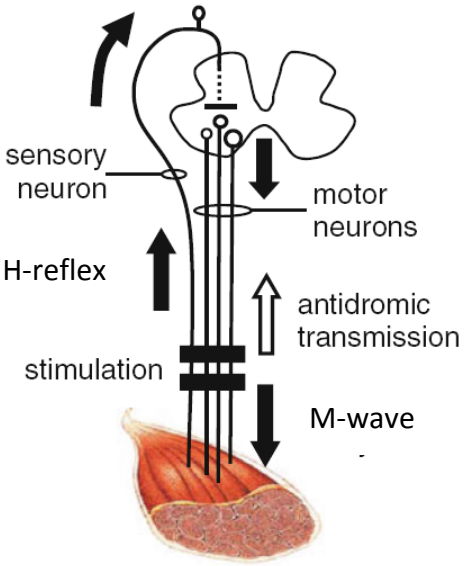



**Figure 1-1** Schematic of ion movements when electrical stimulation is applied over a muscle belly or a nerve trunk. Under the positively charged anode, cations are repelled toward the axonal membrane. Under the negatively charged cathode, anions are repelled toward the axonal membrane. As a result, at the axonal membrane net inward and outward currents are created under the anode and cathode, respectively.

***central pathway:*** motor units are recruited through reflex pathways




***peripheral* pathway:** motor units recruited by activation of motor axons beneath stimulating electrodes

**Figure 1-2** Schematic of the central and peripheral pathways that can contribute to contractions during electrical stimulation. Motor units are recruited by activation of sensory and motor axons under the stimulating electrodes. Motor unit recruitment through central pathways follows Henneman's size principle, whereas recruitment through peripheral pathways is more random (adapted from Collins, 2007).

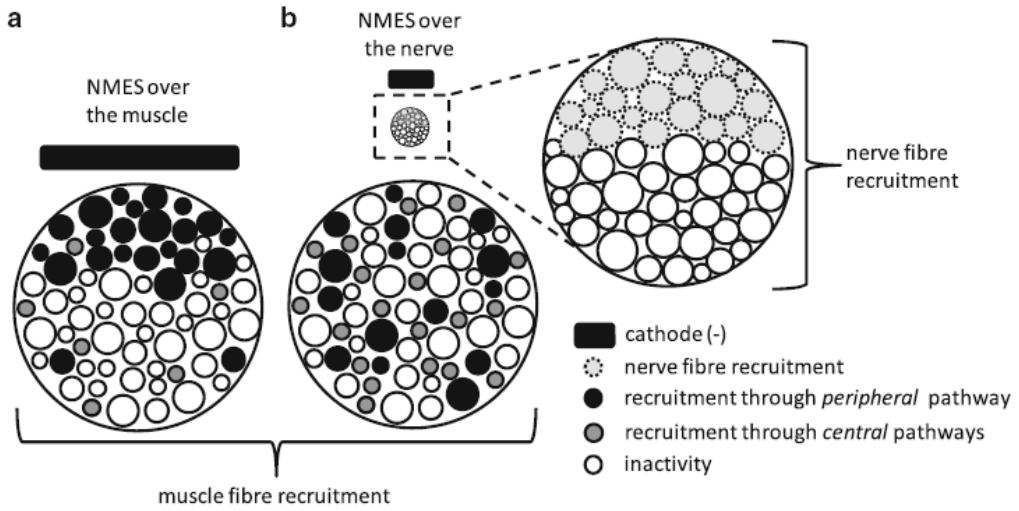



**Figure 1-3** Schematic of recruitment when stimulation is applied over a muscle belly and a nerve trunk. During stimulation over the muscle belly, motor units closest to the stimulating electrodes are preferentially recruited (Panel A). During stimulation over the nerve trunk, axons closest to the stimulating electrodes are preferentially activated. The experiments described in Chapter 3 and 4 show that this recruitment of axons contributes to evenly distributed recruitment of motor units at the level of muscle (adapted from Bergquist et al, 2011b).

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# CHAPTER 2: PATHWAYS CONTRIBUTING TO CONTRACTIONS WHEN NEUROMUSCULAR ELECTRICAL STIMUALTION WAS APPLIED OVER THE TIBIALIS ANTERIOR MUSCLE BELLY COMPARED TO THE COMMON PERONEAL NERVE TRUNK[1]

## 2.1 Introduction

The experiments described in this Chapter comprise the first series of experiments I conducted to compare how neuromuscular electrical stimulation (NMES) generates contractions of the tibialis anterior (TA) muscle when applied over the muscle belly versus the common peroneal (CP) nerve trunk. As such, these experiments were conducted before the experiments described in Chapter 3 and Chapter 4.

NMES is typically delivered repetitively at 20 - 50 Hz (de Kroon et al., 2005) to generate fused or "tetanic" contractions after trauma to sensorimotor pathways in the central nervous system. A common target for such NMES therapies is TA, a muscle that dorsiflexes the ankle and is often affected following CNS trauma (Liberson et al., 1961; Merletti et al., 1978; Chae et al., 2008). During NMES therapy, TA can be activated by delivering NMES over the TA muscle belly (Merletti et al., 1978; Tsang et al., 1994) or over the CP nerve trunk at the head of the fibula (Liberson et al., 1961; Merletti et al., 1978; Stein et al., 2010). Previous studies conducted on the triceps surae (Bergquist et al., 2011a) and quadriceps muscles (Bergquist et al., 2012) have shown that NMES over the muscle belly generated contractions predominantly through peripheral pathways (M-waves) by depolarising motor axons and there was a relatively small

[1] The authors that contributed to this work were Okuma Y, Bergquist AJ, Zhou R, Collins DF.

contribution from transmission along central pathways. In contrast, NMES over the nerve trunk generated contractions primarily through central pathways (H-reflexes and asynchronous activity) by depolarising sensory axons (Bergquist et al., 2011a; Bergquist et al., 2012). Whether the location of where NMES is delivered affects how the contractions are generated has not been explored for TA. H-reflexes in TA are typically smaller and less frequent (Schieppati, 1987; Klakowicz et al., 2006; Zehr, 2002) and electrically-evoked contractions of TA typically have a smaller central contribution (Klakowicz et al., 2006; Nickolls et al., 2004) than has been shown for the triceps surae and quadriceps muscles (Bergquist et al., 2011a; Bergquist et al., 2012). The experiments described in this Chapter were designed to compare the pathways that contribute to contractions when NMES was applied over the TA muscle belly versus the CP nerve trunk.

Based on previous studies (Bergquist et al., 2011a; Bergquist et al., 2012), we hypothesized that NMES over the TA muscle belly would generate contractions through M-waves with little if any contribution from H-reflexes and asynchronous activity. In addition, we hypothesized that NMES over the CP nerve trunk would also generate contractions predominantly through M-waves, but the M-waves would be significantly smaller while H-reflexes and asynchronous activity would be significantly larger than when NMES was applied over the TA muscle belly. The results of the experiments described in this Chapter have not been published, but they led to the development of the experiments described in Chapter 3 and 4.

## 2.2 Methods

Each experimental session lasted ~2 hrs. Ten human participants (6 males and 4 females) aged 25-48 years participated. All participants were free from any known history of neurological or musculoskeletal impairments and provided written informed consent. This project was approved by the Human Research Ethics Board at the University of Alberta.

### 2.2.1 Protocol

*2.2.1.1.Postition*

Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York). All procedures were performed on the right leg with the hip at ~120˚, the knee at ~90˚ and the ankle at ~90˚. The right foot was securely strapped to the footplate of the dynamometer.

*2.2.1.1 Electromyography*

Surface EMG was recorded through two adhesive gel electrodes (2.25 $cm^2$; Vermed Medical, Bellows Falls, VT) placed over the distal portion of the TA muscle (see Figure 2-1). The electrodes were placed parallel to the predicted path of the muscle fibers with ~0.5 cm inter-electrode distance. A common reference electrode was placed over the tibial shaft. All EMG signals were amplified 500 times and band-pass filtered between 10 and 1,000 Hz.

*2.2.1.2 Maximum Voluntary Isometric Contractions (MVICs)*

Maximum voluntary isometric contractions (MVIC) were measured at the beginning of each experiment. During the trials, participants were given visual feedback for their torque displayed on a computer screen and verbal

encouragement. The maximum torque recorded for each participant was used to calculate the target torque value for the NMES trials.

*2.2.1.3 M-wave and H-reflex Recruitment Curve*

Single pulses of stimulation (1 ms pulse duration, DS7A; Digitimer, Welwyn Garden City, UK) were delivered through two adhesive gel electrodes placed over either the TA muscle belly or the CP nerve trunk at the head of the fibula (see Figure 2-1). Stimulation over the TA muscle belly was delivered through electrodes (7.5 x 12.5 cm, model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) trimmed to fit over the middle third of each participants TA with the anode positioned ~1 cm proximal to the cathode. This site is consistent with recommendations for stimulating the motor points of TA (Hang and Joel, 2005; Botter et al., 2011). For stimulation over the CP nerve trunk, the electrodes (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) were positioned at a site that generated ankle dorsiflexion with minimal or no eversion. Typically, the cathode was placed just distal to the fibular head and the anode was positioned ~1 cm distally along the anticipated path of the CP nerve. At each stimulation site, forty stimulation pulses were delivered pseudo-randomly every 8 to 10 s at amplitudes ranging from below M-wave and H-reflex threshold up to (when possible) ~1.5 times the current required to elicit $M_{max}$. In two of the ten participants, the M-wave did not reach a maximum (i.e. M-wave amplitude did not "plateau", despite increases in stimulus amplitude) even at maximum stimulator output (100 mA) during NMES over the TA muscle belly.

*2.2.1.4 Neuromuscular Electrical Stimulation (NMES)*

*Stimulation Patterns.* Two patterns of NMES ("constant frequency" and "step frequency") were delivered at each stimulus site and, when possible, at each of two stimulation amplitudes (described below). The constant frequency pattern consisted of 20 Hz for 8 s. This stimulus frequency was chosen because it is within the recommended range of frequency for lower limb stimulation for rehabilitation (Sheffler and Chae, 2007) and allowed us to measure H-reflex and asynchronous activity between successive stimulus artifacts. The step frequency pattern comprised of 20-100-20 Hz pattern for 3-2-3 s, respectively. This pattern was delivered to examine the effect of high frequency (100 Hz) stimulation on motor unit recruitment by comparing EMG recorded before and after the burst of 100 Hz stimulation. High frequency input increases the sensory volley and the central contribution to the electrically-evoked contractions (Collins et al., 2002; Klakowicz et al., 2006; Dean et al., 2007; Bergquist et al., 2011b). Each trial included 3 repetitions of one stimulation pattern with 60 s rest between each repetition of the stimulation pattern. The order of trials was randomized for each participant. Throughout the trials participants were asked to remain relaxed and ignore the NMES.

*Stimulation Amplitudes.* Stimulus amplitude was adjusted to evoke 10 and 20% MVIC torque at the interval 2 to 3 s into the stimulation ($Time_1$; see Figure 2-2). If participants found the stimulation uncomfortable, the stimulus amplitude was lowered or the experiment was halted. All participants received NMES to

generate 10% MVIC and 6 participants (5 males and 1 female) received NMES to generate 20% MVIC.

### 2.2.2 Data acquisition and Analyses

Data were sampled at 5 kHz using custom written Labview software (National Instruments, Austin, TX) and were stored on a computer for later analyses. Data analyses were performed using custom-written Matlab software (The Mathwork, Natick, MA, USA).

#### *2.2.2.1 MVIC*

Maximum torque was calculated by averaging data over a 500 ms window centered on the peak torque during the MVIC. The MVIC torque value from each participant was used to normalize torque generated during the NMES trials. Maximum root-mean-square ($RMS_{max}$) was calculated by averaging data over a 500 ms window centered on the peak torque during the MVIC.

#### *2.2.2.2 M-wave and H-reflex Recruitment Curve*

The time windows for M-waves and H-reflexes were defined by visual inspection of data from each participant. M-wave and H-reflex amplitudes were measured peak-to-peak.

#### *2.2.2.3 NMES*

*M-waves and H-reflexes.* Peak-to-peak amplitudes of M-waves and H-reflexes were measured at the appropriate time window for each response and were normalized to $M_{max}$ collected during the recruitment curves constructed from when the stimulation was applied over the CP nerve trunk.

*Asynchronous activity.* Asynchronous activity was quantified by calculating the root mean square (RMS) of the EMG calculated over a 10 ms time window just before the onset of the H-reflex. Occasionally, the M-wave had a long tail that persisted over the time window used to measure the asynchronous activity. To prevent overestimation of asynchronous activity, the trend associated with the M-wave removed from the baseline around the period used to measure asynchronous activity. Asynchronous activity was normalized to each participant's $RMS_{max}$.

For each participant, twenty M-waves, H-reflexes and asynchronous measures were averaged over two time windows separately in a single stimulation pattern ($T_1$: 2-3 s into the stimulation; and $T_2$: 6-7 s into the stimulation). The mean ankle dorsiflexion torque, M-waves, H-reflexes and asynchronous activity at $T_1$ and $T_2$ from a single stimulation train were averaged over 3 repetitions of one stimulation pattern in a single trial. For the group analyses, these means at $T_1$ and $T_2$ were pooled across participants.

*2.2.2.4 Statistical Analyses*

Statistical analyses were performed using Statistica software (StatSoft, Tulsa, OK). Separate 3-factor repeated measures analyses of variance (rmANOVA) were run on each dependent variable (torque, M-wave, H-reflex, asynchronous activity) at each contraction level (10 and 20% MVIC). The factors for the ANOVA were "Stimulation site" (TA muscle belly vs CP nerve trunk), "Stimulation pattern" (constant vs step) and "Time" ($T_1$ vs $T_2$). Tukey's HSD tests were used for post hoc analysis when appropriate when significant main

effects or interactions were identified. The significance level was set $p<0.05$ for all tests. All data are reported as mean ± standard deviation.

## 2.3 Results

Figure 2-2 shows data collected when NMES was delivered to evoke 10% MVIC torque at $T_1$ during the constant frequency (Panels A & B) and the step frequency (Panels C & D) stimulation patterns. Data collected during NMES over the TA muscle belly are shown in the left panels (Panels A & C) and data from NMES over the CP nerve trunk are shown in the right panels (Panels B & D). The top part of each panel shows 3 torque traces (light gray lines) and their average (thick black line) and the symbols show the mean amplitudes of the 3 measures of EMG activities. The bottom part of each panel shows sixty EMG responses with their mean EMG recorded at $T_1$ and T2. These data show that, in this individual, contractions were generated predominantly through M-waves and there were hardly any H-reflexes nor asynchronous activity, regardless of stimulation site. Surprisingly, even though torque was similar between stimulation sites, M-waves were much larger during NMES over the TA muscle belly compared to NMES over the CP nerve trunk, and there was little or no central contribution in either case.

Figure 2-3 shows the group data (n=10) for the torque and EMG responses when NMES was delivered over the TA muscle belly or the CP nerve trunk to generate 10% MVIC at $T_1$. The data recorded when NMES was delivered to generate 20% MVIC were also consistent with the 10% MVIC data. Therefore, only the results from 10% MVIC are presented in this Chapter. Regardless of the

stimulation site, contractions of ~10% MVIC were generated predominantly through M-waves and both H-reflexes and asynchronous activity were small. The torque developed by NMES at the two sites was not significantly different (Panel A). As shown in Panel B, when NMES was applied over the TA muscle belly, M-waves were significantly larger ~3 times than when the NMES was applied over the CP nerve trunk. M-waves increased significantly with time when NMES was delivered in the step frequency pattern. H-reflexes were not significantly different between the two stimulation sites. H-reflexes were significantly larger at $T_2$ compared to those at $T_1$ during the step frequency pattern. H-reflexes at $T_2$ during the step frequency pattern were significantly larger compared to both at $T_1$ and $T_2$ during the constant frequency. However, these H-reflexes were small. For asynchronous activity, there were no differences between Stimulation Sites, Stimulation Patterns and Time, and there were no interactions.

## 2.4 Discussion

The primary aim of the experiments described in this Chapter was to compare how contractions were generated when NMES was applied over the TA muscle belly versus the CP nerve trunk. I found that, regardless of stimulation site, contractions were generated primarily through M-waves and the contributions from both H-reflexes and asynchronous activity were negligible. Interestingly, although NMES over the CP nerve trunk produced the same amount of torque as NMES over the muscle belly, M-waves were much smaller during NMES over the CP nerve trunk compared to NMES over the TA muscle belly.

Activation of motor units is essential to generate contractions and surface EMG is thought to be a good reflection of the number of motor unit recruited; therefore, generating the same amount of torque with significantly different EMG activity was surprising. I propose two possible explanations for this surprising result; 1) the stimulus artefact may have led to an overestimation of M-wave amplitude during stimulation over the TA muscle belly due to the close distance between the stimulus electrode and the surface recording electrodes. As can be seen in the raw EMG traces in Figure 2-2A and C, during NMES over the TA muscle belly M-waves were confounded by the stimulus artefact. In contrast, during NMES over the CP nerve trunk, M-waves were separated from stimulus artefacts; therefore, overestimation of the M-wave size was unlikely to have been a problem during NMES over the CP nerve trunk (see Figure 2-2B and D). 2) The surface EMG recording might not have accurately captured the activity of all the motor units in the muscle, especially those in the deep portion of the muscle (Mesin et al., 2010). During NMES over the TA muscle belly, superficial muscle fibres were likely to be preferentially recruited (see Chapter 3 and Mesin et al., 2010), and that activity would be accurately captured by the surface EMG electrodes due to their close proximity to the active muscle fibres. On the contrary, during NMES over the CP nerve trunk, the recruitment of motor units was likely more evenly distributed as I confirmed in Chapter 3. If this were the case, active muscle fibres close to the surface of the skin would be captured well by the surface EMG recording but active muscle fibres in the deep portion of the muscle may have contributed less to the surface EMG recording due to further

distance between the active muscle fibres and the surface EMG electrodes. Consistent with this idea, electrical activity recorded using a multielectrode inserted perpendicularly to the fibre direction (Buchthal et al., 1959) showed that the strength of the signal decreased with distance from the active muscle fibres. Therefore, activity in the deep portion of the muscle may not be captured accurately at the superficial or surface electrodes due to attenuation of the signal. Another study showed that $M_{max}$ recorded from the surface "plateaued" but the amount of force generated by the twitches continued to increase as stimulation amplitude was increased further (Mesin et al., 2010). They suggested this may be because the surface electrode did not detect activity in the deep portions of the muscle and additional units were recruited that generated more force but did not cause the M-wave to grow.

To test the two ideas proposed above, the study described in Chapter 3 and Chapter 4 was designed. In this study, which is reported in two parts, stimulus artefacts were muted to avoid overestimation of M-waves and EMG was measured from surface of the skin using surface electrodes, as well as superficial and deep portion of the muscle using intramuscular electrodes.

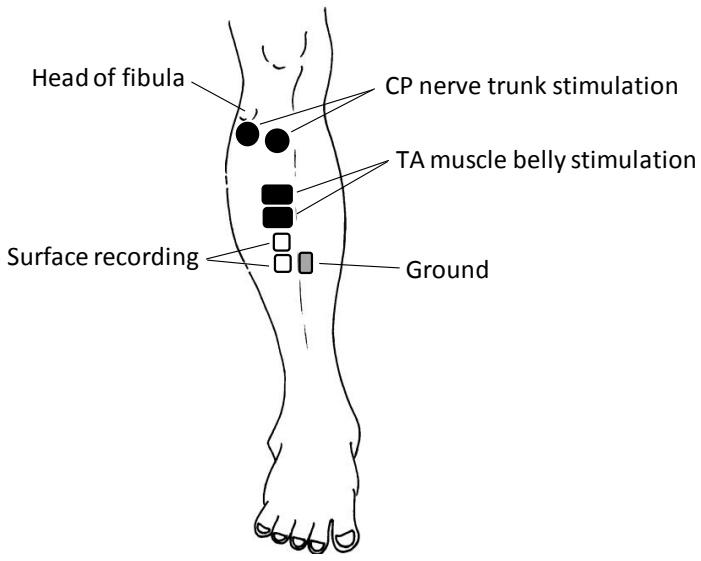


**Figure 2-1** Schematic of the stimulating and recording electrode placements on the right leg.

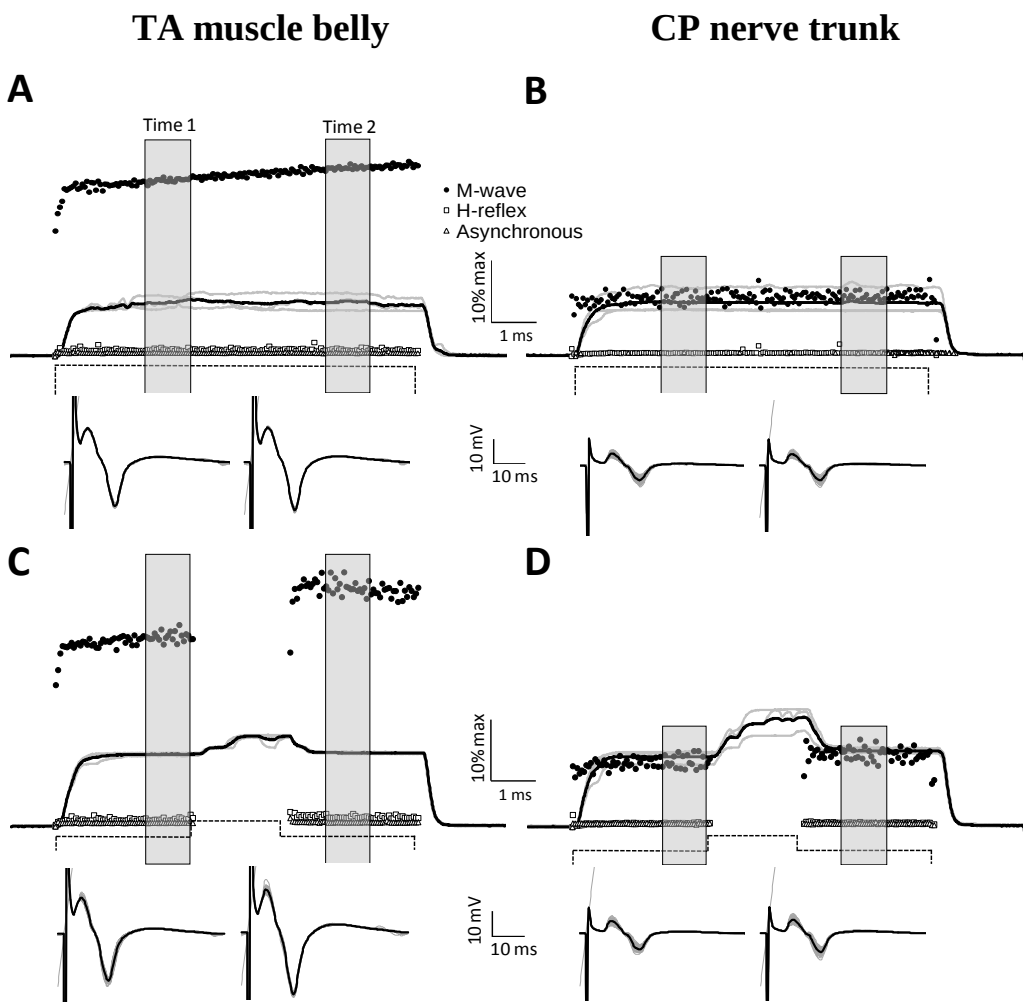


**Figure 2-2** Torque and EMG data recorded from a single participant when constant and step frequency stimulation patterns were delivered to evoke 10% MVIC torque at $T_1$. Regardless of stimulation sites, contractions were generated predominantly through M-waves and there were hardly any H-reflexes or asynchronous activity.

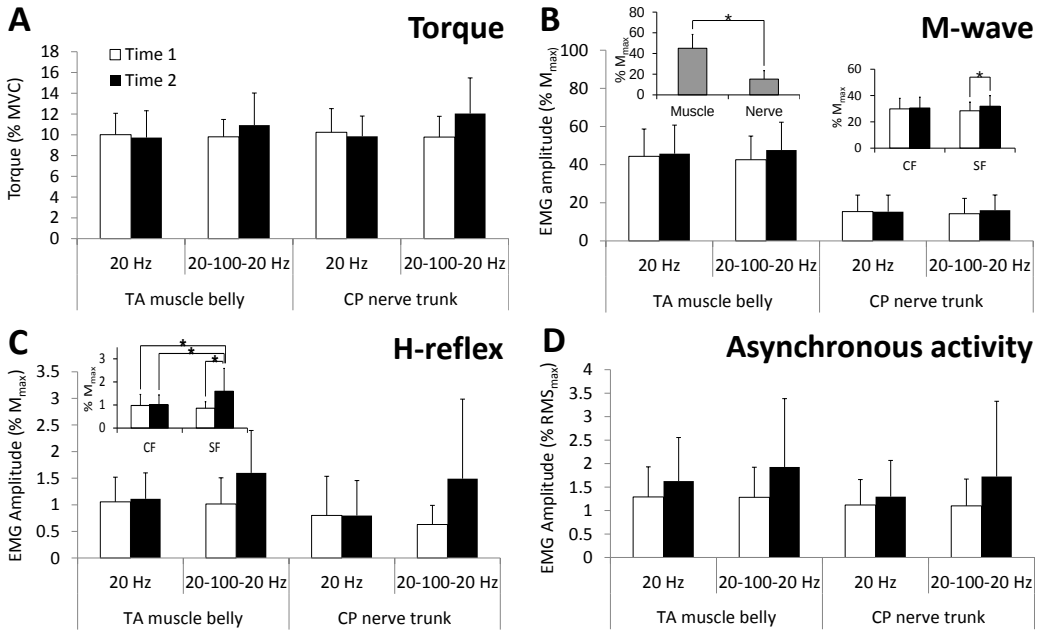


**Figure 2-3** Torque and EMG activity are averaged across the group of 10 participants for each time window, stimulation pattern and stimulation site. When NMES was delivered to generate 10% MVIC torque, contractions were evoked primarily through M-waves and both H-reflexes and asynchronous activity were negligible. M-waves during NMES over the TA muscle belly were significantly larger than when NMES was applied over the CP nerve trunk. M-wave increased with time when NMES was delivered in the step frequency pattern. H-reflexes were significanlty larger at $T_2$ during step frequency compared to at $T_1$ during step frequency and both time windows during constant frequency. Data are depicted as the mean ± standard deviation. Asterisks denote significant differences ($p<0.05$).

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# CHAPTER 3: SPATIAL DISTRIBUTION OF MOTOR UNITS RECRUITED DURING ELECTRICAL STIMULATION OVER TIBIALIS ANTERIOR VERSUS THE COMMON PERONEAL NERVE[2]

## 3.1 Introduction

Neuromuscular electrical stimulation (NMES) is used to restore movement or reduce muscle atrophy after trauma to sensorimotor pathways in the central nervous system (CNS). A common target for such NMES therapies is tibialis anterior (TA), a muscle that dorsiflexes the ankle and is often affected following CNS trauma (Liberson et al., 1961; Merletti et al., 1978; Chae et al., 2008). To activate TA, NMES can be applied over the muscle belly (Merletti et al., 1978; Tsang et al., 1994) or over the common peroneal (CP) nerve trunk near the head of the fibula (Liberson et al., 1961; Merletti et al., 1978; Stein et al., 2010). Regardless of the stimulation site, contractions are generated by the activation of motor axons beneath the stimulating electrodes. The primary aim of this study was to investigate whether there are differences in the spatial distribution of motor units recruited by the activation of motor axons during stimulation over the TA muscle belly versus the CP nerve trunk.

Several studies have investigated the spatial distribution of motor units recruited when NMES is applied over a muscle belly (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010). Vanderthommen et al. (2000) studied blood flow using positron emission tomography of the quadriceps muscle,

[2] A version of this chapter was submitted to Clinical Neurophysiology and rejected with an invitation for resubmission.
The authors that contributed to this work were Okuma Y, Bergquist AJ, Hong M, Chan KM, Collins DF.

Farina et al. (2004) and Mesin et al. (2010) used computer simulations and experimental data for the biceps and TA muscles, respectively. Regardless of the approach used or the muscle tested, these studies support the contention that superficial motor units are preferentially recruited during stimulation over the muscle belly (for review see Maffiuletti, 2010; Bergquist et al., 2011b). Adams et al. (1993), however, used functional magnetic resonance imaging and showed that in some participants motor units were recruited in deep portions of the quadriceps, even at relatively low stimulus amplitudes, when NMES was applied over the muscle belly. Thus, although there are discrepancies between studies about how recruited motor units are distributed within a muscle during stimulation over a muscle belly, the general consensus is that superficial motor units, those closest to the stimulating electrodes, are recruited preferentially. Currently there are no comparable data on the spatial distribution of motor units recruited when electrical stimulation is applied over a nerve trunk.

In the present study, we recorded electromyographic (EMG) activity (M-waves and H-reflexes) from TA using fine wires inserted into superficial and deep portions of the muscle. H-reflexes were evoked infrequently, and when present were small, consistent with previous literature (Schieppati, 1987; Zehr, 2002; Klakowicz et al., 2006); thus, only descriptive statistics are reported for these data. Rather than deliver the stimulation repetitively, as is done when NMES is used for rehabilitation, we delivered single pulses of stimulation to generate M-wave recruitment curves. In this way we were able to characterize the progression of motor unit recruitment from when the stimulation was below threshold for any

response, to that which evoked a maximal M-wave ($M_{max}$) within the range of the maximum current output of our stimulator (100 mA). We predicted that as stimulus amplitude increased during stimulation over the muscle belly, recruitment would progress from motor units closest to the stimulating electrodes (superficial) to those further away (deep). This prediction is supported by the majority of studies in the literature, although it has not been tested by recording EMG from different depths of the stimulated muscle. For stimulation over the CP nerve trunk, we predicted that recruited motor units would be distributed evenly throughout the muscle regardless of stimulus amplitude. Our rationale for this prediction comes from the finding that stimulation over a nerve trunk *in vivo* recruits motor units randomly in relation to axon diameter (Doherty and Brown, 1993; Major and Jones, 2005). Thus, regardless of the spatial organization of motor unit types in TA (Henriksson-Larsen et al., 1983), motor unit recruitment during stimulation over the CP nerve trunk should be randomly distributed throughout the TA muscle. Based on these 2 predictions, 3 hypotheses were tested. *Hypothesis 1)* When stimulation is applied over the TA muscle belly, significantly less current will be required to reach M-wave threshold ($M_{TH}$), an M-wave of 50% maximum ($M_{50\%max}$) and $M_{max}$ for the superficial compared to the deep recording site. *Hypothesis 2)* When stimulation is applied over the CP nerve trunk, the current required to reach $M_{TH}$, $M_{50\%max}$ and $M_{max}$ will not differ between the superficial and deep recording sites. *Hypothesis 3)* Regardless of stimulation site, the amplitude of $M_{max}$ will not be different between the superficial and deep recording sites. Accordingly, we anticipated that although it

would require more current to activate deep versus superficial portions of TA during stimulation over the muscle belly, we would be able to fully activate all portions of this relatively small muscle before reaching maximal stimulator output for both stimulation sites. The results of this study contribute to the body of knowledge about how electrical stimulation generates muscle contractions and provides further evidence that *where* the stimulation is delivered markedly affects *how* contractions are produced (see also Bergquist et al., 2011a; Bergquist et al. *in revision*).

## 3.2 Methods

### 3.2.1 Participants

Nine human participants (4 males and 5 females; Age range: 25-48), with no known neurological or musculoskeletal impairment, volunteered for this study after providing informed written consent. This project was approved by the Health Research Ethics Board at the University of Alberta.

### 3.2.2 Protocol

#### *3.2.2.1 Position*

Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York). All procedures were performed on the right leg with the hip at ~120˚, the knee at ~90˚ and the ankle at ~90˚. The right foot was securely strapped to the footplate of the dynamometer.

#### *3.2.2.2 Electromyography*

EMG was recorded from the superficial and deep portions of TA (see Figure 3-1) through pairs of stainless steel, teflon coated, fine-wires (0.11 mm

outside diameter, A-M Systems Inc., Carlsborg, WA). These electrodes were not intended to record single motor units, but rather ensemble EMG activity, and thus ~0.2 cm was de-insulated from the tip of each wire. A single wire was threaded through each of four 25 gauge needles such that ~0.3 cm of wire extended from the tip of each needle which was then bent to form a hook. Before the needles were inserted, the boundary of TA was visualized using ultrasound (Acuson Sequoia®512 Ultrasound System; 15L8w Acuson Transducer, Mountain View, CA, USA). Two needles were then inserted into both the superficial (2.5 cm length, JMS injection needle, model JS-N2525RSP, JMS CO., LTD, Hiroshima, Japan) and deep portions (3.8 cm length, PrecisionGlide Needle, model 305127, Becton Dickinson and Company, Franklin Lakes, NJ) of TA using ultrasound visual guidance. The de-insulated wire tips in each portion of the muscle were inserted ~1 cm apart along the predicted path of the muscle fibres. The needle tips were inserted into the superficial portion of TA to a depth of 0.7±0.2 (mean±SD) cm from the surface of TA, anterior to the central tendon that typically separates the superficial and deep portions of TA (Nakhostine et al., 1993). The needle tips were inserted into the deep portion of TA to a depth of 2.0±0.3 cm from the surface of TA, posterior to the central tendon. A common ground electrode was placed over the tibial shaft.

EMG was recorded using a Neurolog system (NL824 pre-amplifiers, NL820A isolator, NeuroLog System; Digitimer, Welwyn Garden City, UK) which enabled us to markedly reduce stimulation artifacts from the EMG signals (Figure 3-2A) during data collection. A trigger signal was sent from the stimulator

(DS7A Digitimer, Welwyn Garden City, UK) to the isolator (NL820A) of the EMG system at the time of each stimulation pulse to mute the input to the EMG amplifiers for the duration of each stimulation pulse. In this way, the M-waves we recorded were not contaminated by the tail of the stimulation artifact. All EMG signals were amplified 200 times and band-pass filtered between 10 to 1,000 Hz.

*3.2.2.3 Electrical Stimulation over the Muscle Belly and the Common Peroneal Nerve*

Electrical stimulation (1 ms pulse duration, DS7A; Digitimer, Welwyn Garden City, UK) was applied through two adhesive gel electrodes placed over either the TA muscle belly or the CP nerve trunk at the head of the fibula (see Figure 3-1). Stimulation over the TA muscle belly was delivered through electrodes (7.5 x 12.5 cm, model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) trimmed to fit over the middle third of each participants TA with the anode positioned ~1 cm proximal to the cathode. This site is consistent with recommendations for stimulating the motor points of TA (Hang and Joel, 2005; Botter et al., 2011). For stimulation over the CP nerve trunk, the electrodes (3.2 cm round; model CF3200, Axelgaard Manufacturing, Lystrup, Denmark) were positioned at a site that generated ankle dorsiflexion with minimal or no eversion. Typically, the cathode was placed just distal to the fibular head and the anode was positioned ~1 cm distally along the anticipated path of the CP nerve. At each stimulation site, between forty to eighty stimulation pulses (46±11 pulses) were delivered pseudo-randomly every 8 to 10 s at amplitudes ranging from below M-wave and H-reflex threshold up to (when possible) ~1.5 times the current required

to elicit $M_{max}$ at the recording site that required the most current to obtain a maximal response. In 3 of the 9 participants, the M-wave recorded from the deep recording site did not reach a maximum (i.e. M-wave amplitude did not "plateau", despite increases in stimulus amplitude) even at maximum stimulator output (100 mA) during stimulation over the TA muscle belly.

### 3.2.3 Data acquisition and analyses

Data were sampled at 5 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for later analyses. Data analyses were performed using custom-written Matlab software (The Mathwork, Natick, MA, USA). M-wave and H-reflex amplitudes were quantified as the area under the curve of the full-wave rectified waveforms for each M-wave and H-reflex.

*M-waves.* Separate M-wave recruitment curves were constructed for data collected during stimulation at the two stimulation sites. M-wave amplitude was plotted against current and these data were fit with a sigmoid curve (see Figure 3-2B). A regression line was then fit through the sigmoid curve and 4 values of interest were calculated using methods described by Klimstra and Zehr (2008); 1) current at $M_{TH}$, 2) current at $M_{50\%max}$, 3) current at $M_{max}$ and 4) $M_{max}$ amplitude. For the 3 participants in whom $M_{max}$ was not reached for the deep recording site during stimulation over the TA muscle belly, the largest M-wave recorded was taken to be $M_{max}$. For each participant, M-wave amplitude was normalized to $M_{max}$ recorded at the corresponding electrodes.

*H-reflexes.* Given the low frequency of occurrence of H-reflexes, H-reflex recruitment curves were not constructed and only descriptive statistics are provided for these data. An H-reflex was considered to be present when there was a clear and consistent waveform at an H-reflex latency (30-40 ms) that increased and then decreased as stimulation amplitude increased, and was absent at $M_{max}$. When H-reflexes satisfied these criteria, the amplitude of the maximal H-reflex ($H_{max}$) was calculated as the mean of the 3 largest H-reflexes. In some trials, particularly when the M-wave was large, the baseline of the EMG signal over the interval used to calculate H-reflex amplitude was elevated by the tail of the M-wave, which, if left uncorrected would lead to an inaccurate quantification of H-reflex amplitude. Thus, from all data, the tail of M-wave was removed over the H-reflex interval before H-reflexes were rectified and their amplitude was calculated (see Figure 3-2A).

*Statistical analyses.* Statistical analyses were performed on group data using Statistica software (StatSoft, Tulsa, OK). Kolmogorov-Smirnov tests showed that all data were normally distributed. Separate two-way repeated measures analysis of variance (rmANOVA) tests were used to identify differences between the "current required to reach a specified M-wave amplitude" ($M_{TH}$, $M_{50\%max}$ and $M_{max}$) for each "recording site" (superficial and deep) for stimulation over the TA muscle belly and the CP nerve trunk. A three-way rmANOVA, that included "stimulation site" (muscle belly vs. nerve trunk) as a factor, would not have been appropriate since the current required to generate a given M-wave amplitude was markedly different between stimulation sites, due in part to

differences in the size of the stimulating electrodes used for the two sites. Our main interest was in the effect of recording site on the current required to generate an M-wave of a given amplitude in the different portions of the muscle and we were not interested in the relationship between increases in current and increases in M-wave amplitude. Thus, only main effects of "recording site" and the interactions between "recording site" and "current required to reach specific M-wave amplitude" are reported, while main effects of "current required to reach specific M-wave amplitude" are not reported. Tukey's HSD tests were used for post hoc comparisons when appropriate. Paired *t*-tests were used to test for differences in the amplitude of $M_{max}$ between stimulation sites for each recording site. The significance level was set $p<0.05$ for all statistical analyses. All data are reported as mean±standard deviation.

## 3.3 Results

### 3.3.1 M-waves

Recruitment curves constructed from data collected from a single participant for stimulation over the TA muscle belly and the CP nerve trunk are shown in Figure 3-3A and B, respectively. The right side of this figure shows all of the single sweeps of EMG (overlaid) used to generate the recruitment curves for each recording site. The flat area around the H-reflex time window is due to the trend removal of the M-wave tail (see Methods). In this participant, during stimulation over the muscle belly, the recruitment curve for the superficial recording site was markedly different from the curve for the deep recording site. Clearly, the current required to generate $M_{50\%max}$ and $M_{max}$ was less for the

superficial recording site compared to the deep recording site. In contrast, when the stimulation was applied over the CP nerve trunk, the recruitment curves were similar between the recording sites and the current required to reach $M_{TH}$, $M_{50\%max}$ and $M_{max}$ was similar for both sites.

Figure 3-4 shows the mean current required to reach $M_{TH}$, $M_{50\%max}$ and $M_{max}$ at each recording site averaged across the group of 9 participants. When stimulation was applied over the TA muscle belly (Figure 3-4A), there was a significant interaction between "recording site" and "current required to reach specific M-wave amplitude" [$F_{(2,16)}$=11.11, p<0.001]. Although the current required to reach $M_{TH}$ did not differ between recording sites (p=0.07), significantly more current was required to reach $M_{50\%max}$ (p<0.001) and $M_{max}$ (p<0.001) at the deep, compared to the superficial, recording site. When stimulation was applied over the CP nerve trunk (Figure 3-4B), there was no main effect of "recording site" [$F_{(1,8)}$=3.32, p=0.11] and no interaction between "recording site" and "current required to reach specific M-wave amplitude" [$F_{(2,16)}$=0.08, p=0.92]. Thus, there were no significant differences between recording sites in the current required to generate any of the three M-wave amplitudes of interest.

Figure 3-5 shows the mean amplitude of $M_{max}$ for each stimulation and recording site for the group. At the superficial recording site, $M_{max}$ was not different between simulation sites [$t_{(8)}$=0.29, p=0.78]. At the deep recording site, $M_{max}$ was significantly smaller during stimulation over the TA muscle belly compared to stimulation over the CP nerve trunk [$t_{(8)}$=3.02, p=0.02]. This

difference at the deep recording site likely reflects, at least in part, our inability to reach $M_{max}$ during stimulation over the muscle belly at maximum current amplitude in 3 participants, as indicated by the lack of a clear "plateau" in M-wave amplitude with increasing stimulus amplitude.

### 3.3.2 H-reflexes

When stimulation was applied over the TA muscle belly, H-reflexes were observed only in 1 participant, were evident only at the superficial recording site and were small ($H_{max}$=5.8% $M_{max}$). When stimulation was applied over the CP nerve trunk, H-reflexes were evoked in 3 participants, were evident at both recording sites in each participant, and were small at both the superficial ($H_{max}$=3.7±2.5% $M_{max}$) and deep ($H_{max}$=8.1±4.9% $M_{max}$) recording sites.

## 3.4 Discussion

The primary aim of this study was to investigate whether there are differences in the spatial distribution of motor units recruited by the activation of motor axons during stimulation over the TA muscle belly versus the CP nerve trunk. Consistent with previous literature (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010), we found that stimulation over the muscle belly recruited superficial motor units first, with deeper portions of the muscle recruited with increasing stimulus amplitude. In contrast, motor units recruited during stimulation over the CP nerve trunk were distributed evenly throughout the muscle, regardless of stimulus amplitude.

### 3.4.1 Spatial distribution of motor units recruited by the depolarization of motor axons

We measured the current required to generate M-waves at 3 points of interest on the M-wave recruitment curve; $M_{TH}$, $M_{50\%max}$ and $M_{max.}$ Comparing the current required to reach each of these 3 points between the superficial and deep recording sites provided information about the spatial distribution of motor units recruited for each stimulation site over the full range of stimulus amplitudes. Consistent with previous literature (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010), the data supported our first hypothesis regarding motor unit recruitment during stimulation over the muscle belly, but only for 2 of these 3 points along the curve. Contrary to our first hypothesis, the current required to reach $M_{TH}$ was not significantly different between the deep and superficial recording sites, suggesting that low stimulus amplitudes applied over the TA muscle belly recruits motor units evenly throughout TA. However, we may have been underpowered to detect a difference at $M_{TH,}$ as almost twice as much current was required to reach $M_{TH}$ at the deep recording site compared to the superficial recording site. In contrast, significantly less current was required to reach $M_{50\%max}$ and $M_{max}$ at the superficial recording site compared to the deep site during stimulation over the muscle belly. Thus, when stimulation is applied over the TA muscle belly at higher stimulus amplitudes (i.e. stimulus amplitudes which generate an M-wave $\geq$50% $M_{max}$), more current is required to activate motor units deep in the muscle than those located more superficially. These data are consistent with the finding that current density is highest close to the stimulating

electrodes and progressively decreases with distance from the stimulation electrodes (Cartee and Plonsey, 1992), resulting in a preferential recruitment of motor units closest to the stimulating electrodes. However, these data contrast with those of Adams et al. (1993), who showed that stimulation over the quadriceps muscle belly recruits motor units in deep portions of the muscle in some participants. Adams et al. described data from individual participants, but not averaged across the group, as was done in other studies and presently. A comprehensive study that compares the spatial distribution of electrically-recruited motor units between individuals may help to address the apparent discrepancies between the study of Adams et al. and other studies.

Our second hypothesis was supported. When stimulation was applied over the CP nerve trunk, the current required to reach $M_{TH}$, $M_{50\%max}$ and $M_{max}$ did not differ between the superficial and deep recording sites. Thus, unlike stimulation over the TA muscle belly, stimulation over the CP nerve trunk recruited motor units evenly throughout the TA muscle, regardless of stimulus amplitude. This even distribution of motor units recruited in TA during stimulation over the nerve trunk indicates that there is no relationship between the order in which axons are recruited in the nerve trunk and the spatial distribution of the motor units they innervate in TA. If there was a relationship between these factors, the recruitment of motor units would have progressed from superficial to deep layers of the muscle as stimulation amplitude increased.

Our third hypothesis, that the amplitude of $M_{max}$ recorded at a given recording site would not differ between stimulation sites, was not fully supported.

This hypothesis was based on the idea that our stimulator would have sufficient current to fully activate all motor units in TA before the 100 mA maximal output of the stimulator was reached for both stimulation sites. Consistent with our hypothesis, we found that $M_{max}$ was not different between stimulation sites for the superficial recording site, indicating that all the motor units in this portion of the muscle were fully recruited by stimulation at both sites. Contrary to our hypothesis, however, $M_{max}$ recorded from the deep portion of the muscle was significantly smaller during stimulation over the TA muscle belly compared to stimulation over the CP nerve trunk. This was likely due, at least in part, to data recorded from 3 of the 9 participants in whom M-waves recorded at the deep recording site did not reach $M_{max}$ at maximal stimulator output, as indicated by the lack of a "plateau" in the M-wave recruitment curve. Thus, the smaller mean $M_{max}$ amplitude during stimulation over the muscle belly can be explained by a limitation of the current in these participants. This inability to reach $M_{max}$ will have led not only to an underestimation of $M_{max}$ amplitude for the deep recording site, but also an underestimation of the current required to reach $M_{50\%max}$ and $M_{max}$ for these participants. Regardless, the fact that stimulation over the TA muscle belly maximally recruited the superficial portion of the muscle, but not the deep, provides further evidence that stimulation over the TA muscle recruits muscle fibres from superficial to deep with increasing stimulus amplitude.

### 3.4.2 Spatial distribution of motor units recruited by the depolarization of sensory axons

Generally, in other lower limb muscles, H-reflexes appear at high incidence rates when stimulation is applied over a nerve trunk. For example, H-reflexes appear with a 100% incidence rate in quadriceps (vastus medialis and rectus femoris), 97-100% in biceps femoris and 77-100% in soleus; however, in TA, H-reflexes appear with only a 0-11% incidence rate (Zehr, 2002). Presently we show that TA has low incidence rates of H-reflexes when stimulation is applied over either the TA muscle belly (11%) or the CP nerve trunk (33%).

### 3.4.3 Clinical implications for NMES

When NMES is applied over a muscle belly, superficial motor units are recruited preferentially, due to the greater distance between the stimulating electrodes and the deeper portions of the muscle (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010). This pattern of spatial recruitment of motor units has been cited as a factor that limits the efficacy of NMES-evoked contractions because the force is being generated by the synchronous and repetitive activation of the same population of motor units (Maffiuletti, 2010). During normal voluntary contractions, motor units discharge asynchronously from each other at lower frequencies than the stimulus frequencies used for NMES and recruitment can rotate between motor units, all of which are thought to help minimize voluntary contraction fatigue. Further, this pattern of spatial recruitment during NMES over the muscle belly means that it may not be possible to activate motor units located furthest from the stimulating electrodes

(Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010; Maffiuletti, 2010). Accordingly, when the stimulation was applied over the muscle belly in the present study, $M_{max}$ was not reached at the deep recording site in 3 participants even at maximal stimulator output and, across the group of participants, $M_{max}$ was smaller in this portion of the muscle than when the stimulation was delivered over the nerve trunk. This has important implications for NMES, since preferentially activating one portion of the muscle makes the contraction of the whole muscle less efficient due to suboptimal force transmission to the tendons (Hill, 1938; Martins et al., 1998). Whether the presently observed effect of stimulation site on the spatial distribution of recruited motor units for this relatively small muscle is generalizable to other muscles requires further investigation; however, we expect the effect to be even more pronounced in larger muscles such as the quadriceps.

To recruit previously inactive motor units during stimulation over a muscle belly, Maffiuletti (2010) has suggested re-locating the stimulating electrodes, varying the joint angle or increasing stimulus amplitude within tolerance levels. Data from the present study indicate that another way to achieve this outcome would be to relocate the stimulating electrodes to over the nerve trunk, at least for muscles such as TA in which the nerve trunk is easily accessible from the surface. Alternatively, one could alternate or interleave stimulus pulses between over the muscle belly and nerve trunk. In addition to recruiting, at least partially, a different population of motor units with every other stimulus pulse, this would have the added benefit of halving the firing frequencies of motor units recruited by only one of the stimulation sites, thus reducing the metabolic demand

on these units. Another option would be to alternate the delivery of stimulation trains (or sets of stimulus trains) between over the muscle belly and over the nerve trunk to, in a crude way, mimic the motor unit rotation observed during voluntary contractions (Bawa et al., 2006; Bawa and Murnaghan, 2009).

Changing the stimulation site should have an effect, not only on the spatial distribution of recruited motor units, but also on the type of motor units that are recruited. Since the spatial distribution of different fibre types varies both within and between muscles (Burke and Tsairis, 1973; Stalberg and Antoni, 1980; Henriksson-Larsen et al., 1983; Knight and Kamen, 2005), the fact that stimulation over the nerve trunk activates motor units more diffusely throughout the muscle than stimulation over the muscle belly suggests that different types of motor units will be recruited by stimulation at the two sites. TA is composed of 75% Type I muscle fibres (Gregory et al., 2001; Jakobsson et al., 1988) with the highest density of these fibres located in superficial portions (Henriksson-Larsen et al., 1983). Data from the present study would suggest that to target these muscle fibres most effectively, stimulation should be applied over the muscle belly. In contrast, vastus lateralis is composed of 48% Type I muscle fibes (Gregory et al., 2001) with a higher density of these fibres located in deeper portions of the muscle (Knight and Kamen, 2005). To target these muscle fibres most effectively, stimulation over the nerve trunk may be more appropriate.

### 3.5 Summary

In this study, single pulses of electrical stimulation were delivered over the TA muscle belly or the CP nerve trunk over a range of stimulus amplitudes to

compare the spatial distribution of motor units recruited between stimulation sites. Consistent with previous studies, we found that stimulation over the muscle belly recruited superficial motor units preferentially, with deeper portions of the muscle recruited with increases in stimulus amplitude. In contrast, during stimulation over the nerve trunk, the recruitment of motor units was evenly distributed between superficial and deep portions, regardless of stimulus amplitude. These results contribute to our understanding of how electrical stimulation generates muscle contractions and provides further evidence that *where* stimulation is delivered markedly affects *how* contractions are produced. Since repetitive stimulation is used to produce functional contractions for rehabilitation, further investigation is required to test whether these findings hold true when repetitive stimulation is delivered.

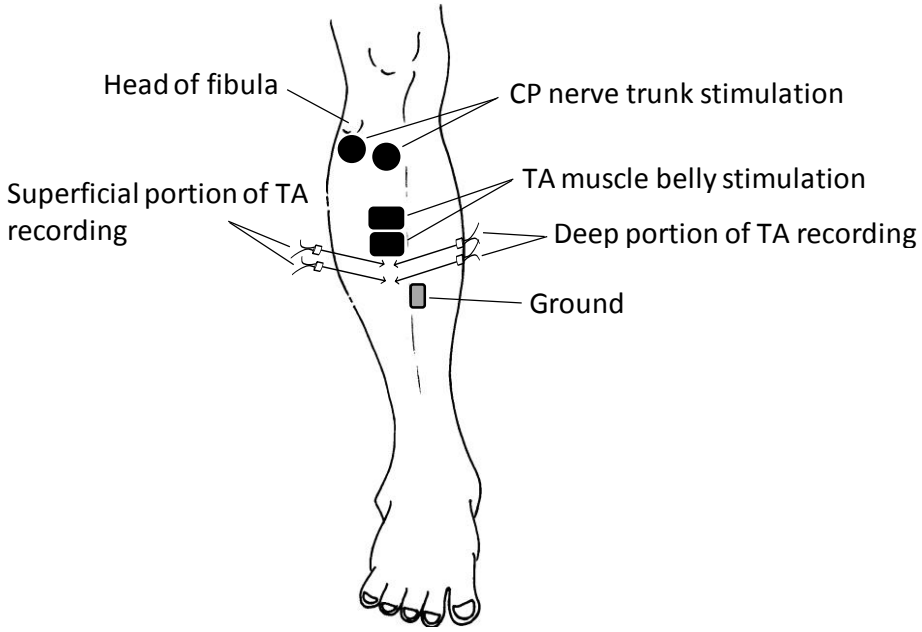


**Figure 3-1** Schematic of the stimulating and recording electrode sites on the right leg.

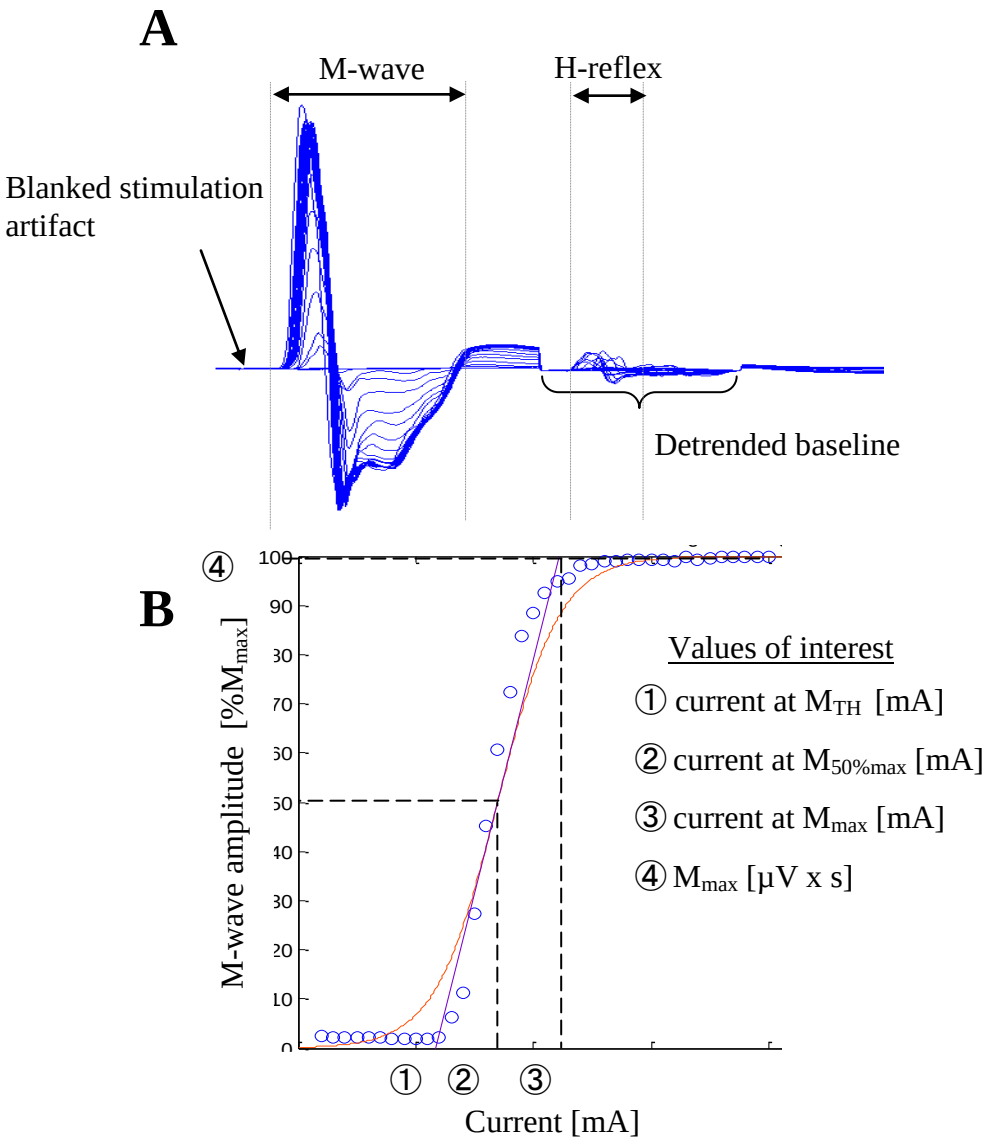


**Figure 3-2** Data analysis procedures. All sweeps of EMG used to generate the recruitment curves were overlaid to define the windows to quantify M-waves and H-reflexes (panel A). The stimulation artifact was blanked during data collection (see Methods). The tail of the M-wave was removed from the H-reflex time window during post hoc analyses. Data were full-wave rectified and the area of each M-wave was plotted against current (panel B). These data were fit with a sigmoid curve and a regression line was calculated. Four values of interest from each M-wave recruitment curve were calculated using methods adopted from Klimstra and Zehr (2008).

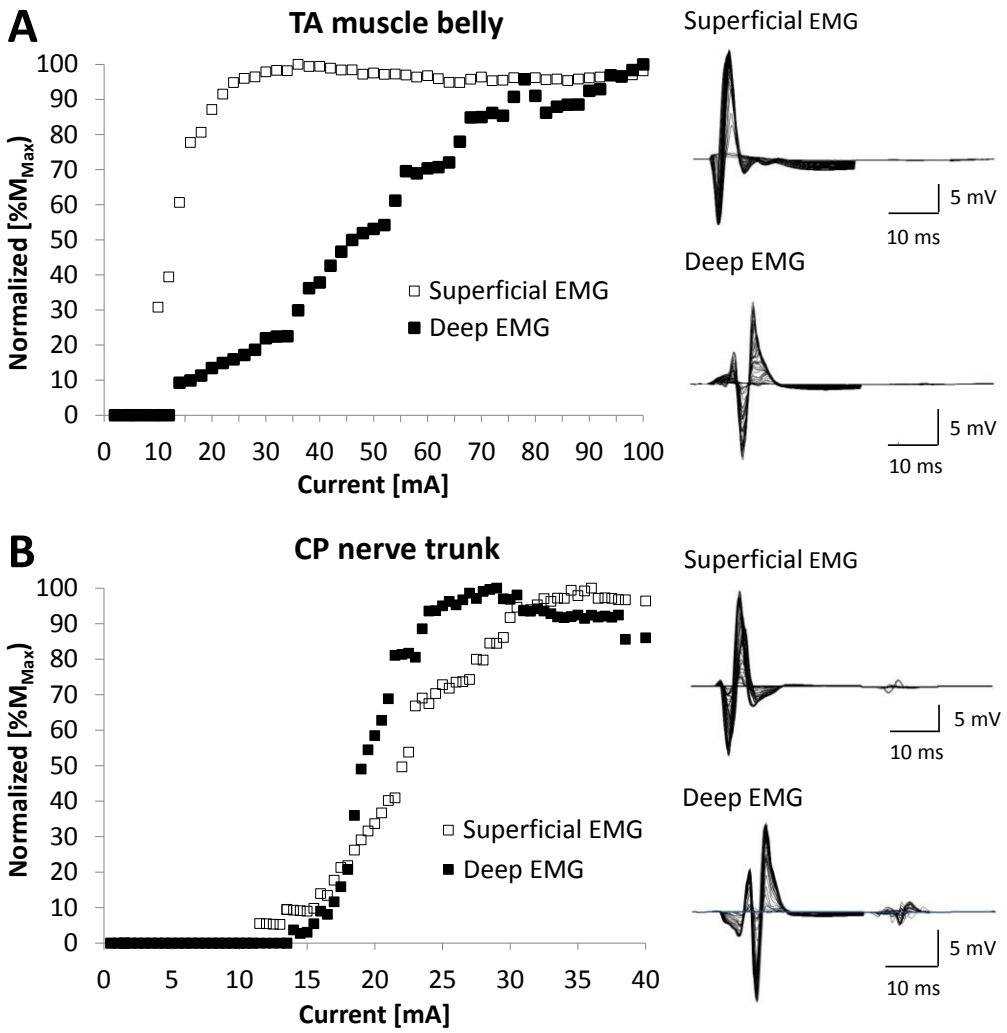


**Figure 3-3** Recruitment curves constructed from data recorded from a single participant for each recording site when stimulation was applied over the TA muscle belly (panel A) and CP nerve trunk (panel B). Overlaid sweeps of the EMG used to generate each recruitment curve are shown on the right.

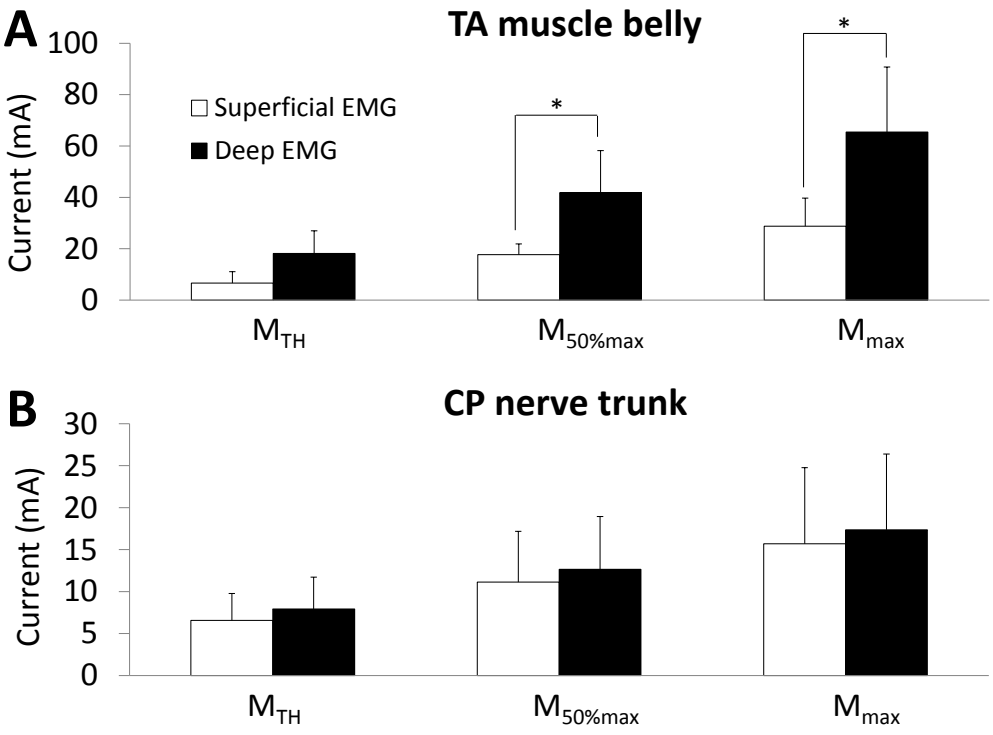


**Figure 3-4** Current required to generate M-waves at three key points along the M-wave recruitment curves ($M_{TH}$, $M_{50\%max}$ and $M_{max)}$ averaged across the group of 9 participants. Data are shown for M-waves recorded from the superficial and deep recording sites for stimulation over the TA muscle belly (panel A) and CP nerve trunk (panel B). Data are depicted as the mean±standard deviation. Asterisks denote significant differences ($p<0.05$). Note that the y-axis scales are different in panels A and B.

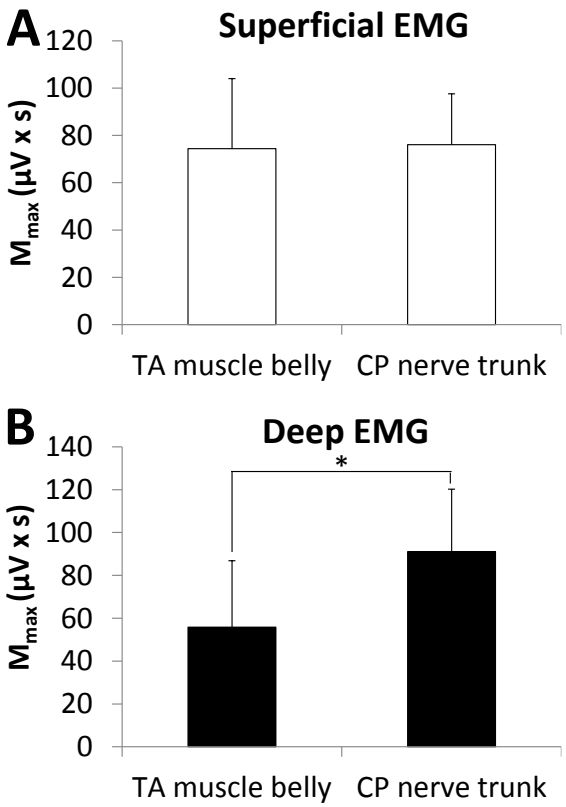


**Figure 3-5** Amplitude of $M_{max}$ averaged across the group of 9 participants for the superficial (panel A) and deep (panel B) recording sites during stimulation over the TA muscle belly and CP nerve trunk. Data are depicted as the mean±standard deviation. Asterisks denote significant differences ($p<0.05$).

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# CHAPTER 4: THE SPATIAL DISTRIBUTION OF MOTOR UNITS RECRUITED WHEN NEUROMUSCULAR ELECTRICAL STIMULATION IS APPLIED OVER THE TIBIALIS ANTERIOR MUSCLE BELLY COMPARED TO THE COMMON PERONEAL NERVE TRUNK[3]

## 4.1 Introduction

The results of the experiments described in Chapter 2 showed that electrically evoked contractions of tibialis anterior (TA) were generated almost exclusively through peripheral pathways regardless of whether neuromuscular electrical stimulation (NMES) was delivered over the TA muscle belly or the common peroneal (CP) nerve trunk. Interestingly, in that study, even though NMES over the CP nerve trunk produced a similar amount of torque as NMES over the TA muscle belly, M-waves were much smaller with NMES over the CP nerve trunk and there were hardly any H-reflexes or asynchronous activity to compensate for the apparently fewer motor units recruited as M-waves. The experiments described in Chapter 3 were designed to address two possible explanations for this surprising finding; 1) M-waves recorded during NMES over the TA muscle belly may have been overestimated due to the stimulus artifact, 2) M-waves recorded during NMES over the CP nerve trunk may have underestimated the activation of motor units in TA because the surface electromyographic (EMG) recordings may not have accurately captured activity from the deep muscle fibres (Mesin et al., 2010)**.** In the present experiments stimulus artifacts were muted to enable us to measure M-waves more accurately and EMG activity in TA was recorded not only from the surface of the skin but

[3] Authors contributing to this work were Okuma Y, Bergquist AJ, Hong M, Chan KM, Collins DF.

also from superficial and deep portions of the muscle using intramuscular EMG electrodes. Even though further investigation is required to confirm by comparing EMG activity recorded from surface and intramuscular electrodes, the data in Chapter 3 may support my idea that the reason M-waves were larger during stimulation over the TA muscle belly was because surface EMG recording did not capture activity in deep portions of the muscle during stimulation over the CP nerve trunk. When single pulses were delivered over the CP nerve trunk, both superficial and deep portions of muscle fibres were equally activated, regardless of stimulation amplitude. When single pulses were delivered over the TA muscle belly, superficial motor units were activated preferentially. Thus, it is possible that stimulation over the TA muscle belly and the CP nerve trunk recruited the same number of motor units (and generate the same torque), but M-waves were larger during stimulation over the TA muscle belly because the activated motor units were closer to the recording electrodes. The primary aim of the experiments reported in this Chapter was to investigate whether the results described in Chapter 3 held true when the stimulation was delivered repetitively (i.e. NMES) over the TA muscle belly and the CP nerve trunk.

During voluntary contractions, motor unit recruitment follows Henneman's size principle (Milner-Brown et al., 1973). Thus, the spatial distribution of voluntarily recruited muscle fibres will depend on the spatial distribution of the different muscle fibre types within the muscle (Lexell et al., 1983). In TA, more type II muscle fibers are located deep within the muscle than in superficial layers (Henriksson-Larsen et al., 1983). A secondary aim of this

study was to investigate the spatial distribution of muscle fibres recruited during voluntary contractions.

In the present experiments we tested 3 hypotheses; 1) When NMES is delivered over the TA muscle belly to generate 10 and 20% maximum voluntary isometric contraction (MVIC), there will be more EMG (as a percent of maximum) recorded from superficial portions of TA than the deep portions, 2) When NMES is delivered over the CP nerve trunk to generate 10 and 20% MVIC, EMG recorded from the superficial and deep portions of TA will not be different, 3) During voluntary contractions between 5-20% MVIC, there will be more EMG activity in superficial than deep portions of TA.

## 4.2 Methods

The experiments described in this Chapter were conducted during the same experimental sessions as those described in Chapter 3 and thus these experiments used the same EMG recording methodology as described for that Chapter. Similarly, these experiments used a similar stimulation methodology as described in Chapter 2. Thus, details of the methodology that were common to Chapter 2 or 3 are described only briefly here.

### 4.2.1 Participants

Data were collected for the NMES trials from four out of the nine participants (3 males and 1 female, aged 25-48 years) who took part in the study described in Chapter 3. Three participants withdrew due to discomfort associated with the intramuscular recording electrodes during the repetitive stimulation and two were withdrawn because we were unable to evoke dorsiflexion without strong

eversion. Data were collected from eight of the nine participants for the voluntary contraction trials. All participants were free from any known history of neurological or musculoskeletal impairments and provided written informed consent. This project was approved by the Human Research Ethics Board at the University of Alberta.

### 4.2.2 Protocol

#### *4.2.2.1 Position*

Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, New York).

#### *4.2.2.2 Electromyography*

Surface EMG was recorded from TA using two adhesive gel electrodes (2.25 $cm^2$; Vermed Medical, Bellows Falls, VT; see Figure 2-1). A common reference electrode was placed over the tibial shaft. EMG signals were amplified 500 times. Intramuscular EMG was recorded using fine wires inserted in to the superficial and deep portions of TA as described in Chapter 3. EMG signals were amplified 200 times. EMG signals from surface and intramuscular electrodes were band-pass filtered between 10 to 1,000 Hz.

EMG was recorded using a Neurolog system (NL824 pre-amplifiers, NL820A isolator, NeuroLog System; Digitimer, Welwyn Garden City, UK) which enabled us to markedly reduce stimulation artifacts from the EMG signals during data collection (see Figure 2A and Chapter 3 for more information).

*4.2.2.3 Maximum Voluntary Isometric Contractions*

Maximum voluntary isometric contractions (MVICs) were recorded at the beginning and end of each experimental session.

*4.2.2.4 Neuromuscular Electrical Stimulation*

Electrical stimulation (1 ms pulse duration, DS7A; Digitimer, Welwyn Garden City, UK) was applied through two adhesive gel electrodes placed over either the TA muscle belly or the CP nerve trunk (see Figure 3-1 and 2-1). Two patterns of NMES ("constant frequency" and "step frequency") were delivered at each stimulus site. Stimulation amplitude was set to evoke 10% (n=4) and 20% (n=3) MVIC torque at $T_1$ (2-3s into the stimulation pattern). If participants found the stimulation uncomfortable the stimulus intensity was lowered or the experiment was halted.

*4.2.2.5 Voluntary Contractions*

Participants were asked to hold 5, 10 and 20% MVIC torque using visual feedback of the target and contraction torque on a computer monitor. Each participant performed one contraction of approximately 3 to 5 s at each contraction amplitude with approximately 5 s rest between contractions.

**4.2.3 Data acquisition and analyses**

Data were sampled at 5 kHz using custom written Labview software (National Instruments, Austin, TX) and stored on a computer for later analysis. Data analyses were performed using custom-written Matlab software (The Mathwork, Natick, MA, USA).

*4.2.3.1 MVIC*

MVIC torque and maximum root-mean-square of the EMG ($RMS_{max}$) were calculated by averaging data over a 500 ms window centered on the peak torque during MVIC. The MVIC torque recorded from each participant at the beginning of the session was used to normalize torque generated during the NMES trials and set the target value for the voluntary contraction trials. The $RMS_{max}$ from each participant recorded at the end of each session was used to normalize asynchronous activity generated during the NMES and the voluntary contraction trials from the corresponding recording site.

*4.2.3.2 NMES*

M-waves, H-reflexes and asynchronous activity were analyzed as described in Chapter 3. Both M-waves and H-reflexes were rectified and quantified as the area under the curve. Each response was normalized to the maximum M-wave ($M_{max}$) recorded during NMES over the CP nerve trunk during collection of the recruitment curves described in Chapter 3. To analyze asynchronous activity, the RMS of the EMG was calculated over a 10 ms time window taken just before the onset of the H-reflex. The trends associated with M-wave tail were removed from the baseline for more accurate measurement of H-reflexes and asynchronous activity.

For each participant, twenty M-waves, H-reflexes and asynchronous measures were quantified for each stimulation train and were averaged separately over two time windows ($T_1$: 2-3 s and $T_2$: 7-8 s into the stimulation). The mean ankle dorsiflexion torque, M-waves, H-reflexes and asynchronous activity at $T_1$

and $T_2$ from a single stimulation train were averaged over 3 repetitions of one stimulation pattern in a single trial. For the group analyses, these means calculated for $T_1$ and $T_2$ were pooled across participants.

*4.2.3.3 Voluntary contractions*

Torque and RMS were calculated by averaging data over a 500 ms window centered around the point where the torque remained stable at, or near, the target amplitude. Data were expressed as %MVIC or $\%RMS_{max}$ for the torque and EMG, respectively

*4.2.3.4 Statistical Analyses*

Statistical analyses were performed using Statistica software (StatSoft, Tulsa, OK) on group torque and EMG activity recorded during voluntary contraction trials. The data from the NMES trials were not analyzed due to the small sample size (n=4 for 10% MVIC and n=3 for 20% MVIC). To test for differences in the torque recorded during different amplitudes of voluntary contraction, a one-factor repeated measures analyses of variance (rmANOVA) was run with 3 levels of "contraction amplitude" (5, 10, and 20% MVIC). For the EMG data from the voluntary contraction trials, a two-factor repeated measures ANOVA was run with 3 levels of "contraction amplitude" (5, 10 and 20% MVIC) and 3 levels of "recording site" (surface, superficial and deep recording sites). Tukey's HSD tests were used for post hoc analyses when significant main effects or interactions were identified. The significance level was set $p<0.05$ for all statistical analyses. All data are reported as the mean ± standard deviation.

## 4.3 Results

### 4.3.1 The spatial distribution of motor units recruited during NMES

Figure 4-1 shows data collected when the stimulation was delivered to evoke 10% MVIC torque at $T_1$ during the constant frequency stimulation patterns. The data collected during step frequency stimulation pattern showed similar trends as these data from the constant frequency trials, therefore, only data recorded during the constant frequency patterns are reported in this Chapter. Data collected during NMES over the TA muscle belly are shown in the left panels of Figure 4-1 (Panel A, C & E) and data during NMES over the CP nerve trunk are shown in the right panels (Panel B, D & F). The top two panels show data recorded from the surface EMG (Panel A & B), the middle two panels show data from the superficial EMG (Panel C & D) and the bottom two panels show data from the deep EMG (Panel E & F). The top part of each panel shows 3 torque traces (light gray lines) and their average (a thick black line), and the symbols show the mean amplitude of the 3 measures of EMG activity. The bottom part of each panel shows sixty EMG responses and their mean recorded at $T_1$ and $T_2$. These data show that, regardless of stimulation site, contractions were generated primarily through M-waves and there was little if any H-reflex or asynchronous activity for all 3 recording sites. Surprisingly, the data recorded at all three sites were similar to the data recorded from the surface as described in Chapter 2, that is, even though NMES over the CP nerve trunk generated a similar amount of torque as NMES over the TA muscle belly, M-waves were smaller with NMES

over the CP nerve trunk compared to NMES over the TA muscle belly and there were hardly any H-reflexes or asynchronous activity.

Figure 4-2 shows group results from the three EMG recording sites during constant frequency NMES delivered to generate 10 (left panels; n=4) and 20% (right panels; n=3) MVIC torque. Due to limited sample size, statistical analyses were not performed on these data and only a qualitative description is provided here. For both stimulation sites, M-waves dominated the EMG and H-reflexes and asynchronous activity were negligible. Regardless of recording sites, M-waves were larger during NMES over the TA muscle belly than the CP nerve trunk when stimulus amplitude was set to evoke 10% MVIC torque (Figure 4-2C). As stimulus amplitude was increased to evoke 20% MVIC torque, this difference in M-wave amplitude between stimulation sites appeared to be reduced (Figure 4-2D). When NMES was delivered over the TA muscle belly, M-waves recorded at the superficial site were larger compared to the surface and deep recording sites at both stimulus amplitudes. When NMES was applied over the CP nerve trunk, M-waves recorded at the superficial site were larger than the surface and deep recording sites at 10% MVIC torque. When stimulus amplitude applied over the CP nerve trunk was increased to evoke 20% MVIC torque, EMG activity recorded at the three recording sites seemed to be become more similar to each other. H-reflexes and asynchronous activity were small regardless of stimulus site or amplitude (Figure 4-2E to H).

### 4.3.2 Voluntary contractions

Figure 4-3 shows torque and EMG activity recorded from all three EMG recording sites while participants held voluntary contractions to generate 5, 10 and 20% MVIC torque. As presented in panel A, for the torque data there was a significant main effect of "contraction amplitude" [$F_{(2,14)}$=598.41, p<0.001] and the torque recorded during each of the three levels of voluntary contraction was significantly different from each other (p<0.001). For the EMG data there was a significant main effect of "contraction amplitude" [$F_{(2,14)}$=36, p<0.001] and "recording site" [$F_{(2,14)}$=14.34, p<0.001] but there was no significant interaction [$F_{(4,28)}$=0.11, p=0.98]. As shown by the inset in the left of panel B, when the data were collapsed across the 3 recording sites there was significantly more EMG when participants held 20% MVIC torque compared to 5% MVIC (p<0.001) and 10% MVIC (p<0.001). The inset in the right of panel B shows that the EMG recorded at the surface was significantly smaller than that recorded from the superficial (p<0.001) and deep (p=0.03) portions of the muscle, when the data were collapsed across contraction amplitudes.

## 4.4 Discussion

The goals of this study were to characterize the spatial distribution of motor units recruited in TA under two conditions; 1) when NMES was applied over the TA muscle belly versus the CP nerve trunk and 2) during voluntary contractions. Due to the limited sample size for the NMES trials, only a qualitative description is provided for those data.

### 4.4.1 The spatial distribution of motor units recruited during NMES

Superficial motor units were preferentially recruited during NMES over the TA muscle belly. This fits with the data from Chapter 3 and the idea that current density is highest close to the stimulating electrodes and progressively decreases with distance from the stimulation electrodes (Cartee and Plonsey, 1992). This difference in current density leads to a preferential recruitment of motor units closest to the stimulating electrodes. In contrast, unlike the data presented in Chapter 3 where single pulses of stimulation applied over the CP nerve trunk recruited motor units evenly throughout the muscle, when NMES was applied over the nerve trunk it appeared to preferentially recruit superficial motor units. The only obvious difference in methodology between the experiments described in Chapter 3 and those in this Chapter is the difference in stimulation frequency. The stimulation was delivered between 0.13 to 0.1 Hz pseudo-randomly in the experiments described in Chapter 3, and at either 20 or 100 Hz in the experiments described in this Chapter. Therefore, it may be that somehow stimulation frequency affected the spatial distribution of recruited motor units.

Consistent with the data presented in Chapter 2, when similar torque was generated by stimulation at both sites, M-waves recorded at the surface were smaller during NMES over the CP nerve trunk than over the TA muscle belly, and both H-reflexes and asynchronous activity were negligible. The data in Chapter 3 suggested this may be because stimulation over the TA muscle belly preferentially activates superficial motor units and the surface EMG recordings easily capture this activity due to close distance between the active muscle fibres

and the surface electrodes; whereas stimulation over the CP nerve trunk recruited both superficial and deep motor units, and the surface EMG recordings may not accurately capture the activity in the deep portions of the muscle due to relatively further distance between the active muscle fibres and the surface electrodes. Surprisingly, when we delivered the stimulation repetitively as described in this Chapter, different results were obtained than in Chapter 3. When similar torque was generated by the two stimulation sites, NMES over the CP nerve trunk evoked smaller M-waves at both superficial and deep recording sites than NMES over the TA muscle belly and there was little if any H-reflex or asynchronous activity. One possible explanation for this finding is that when NMES was applied over the CP nerve trunk it activated other muscles that dorsiflex the ankle but the EMG activity from those muscles was not captured due to their further distance from recording electrodes. There are four ankle dorsiflexors that are innervated by the CP nerve or its branch, the deep peroneal nerve; these are extensor digitorum longus, TA, extensor halluces longus and peroneus tertius. Therefore, even though the TA muscle was strongly activated with single pulses (Chapter 3), it is possible that additional dorsiflexors were activated during NMES over the CP nerve trunk. Such an effect may explain why, when a similar amount of torque was produced, EMG activity recorded during stimulation over the CP nerve trunk tended to be smaller at all three recording sites compared to stimulation over the TA muscle belly.

### 4.4.2 Voluntary contractions

Given that during voluntary contractions motor unit recruitment follows Henneman's size principle (Milner-Brown et al., 1973) and that in TA more type I fibres are located in superficial than in deep portions of the muscle (Henriksson-Larsen et al., 1983), we hypothesised that there would be more EMG activity in the superficial portions of the muscle than the deep portions over the range of voluntary contraction amplitudes studied. However, our data showed that EMG activity recorded at the superficial and deep recording sites were not significantly different for contractions over the range of 5 to 20% MVIC. Further, when EMG from all three recording sites was pooled, EMG activity was not different when participants held 5 or 10% MVIC torque. The relationship between EMG and force is influenced by many factors and is not always linear (Disselhorst-Klug et al., 2009). Since TA has three synergist muscles, it is possible that these synergists assisted the dorsiflexion movement during stimulation over the CP nerve trunk but the EMG activity from these synergist muscles was not captured. EMG activity recorded at the surface recording sites was smaller than both the superficial and deep recording sites. This result may be explained by the larger distance between the active muscle fibres and the surface recording electrodes compared to the intramuscular electrodes. EMG activity from the active muscle fibres attenuates as the signals travel through the subcutaneous tissue.

### 4.4.3 Future directions

*4.4.3.1. NMES*

Due to discomfort from delivering NMES to individuals who have intramuscular electrodes in TA and the inability to generate dorsiflexion without large eversion movement, only four (10% MVIC) and three (20% MVIC) out of the nine participants completed the NMES trials. More data need to be collected to provide sufficient statistical power to adequately assess the spatial distribution of muscle fibres recruited during NMES over the TA muscle belly and the CP nerve trunk.

The differences in the spatial distribution of motor units between stimulation sites is likely to be largest at low stimulus amplitudes, thus, in future experiments it may be advantageous to test at lower stimulus amplitudes (i.e. 1% and 5% MVIC) which should be tolerable for a greater number of participants. To identify whether some of the dorsiflexion torque during CP nerve stimulation is being generated by muscles other than TA, we could also monitor the amount of toe extension since the extensor digitorum longus and extensor halluces longus extend toes but TA and peroneus tertius do not. It may not be possible to localise activity in peroneus tertius since this muscle everts the ankle as peroneus longus and brevis also do.

To investigate the discrepancy between the results from Chapter 3 and this Chapter, a study of the possible influence of stimulus frequency on the spatial distribution of recruited motor units is needed. We could record EMG activity from the superficial and deep portions of the muscle while altering stimulus

frequency, either slowly and smoothly ramping up and down from 0.2 to 20Hz or slowly stepping up and down from 0.2 to 20 Hz in 1-2 s intervals.

*4.4.3.2. Voluntary contractions*

The protocol we used to assess the spatial distribution of motor unit recruited during voluntary contraction could be refined by measuring EMG activity within the muscle while people hold a wider range of contraction levels (1-99% MVIC torque). In this way, we would be able to capture the progression of EMG activity within the muscle over a wider range of contraction levels.

## 4.5 Summary

The preliminary study described in this Chapter showed that NMES over the TA muscle belly recruited superficial motor units preferentially, similar to when single pulses were delivered to generate the recruitment curves described in Chapter 3. Unexpectedly, NMES over the CP nerve trunk did not recruit motor units evenly throughout the muscle, as occurred when single pulses were delivered to generate the recruitment curves described in Chapter 3. Stimulation over the CP nerve trunk, rather, seemed to also recruit superficial motor units preferentially. Unexpectedly, during voluntary contractions, motor units were recruited evenly throughout the muscle. To confirm these findings, more data needed to be collected for both the NMES and voluntary contraction trials.

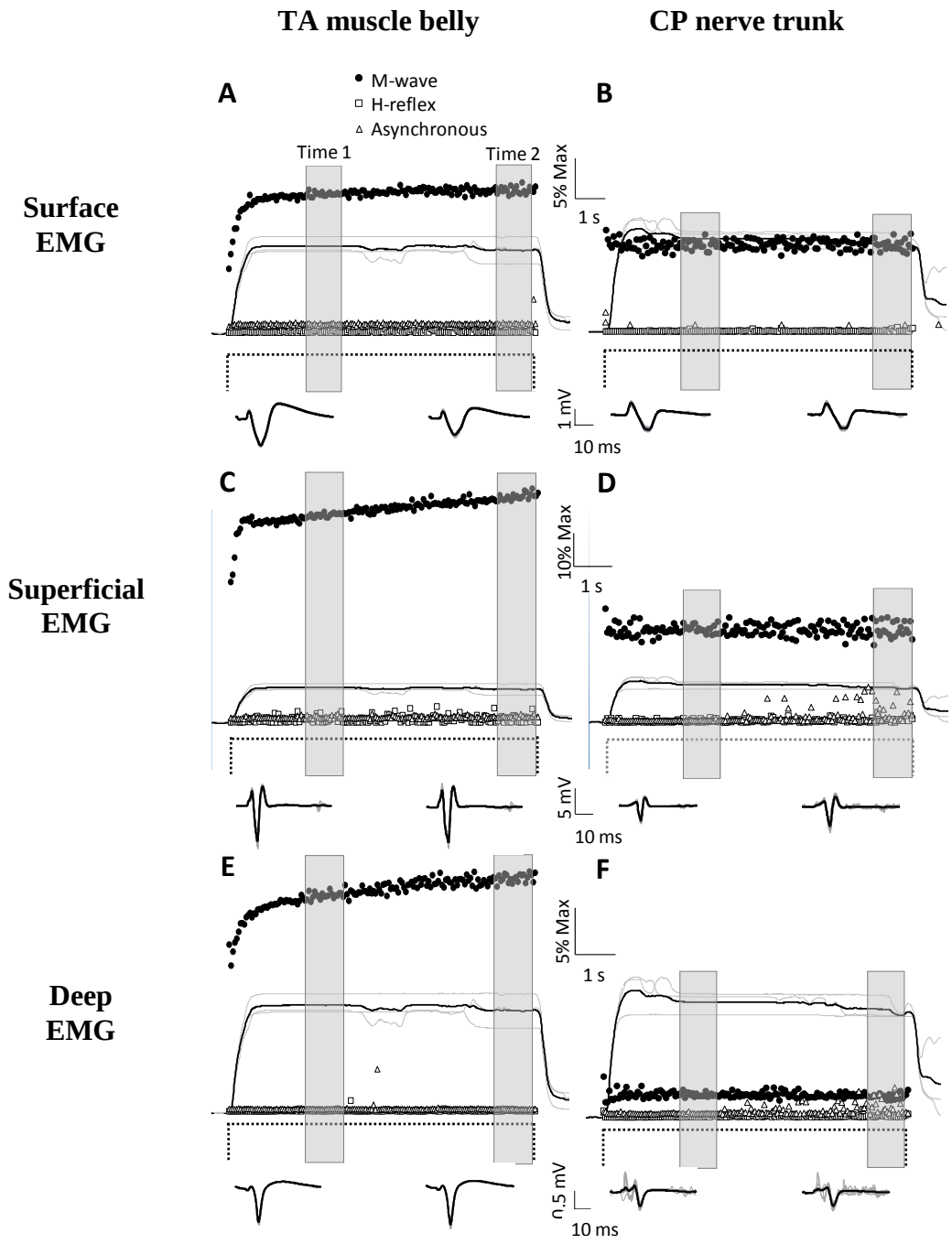


Figure 4-1 Torque and EMG data from a single participant recorded from each electrode when the constant frequency stimulation pattern was delivered over the TA muscle belly and the CP nerve trunk. Contractions were primarily generated through M-waves and both H-reflexes and asynchronous activity were negligible, regardless of stimulation site. M-waves were larger with stimulation over the TA muscle belly compared to stimulation over the CP nerve trunk.

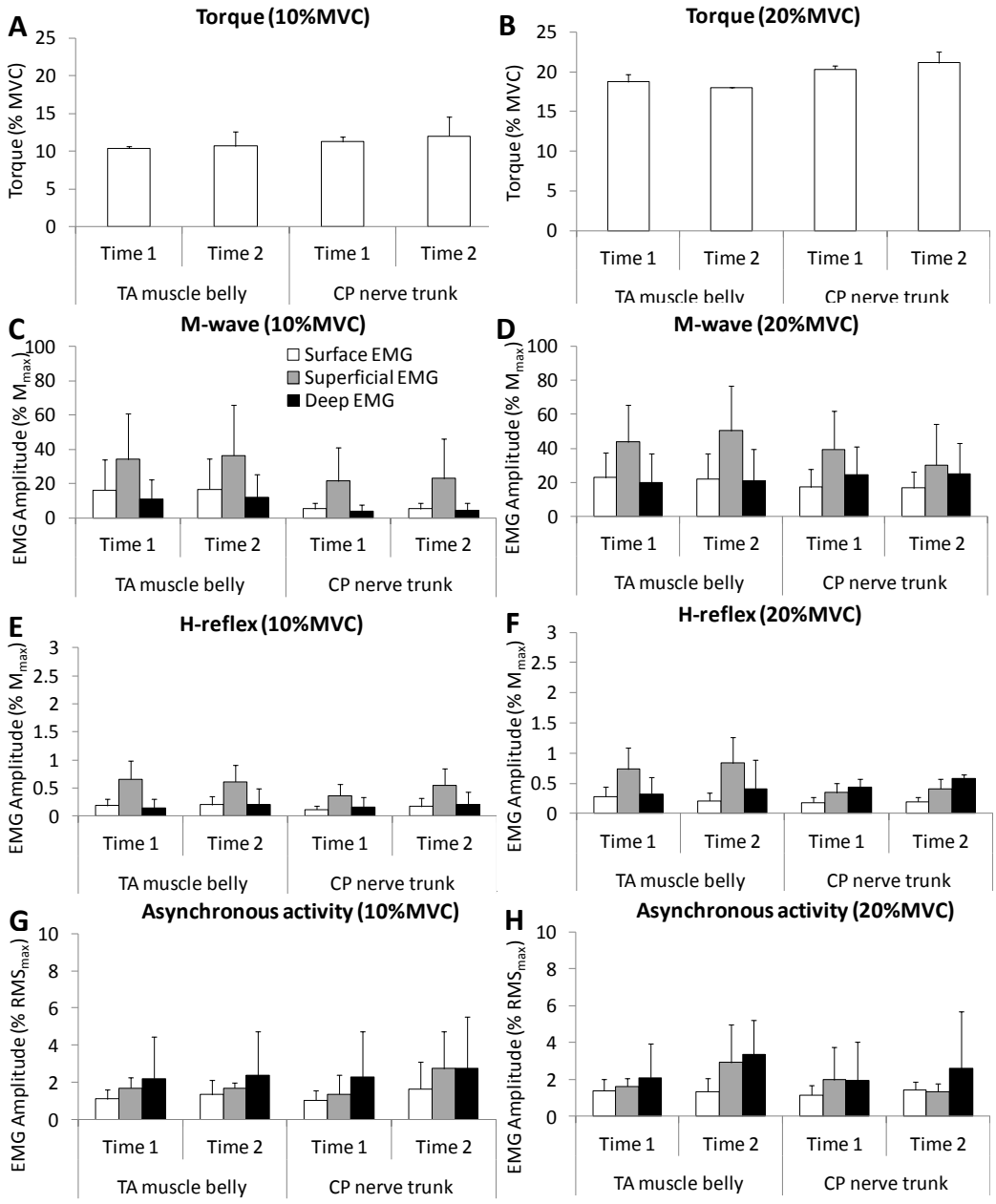


**Figure 4-2** Group data for torque and EMG results recorded at the surface, superficial and deep EMG recording sites when constant frequency pattern was delivered to induce 10 (n=4) and 20% (n=3) MVIC torque. For both stimulation sites, contractions were generated predominantly through M-waves, and there were hardly any H-reflexes and asynchronous activity. Regardless of the stimulation site, EMG activity recorded at the superficial EMG recording site was larger than the surface and deep EMG recording site.

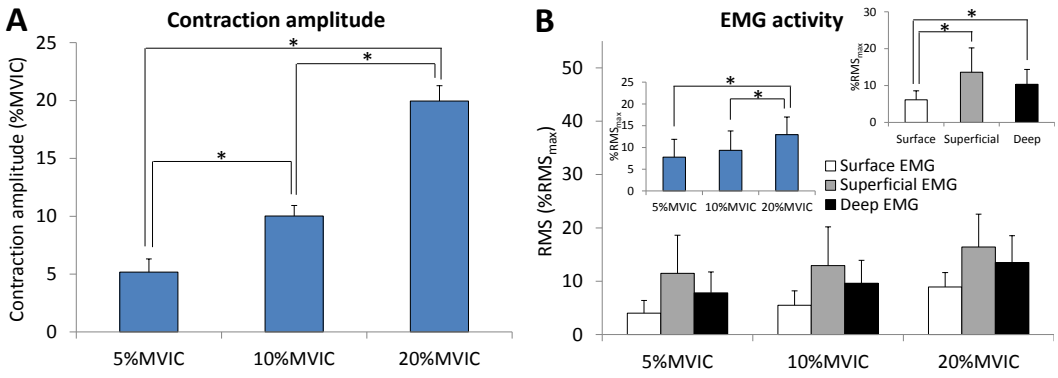


**Figure 4-3** Torque and EMG activity from the voluntary contraction trials (n=8). All three contraction amplitudes were different from each other (Panel A). EMG activity recorded at 5 and 20% MVIC contraction amplitudes were different and 10 and 20% MVIC contraction amplitude were different. EMG activity recorded at superficial recording site was different from the surface and deep recording sites (Panel B). Asterisks denote significant differences ($p<0.05$).

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# CHAPTER 5: GENERAL DISCUSSION

The overall purpose of the experiments described in this thesis was to investigate whether the location *where* stimulation is delivered affects *how* contractions are generated in the tibialis anterior (TA) muscle in healthy, neurologically intact humans. A main focus was to determine how motor units are spatially distributed in TA when they are recruited by electrical stimulation and during voluntary contractions. In this chapter, first I discuss how the stimulation site affects pathways contributing to contractions (Chapter 2). Secondly, I discuss how the stimulation site affects the spatial distribution of recruited motor units recorded using intramuscular EMG when single pulses (Chapter 3) or repetitive stimulation (i.e. neuromuscular electrical stimulation: NMES) was delivered (Chapter 4). Next, the spatial distribution of motor units recruited during voluntary contractions is discussed (Chapter 4). Last but not least, clinical implications and future directions for this work are presented.

## 5.1 The Pathways Contributing to Contractions During NMES

Previously, Bergquist et al. (2011; 2012) had shown that NMES applied over the triceps surae or quadriceps muscle bellies generates contractions predominantly through peripheral pathways (M-waves), while NMES over the tibial or femoral nerve trunk can generate contractions primarily through central pathways (H-reflexes and asynchronous activity). The purpose of the study described in Chapter 2 was to extend this line of the investigation to TA, which, unlike the muscles tested in the previous studies (Bergquist et al., 2011; Bergquist et al., 2012), has relatively small H-reflexes (Schieppati, 1987; Zehr, 2002;

Klakowicz et al., 2006). I hypothesized that contractions evoked by stimulation over the TA muscle belly would be generated through larger M-waves, and smaller H-reflexes and asynchronous activity compared to contractions evoked by stimulation over the CP nerve trunk. Electromyographic (EMG) activity was measured using surface electrodes placed over TA. The results showed that, regardless of the stimulation site, contractions were evoked predominantly through M-waves and there were hardly any H-reflexes or asynchronous activity. Interestingly, when the same amount of torque was generated by delivering stimulation at the two sites, M-waves were significantly smaller with stimulation over the CP nerve trunk compared to over the TA muscle belly, and central contributions were similarly small for both stimulation sites. Two possible explanations for these findings are that; 1) during stimulation over the TA muscle belly, M-waves may have been overestimated due to the stimulus artifacts and, 2) during stimulation over the CP nerve trunk, EMG activity may have been underestimated because the surface EMG recordings may not have accurately captured activity from the deep muscle fibres (Mesin et al., 2010). To test these ideas, in the experiments described in Chapter 3, EMG activity was measured from different portions of the muscle using intramuscular electrodes, and stimulus artifacts were muted to enable us to measure M-waves more accurately.

## 5.2 The Spatial Distribution of Motor Units Recruited by Single Pulse Stimulation

In the experiments described in Chapter 3, EMG activity was measured using two pairs of intramuscular electrodes inserted into superficial and deep

portions of TA and single pulses of stimulation were delivered to generate M-wave recruitment curves. Consistent with previous literature (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010), our results showed that stimulation over the TA muscle belly recruited motor units from superficial to deep as stimulus amplitude increased. On the contrary, stimulation over the CP nerve trunk recruited motor units evenly throughout the muscle, regardless of stimulus amplitude. These results could explain the surprising findings of Chapter 2. During stimulation over the TA muscle belly, EMG activity might be well captured by the surface EMG recordings due to close distance between the active muscle fibres and the surface recordings. During stimulation over the CP nerve trunk, EMG activity in the superficial portions of the muscle might be captured well by the surface EMG recordings but not in the deep portions of the muscle. As shown in previous literature (Schieppati, 1987; Zehr, 2002; Klakowicz et al., 2006), H-reflexes were evoked infrequently and when present were small.

These data showed that different, but not completely different, populations of motor units were activated by stimulation at the two sites and the deep portion of the muscle was activated by stimulation over the CP nerve trunk even at relatively low stimulus amplitudes. During stimulation over the TA muscle belly, more current was required to activate the deep portion of the muscle and it could not be fully activated in all participants even for a relatively small muscle like TA. Further investigation is required but we speculate more clear effects of stimulation sites on the spatial distribution of recruited motor units would be seen in a relatively larger muscle like the quadriceps muscle. However, using

functional magnetic resonance imaging, Adams et al. (1993) showed that in some participants motor units were recruited in deep portions of the quadriceps during stimulation over the quadriceps muscles belly, even at relatively low stimulus amplitude. Unlike other studies, Adams et al. were interested in differences in the spatial distribution of motor units within each participant and did not average data across the group. Therefore, it would be interesting to investigate *how* the spatial distribution of recruited motor units in the quadriceps muscles is affected by *where* NMES is delivered between people.

### 5.3 The Spatial Distribution of Motor Units Recruited by NMES

As a next step from the study described in Chapter 3, repetitive stimulation (i.e. NMES) was used to investigate how recruited motor units are spatially distributed during NMES over the TA muscle belly and the CP nerve trunk. EMG activity was measured from the surface of the skin using surface electrodes, as well as from the superficial and deep portions of the muscle using two pairs of intramuscular electrodes. Due to the small sample size, only a qualitative description was reported for these data. Consistent with single pulse stimulation reported in Chapter 3, NMES over the TA muscle belly preferentially recruited superficial motor units. In contrast, unexpectedly, NMES over the CP nerve trunk did not recruit motor units evenly throughout the muscle as reported in Chapter 3, rather, it preferentially recruited superficial motor units. Surprisingly, even muting the stimulus artifacts and recording EMG within the muscle did not explain why the two stimulation sites generated a similar torque but evoked significantly different EMG activity. When similar torque was

generated by the two stimulation sites, NMES over the CP nerve trunk evoked smaller M-waves compared to NMES over the TA muscle belly and H-reflexes and asynchronous activity were negligible for both stimulation sites.

Preferential recruitment of superficial motor units during NMES over the TA muscle belly fits with the idea that current density is highest close to the stimulating electrodes and progressively decreases with distance from the stimulating electrodes (Cartee and Plonsey, 1992), which results in recruiting superficial motor units preferentially. The inconsistencies between the results presented in Chapter 3 and 4 may be explained by different stimulation frequencies used in these two studies. The low frequency stimulation (i.e. 0.13-0.1 Hz) over the CP nerve trunk recruited motor units evenly throughout the muscle but the high frequency stimulation (i.e. 20 Hz) seemed to preferentially recruit superficial motor units. Thus, further investigation is required to investigate the effects of stimulation frequency on the spatial recruitment of motor units during stimulation over the CP nerve trunk. One possible reason for generating the same torque but evoking different EMG activity by the two stimulation sites is that NMES over the CP nerve trunk may have activated other dorsiflexors that contributed to the dorsiflexion torque produced by TA but EMG activity from the additional dorsiflexors was not captured due to their further distance from the surface recordings.

Even though the study described in Chapter 4 was not completed, the results provided some insight into motor unit recruitment when NMES was delivered at two different sites. Further investigation is required to investigate the

spatial distribution of motor units when NMES is applied at the two stimulation sites and the possible origin of dorsiflexion torque from muscles other than TA during NMES over the CP nerve trunk.

## 5.4 The Spatial Distribution of Motor Units Recruited During Voluntary Contractions

In the experiments described in Chapter 4, the spatial distribution of motor units recruited during voluntary contractions was investigated. During voluntary contractions, motor unit recruitment follows Henneman's size principle (Milner-Brown et al., 1973). Therefore, the spatial distribution of motor units recruited during voluntary contractions depends on how muscle fibre types are organized within a muscle (Lexell et al., 1983). In TA, more type II muscle fibers are found in the deep portion of the muscle (Henriksson-Larsen et al., 1983). Thus, we expected to see more EMG activity in the superficial portions of the muscle than the deep portions over the range of contraction amplitudes studied (5-20% MVIC), similar to when NMES was applied over the TA muscle belly as described in Chapter 3 and 4. However, EMG recorded at the superficial and deep recording sites were not different. Further, EMG activity recorded when participants held 5% and 10% MVIC torque was not different, suggesting possible contributions from synergist muscles to produce the dorsiflexion movement. Further investigation is required to more accurately characterize the spatial distribution of motor units recruited during voluntary contractions.

## 5.5 Clinical Implications

NMES can be used to generate contractions for rehabilitation. Based on the data presented in this thesis, it is clear that *where* the stimulation is delivered influences *how* muscle contractions are generated. In this section, 5 clinical implications for using NMES for rehabilitation are discussed.

Firstly, stimulation over the TA muscle did not activate the deep portions of TA in some participants, despite being a relatively small muscle, but stimulation over the CP nerve trunk did completely activate both superficial and deep portions of the muscle. In a clinical setting, the limitation of current is a critical issue. Activating as large a portion of muscle as possible without discomfort would be beneficial for rehabilitation. Therefore, one should consider applying the stimulation over a nerve trunk to activate the muscle more completely, especially for larger muscles such as the quadriceps when NMES is used to generate contractions for rehabilitation.

Secondly, from a biomechanical point of view, due to the structure of the TA being divided in half by its tendon, stimulation over the TA muscle belly may have a disadvantage by preferentially activating only one side of this muscle (superficial). In this way, contractions generated by stimulation over the TA muscle belly may be less efficient due to suboptimal transmission of force to the tendons (Hill, 1938; Martins et al., 1998). In contrast, evenly distributed recruitment of motor units during stimulation over the nerve trunk may be preferential due to activating both sides of muscle (superficial and deep) and more efficiently transmitting force to the tendon.

Thirdly, based on the preliminary data described in Chapter 4, contractions generated during stimulation over the TA muscle belly seem to arise from predominantly superficial portions of the TA muscle. On the contrary, contractions generated during stimulation over the CP nerve trunk may arise not only from TA but possibly other synergists as well. In a clinical setting, TA is a common target for NMES therapies to overcome foot drop after trauma to the central nervous system. If activation of muscles during stimulation over the CP nerve trunk is not limited to TA, stimulation over the CP nerve trunk may more effectively generate dorsiflexion by activating more dosiflexors than stimulation over the TA muscle belly. Stimulation over the CP nerve trunk also activates muscles that evert the ankle, the peroneus longus and brevis. People with foot drop tend to invert their ankle and land on the lateral side of their foot at heel strike; therefore, combining an eversion movement with dorsiflexion helps to stabilize the ankle at heel strike (Stein et al., 2010).

Fourthly, the organization of muscle fibre types depends on the muscle. TA is composed of 75% Type I muscle fibres (Jakobsson et al., 1988; Gregory et al., 2001) with the highest density of these fibres located in superficial portions (Henriksson-Larsen et al., 1983). Data from our study suggest that to target type I muscle fibres most effectively, stimulation should be applied over the muscle belly. In contrast, vastus lateralis is composed of 48% Type I muscle fibres (Gregory et al., 2001) with a higher density of these fibres located in deeper portions of the muscle (Knight and Kamen, 2005). To target type I muscle fibres most effectively for the quadriceps, stimulation over the nerve trunk may be more

appropriate. Further investigation is required to investigate the spatial distribution of motor units recruited during NMES over the two stimulation sites.

Lastly, one of factor that limits the efficacy of NMES-evoked contractions is the localized spatial distribution of recruited motor units (Maffiuletti, 2010). During NMES, the contractions are generated by the synchronous and repetitive activation of the same population of motor units, which contribute to development of fatigue and activating limited portion of a muscle. As solutions for the localized muscle activation during NMES over a muscle belly and to activate different populations of motor units, Maffiuletti (2010) suggested increasing the stimulus amplitude, moving the stimulating electrodes or changing the joint ankle. Based on our data, another possible solution would be adding stimulating electrodes to over the nerve trunk, at least for muscles such as TA in which the nerve trunk is easily accessible from the surface. If the stimulation is alternated between over the muscle belly and the nerve trunk, the stimulation at each site will recruit different, albeit not completely different, populations of motor units. This NMES paradigm may potentially reduce the metabolic demand on the active units and minimize fatigue.

## 5.6 Future Directions

One critical limitation of the NMES trials is the small sample size. Only four and three out of the nine people completed the NMES trial at 10 and 20% MVIC torque, respectively, because of discomfort from the stimulation and intramuscular recordings, and ability to evoke dorsiflexion without large eversion movement. For future NMES experiments, it may be a good idea to test at lower

stimulus amplitude (i.e. 1-5% MVIC) to collect more data since differences in the spatial distribution of motor units recruited at the two stimulation sites should be most apparent at these lower stimulus amplitudes. To identify the origin of dorsiflexion torque from muscles other than TA, we could monitor toe extension since activation of extensor digitorum longus and extensor halluces longus will extend the toes but activation of the TA and peroneus tertius will not. It may not be possible to identify activity in peroneus tertius since this muscle everts at ankle joint as peroneus longus and brevis do. To explain the contradictory data between Chapters 2 and 4, the effects of stimulation frequency on the spatial distribution of motor units recruited during NMES over the CP nerve trunk needs to be tested. We could record EMG activity from the superficial and deep portions of the muscle while stimulation frequency is manipulated slowly and smoothly ramping up and down from 0.2 to 20 Hz or slowly stepping up and down from 0.2 to 20 Hz. If NMES over a nerve trunk recruits motor units evenly throughout the muscle, like single pulse stimulation, slightly different population of motor units would be activated during both stimulation sites. If this is the case, it would be interesting to test whether fatigue during NMES would be minimized by delivering NMES over a muscle belly and a nerve trunk alternately pulse by pulse.

The protocol for the voluntary contraction trials may be improved by measuring EMG activity at the superficial and deep portions of the muscle while participants are holding wide range of contractions (1-99% MVIC torque). All the studies described in this thesis were performed on this relatively small muscle,

TA. It would be interesting to repeat these experiments in larger muscles such as the quadriceps.

## 5.7 Summary

The main goal of this thesis was to investigate whether the location *where* the stimulation is delivered influences *how* muscle contractions are generated. A secondary goal was to investigate the spatial distribution of motor units recruited during electrically-evoked and voluntary contractions. The data from Chapter 2 showed that electrically-evoked contractions were generated predominantly through M-waves and there were hardly any H-reflexes or asynchronous activity. The data from Chapter 3 showed that single pulse stimulation over the TA muscle belly recruited motor units from superficial to deep within the muscle as stimulation amplitude increased. In contrast, single pulse stimulation over the CP nerve trunk recruited motor units evenly throughout the muscle, regardless of stimulation amplitude. The data described in Chapter 4 indicated that these results may not hold true when the stimulation is delivered repetitively (i.e. NMES) over the two stimulation sites; however, this requires further investigation. For the voluntary contraction trials, investigating the progression of EMG activity within the muscle throughout the wide range of contraction amplitude will give us a better understanding of the spatial distribution of motor units recruited during voluntary contractions. All the studies described in this thesis were performed on a relatively small muscle, the TA muscle. We speculate that the results will be more robust in a larger muscle such as quadriceps.

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