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Electrical stimulation site influences the spatial distribution of motor units recruited in tibialis anterior

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HIGHLIGHTS

- M-wave recruitment curves were constructed from responses to electrical stimulation applied over the tibialis anterior muscle belly or common peroneal nerve.
- Stimulation over the muscle belly recruited motor units progressively from superficial to deep as stimulation amplitude increased.
- Stimulation over the nerve trunk recruited superficial and deep motor units equally, regardless of stimulation amplitude.

ABSTRACT

Objective: To compare the spatial distribution of motor units recruited in tibialis anterior (TA) when electrical stimulation is applied over the TA muscle belly versus the common peroneal nerve trunk.
Methods: Electromyography (EMG) was recorded from the surface and from fine wires in superficial and deep regions of TA. Separate *M*-wave recruitment curves were constructed for muscle belly and nerve trunk stimulation.
Results: During muscle belly stimulation, significantly more current was required to generate *M*-waves that were 5% of the maximal *M*-wave (M_{max}; $M_{5\%max}$), 50% M_{max} ($M_{50\%max}$) and 95% M_{max} ($M_{95\%max}$) at the deep versus the superficial recording site. In contrast, during nerve trunk stimulation, there were no differences in the current required to reach $M_{5\%max}$, $M_{50\%max}$ or $M_{95\%max}$ between deep and superficial recording sites. Surface EMG reflected activity in both superficial and deep muscle regions.
Conclusions: Stimulation over the muscle belly recruited motor units from superficial to deep with increasing stimulation amplitude. Stimulation over the nerve trunk recruited superficial and deep motor units equally, regardless of stimulation amplitude.
Significance: These results support the idea that *where* electrical stimulation is applied markedly affects *how* contractions are produced and have implications for the interpretation of surface EMG data.

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 trauma to the CNS (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 predominantly by the activation of motor axons beneath the stimulating electrodes; although the activation of sensory axons can also contribute to contractions of soleus (Klakowicz et al., 2006; Lagerquist and Collins, 2010; Bergquist et al., 2011a), vastus medialis and vastus lateralis (Bergquist et al., 2012). 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

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stimulation over the TA muscle belly versus the CP nerve trunk. Our goal was not to distinguish between the territories of single motor units, but rather to compare the spatial distribution of populations of motor units recruited by electrical stimulation applied at these two sites. Our approach also provided insight into how electromyographic (EMG) signals recorded from the surface of the skin reflect activity in the deep and superficial regions of the TA muscle.

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). 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 regions of the quadriceps, even at relatively low stimulation 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 EMG activity (*M*-waves and H-reflexes) from TA using surface EMG and fine wires inserted into superficial and deep regions of the muscle. H-reflexes were evoked infrequently and when present were small, consistent with previous literature for TA (Schieppati, 1987; Zehr, 2002; Klakowicz et al., 2006); thus, these data are not reported. 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 characterise 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}). We predicted that as stimulation amplitude increased during stimulation over the muscle belly, recruitment would progress from motor units closest to the stimulating electrodes (superficial) to those farthest 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 stimulation 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 two predictions, three hypotheses were tested. *Hypothesis* (1) When stimulation is applied over the TA muscle belly, significantly less current will be required to achieve an *M*-wave of 5% M_{max} ($M_{5\%max}$), an *M*-wave of 50% M_{max} ($M_{50\%max}$) and 95% M_{max} ($M_{95\%max}$) for the superficial compared to the deep recording site. *Hypothesis* (2) When stimulation is applied over the CP nerve trunk, the current required to achieve $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ will not differ between the superficial and deep recording sites. *Hypothesis* (3) Regardless of stimulation site, the area of either M_{max} or the largest evocable *M*-wave within the range of stimulator output 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 regions of TA during stimulation over the muscle belly, we would be able to fully activate all regions 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 applied markedly affects *how* contractions are produced (see also Bergquist et al., 2011a, 2012).

2. Methods

2.1. Participants

Nine human participants (4 males and 5 females; age range: 20–48, 27.4 ± 8.4 [mean ± SD]), 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.

2.2. Protocol

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 approximately 120°, the knee at approximately 90° and the ankle at approximately 90°. The right foot was securely strapped to the footplate of the dynamometer.

2.2.2. Electromyography

EMG was recorded at the surface of the skin using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls. VT) placed over the distal portion of TA and from superficial and deep regions of TA (see Fig. 1) using pairs of stainless steel, Teflon coated, fine-wires (0.11 mm outside diameter, A-M Systems Inc., Carlsborg, WA). These fine-wire electrodes were not intended to record single motor units, but rather ensemble EMG activity, and thus approximately 0.2 cm was de-insulated from the tip of each wire. A single wire was threaded through each of four needles (25 Gauge) such

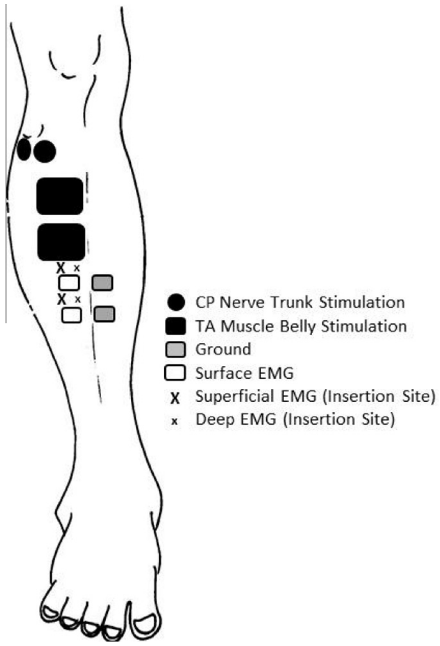

Fig. 1. Schematic of the stimulating and recording electrode sites on the right leg.

that approximately 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 regions (3.8 cm length, PrecisionGlide Needle, model 305127, Becton Dickinson and Company, Franklin Lakes, NJ) of TA using ultrasound for visual guidance. The de-insulated wire tips in each region of the muscle were inserted approximately 1 cm apart along the predicted path of the muscle fibres. The needle tips were inserted into the superficial region of TA to a depth of 0.7 ± 0.2 cm from the surface of TA, anterior to the central tendon that typically separates the superficial and deep regions of TA (Nakhostine et al., 1993). The needle tips were inserted into the deep region of TA to a depth of 2.0 ± 0.3 cm from the surface of TA, posterior to the central tendon. Common ground electrodes were 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 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 or 500 times and band-pass filtered between 10 and 1000 Hz.

2.2.3. 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 at the head of the fibula (see Fig. 1). Stimulation over the TA muscle belly was applied through electrodes (7.5 × 12.5 cm, model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) trimmed to fit over the middle third of each participants TA with the anode positioned approximately 1 cm proximal to the cathode. This site is consistent with recommendations for stimulating the main motor point of TA (Hang and Joel, 2005; Botter et al., 2011; Gobbo 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 approximately 1 cm distally along the anticipated path of the CP nerve. At each stimulation site, between 40 and 80 stimulation pulses (46 ± 11 pulses) were applied randomly every 8–10 s at amplitudes ranging from below *M*-wave and H-reflex threshold up to (when possible) approximately 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 three of the nine participants, the *M*-wave recorded from the deep recording site did not reach a maximum (i.e. *M*-wave area did not "plateau", despite increases in stimulation amplitude) even at maximum stimulator output (100 mA) during stimulation over the TA muscle belly.

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 areas were quantified as the area under the curve of the full-wave rectified waveform.

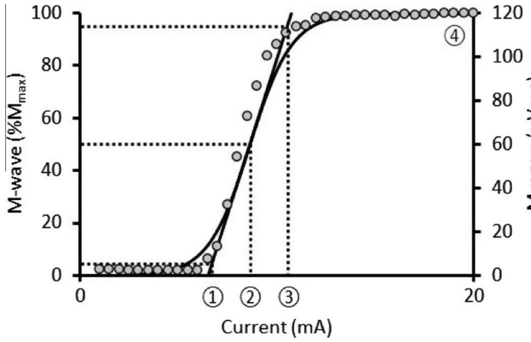

Fig. 2. Points of interest on the *M*-wave recruitment curve (① Current at $M_{5\%max}$, ② Current at $M_{50\%max}$, ③ Current at $M_{95\%max}$, ④ Largest evocable *M*-wave). *M*-wave data were fit with a sigmoid curve and a regression line was calculated. These four points of interest were determined from each *M*-wave recruitment curve using methods adopted from Klimstra and Zehr (2008).

Separate *M*-wave recruitment curves were constructed for data collected during stimulation at the two stimulation sites. *M*-wave area was plotted against current and these data were fit with a sigmoid curve (see Fig. 2) according to $M(s) = (M_{max})/(1 + e^{m}[s50 - s])$, where "$M_{max}$" is the upper limit of the curve, "m" is the slope parameter of the function, "$s50$" is the stimulation amplitude at $M_{50\%max}$, and "$M(s)$" is the *M*-wave area at a given stimulation amplitude (s). The upper limit of the sigmoid function is defined as the area of the M_{max} in a manually selected region. A sigmoid function with least squares error in current was determined from these parameters. A regression line was then fit through the sigmoid curve (Klimstra and Zehr, 2008) and four points of interest consistent with previous literature were calculated (Klimstra and Zehr, 2008; Maathuis et al., 2011; see Fig 2); (1) current at $M_{5\%max}$, (2) current at $M_{50\%max}$, (3) current at $M_{95\%max}$ and, (4) either M_{max} or the largest *M*-wave response within the range of stimulator output. When possible, for each participant, *M*-wave area was normalized to M_{max} recorded at the corresponding electrodes.

2.4. 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 2-way repeated measures analysis of variance (rmANOVA) tests were used to identify differences between the current required to achieve a specified "*M*-wave area" ($M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$) for each "recording site" (surface, superficial and deep) for stimulation over the TA muscle belly and the CP nerve trunk. A 3-way rmANOVA, that included "stimulation site" (muscle belly and nerve trunk) as a factor, would not have been appropriate since the current required to generate a given *M*-wave area was markedly different between stimulation sites, due in large part to differences in the size of the stimulating electrodes used for the two sites. For each stimulation site, since our main interest was in the current required to generate an *M*-wave of a given area for motor units located in the different regions of the muscle (i.e. recording site), only main effects of "recording site" and interactions between "recording site" and "*M*-wave area" are reported and main effects of "*M*-wave area" are not reported. Tukey's HSD tests were used for post hoc comparisons when appropriate. The three participants in whom M_{max} was not reached for the deep recording site during stimulation over the TA muscle belly were excluded from the rmANOVA analyses ($n = 6$). Paired t-tests were used to test for differences in the area of the largest evocable *M*-wave (in mV) within the range of stimulator output, between stimulation sites for each recording site. The 3 participants in whom M_{max} was not reached for the deep recording site during stimulation over the TA muscle belly were retained for the paired *t*-test analyses ($n = 9$), since these data were not normalized to M_{max}. The significance level was set $p < 0.05$ for all statistical analyses. All data are reported as mean ± standard deviation.

3. Results

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 Fig. 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. In this participant, during stimulation over the muscle belly, the recruitment curve for the surface and superficial recording sites were similar, however both were markedly different from the curve for the deep recording site. Clearly, the current required to generate $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ was less for the surface and superficial recording sites 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 achieve $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ was similar for all three recording sites.

Fig. 4 shows the mean current required to achieve $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ at each recording site averaged across the group ($n = 6$). When stimulation was applied over the TA muscle belly (Fig. 4A), there was a significant interaction between "recording site" and "*M*-wave area" [$F_{(4,20)} = 6.9$, $p < 0.01$]. Significantly more current was required to achieve $M_{5\%max}$ ($p = 0.04$), $M_{50\%max}$ ($p < 0.01$) and $M_{95\%max}$ ($p < 0.01$) at the deep, compared to the superficial, recording site. Similarly, significantly more current was required to achieve $M_{5\%max}$ ($p < 0.01$), $M_{50\%max}$ ($p < 0.01$) and $M_{95\%max}$ ($p < 0.01$) at the deep, compared to the surface recording site. Interestingly, although there were no differences in the

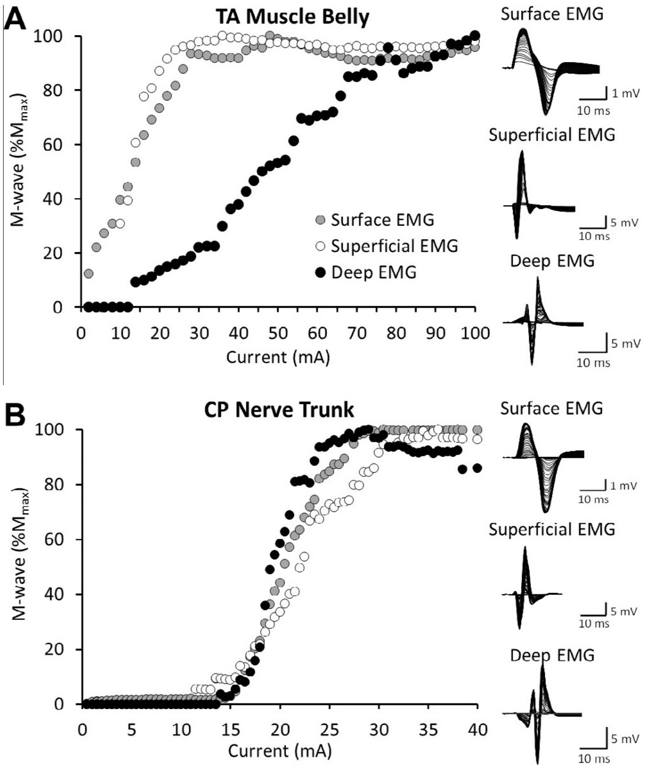

Fig. 3. Recruitment curves constructed from data recorded from a single participant for each recording site when stimulation was applied over the TA muscle belly (panel A) or CP nerve trunk (panel B). Overlaid sweeps of the EMG used to generate each recruitment curve are shown on the right of each panel.

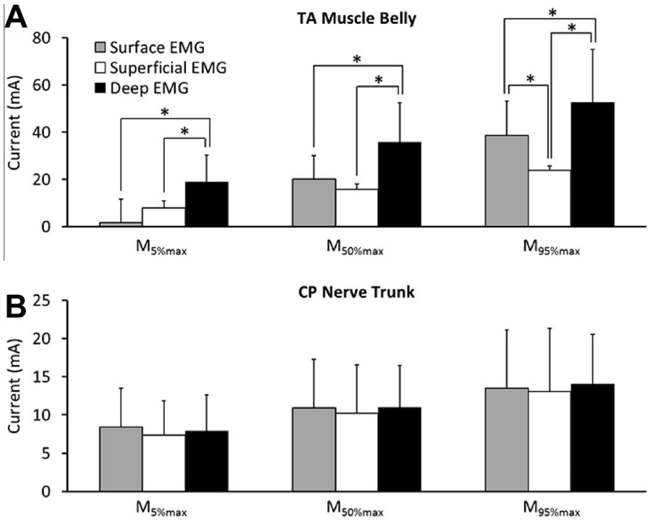

Fig. 4. Current required to generate *M*-waves of $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ averaged across six participants. Data are shown for *M*-waves recorded from the surface, superficial and deep recording sites for stimulation over the TA muscle belly (panel A) and CP nerve trunk (panel B). Note that the *y*-axis scales are different in panels A and B. Asterisks denote significant differences at $p < 0.05$.

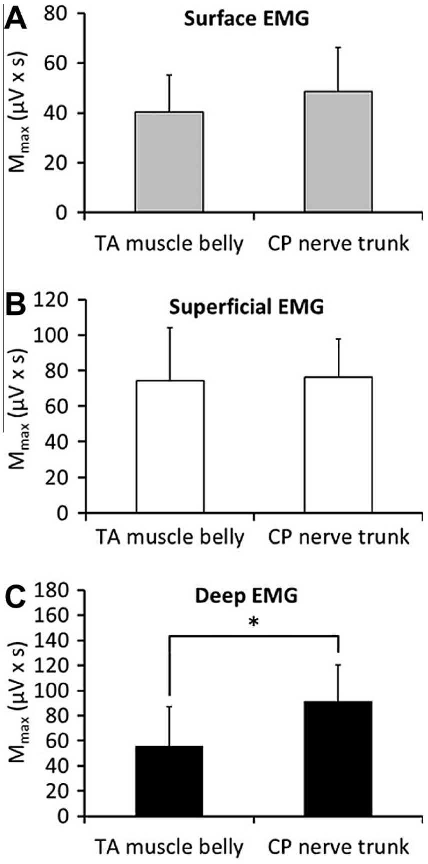

Fig. 5. Area of M_{max} or the largest evocable *M*-wave response within the range of stimulator output averaged across nine participants for the surface (panel A), superficial (panel B) and deep (panel C) recording sites during stimulation over the TA muscle belly and CP nerve trunk. Asterisk denotes significant difference at $p < 0.05$.

current required to reach $M_{5\%max}$ ($p = 0.56$) or $M_{50\%max}$ ($p = 0.87$) between the surface and superficial recordings sites, significantly more current was required to reach $M_{95\%max}$ ($p < 0.01$) at the surface compared to the superficial site. In contrast, when stimulation was applied over the CP nerve trunk (Fig. 4B), there was no main effect of "recording site" [$F_{(2,10)} = 0.94$, $p = 0.42$] and no interaction between "recording site" and "M-wave area" [$F_{(4,20)} = 0.42$, $p = 0.79$]. Thus, there were no significant differences between recording sites in the stimulation current required to generate $M_{5\%max}$, $M_{50\%max}$ or $M_{95\%max}$.

Fig. 5 shows the mean area of the largest evocable *M*-wave, within the range of stimulator output, for each stimulation site and recording site for the group ($n = 9$). At the surface [$t_{(8)} = 2.1$, $p = 0.07$] and superficial [$t_{(8)} = 0.29$, $p = 0.78$] recording sites, the largest evocable *M*-wave was not different between stimulation sites. In contrast, at the deep recording site, the largest evocable *M*-wave 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 three participants, as indicated by the lack of a clear "plateau" in *M*-wave area with increasing stimulation amplitude.

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 regions of the muscle recruited with increasing stimulation amplitude. In contrast, motor units recruited during stimulation over the CP nerve trunk were distributed evenly throughout the muscle, regardless of stimulation amplitude. We also found that EMG recorded from the surface reflected activity in both superficial and deep muscle regions.

4.1. Spatial distribution of motor units recruited by the depolarisation of motor axons

We measured the current required to generate *M*-waves at three points on the *M*-wave recruitment curve; $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max.}$ A comparison of the current required to achieve each of these three 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 stimulation amplitudes. As anticipated from 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; significantly less current was required to achieve $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ at the superficial compared to the deep recording site during stimulation over the muscle belly. Thus, when stimulation was applied over the TA muscle belly more current was required to activate motor units deep in the muscle than those located more superficially. Given the previous results (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010) and that fact that current density decreases with distance from the stimulation electrodes (Cartee and Plonsey, 1992), this result is perhaps not surprising. However, Adams et al. (1993) showed that in some participants, stimulation over the quadriceps muscle belly recruited motor units in deep regions of the muscle even at relatively low amplitudes. The fMRI technique used in that study, however, could not differentiate between activity in motor units recruited by the depolarisation of motor axons (i.e. as *M*-waves) and those recruited by reflex pathways through the spinal cord (see Bergquist et al., 2012), the latter of which would preferentially activate slow-twitch muscle fibres (Henneman, 1957; Henneman et al., 1965; Bawa et al., 1984) that are located deep in the quadriceps muscle (Knight and Kamen, 2005). Our approach, which involved the direct recording of EMG from different regions of the muscle, unlike that of Adams et al. (1993) or any of the other previous studies (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010), permits us to confirm that when stimulating over the muscle belly the depolarisation of motor axons recruits superficial motor units preferentially. Further, by comparing recruitment curves recorded at different depths of the muscle, these are the first data to show the progression of motor unit recruitment over a wide range of stimulation amplitudes applied over the muscle belly.

When the stimulation was applied over the nerve trunk, the current required to achieve $M_{5\%max}$, $M_{50\%max}$ and $M_{95\%max}$ did not differ between the superficial and deep recording sites, supporting our second hypothesis. Thus, unlike stimulation over the muscle belly, stimulation over the nerve trunk recruited motor units evenly throughout TA, regardless of stimulation amplitude. This even distribution of motor unit recruitment indicates that when electrical stimulation is applied through the skin over the CP nerve trunk, there is no relationship between the order in which axons are recruited in the nerve trunk and the spatial distribution of the muscle fibres they innervate in TA.

Our third hypothesis, that the area of the largest *M*-wave recorded at a given recording site would not differ between stimulation sites, was not fully supported. This hypothesis was based on the idea that, for both stimulation sites, 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. Consistent with our hypothesis, we found that the area of the largest evocable *M*-wave was not different between stimulation sites for the superficial recording site. Contrary to our hypothesis, however, the largest evocable *M*-wave recorded from the deep region 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 three of the nine participants in whom *M*-waves recorded at the deep recording site did not reach M_{max} at maximum stimulator output, as indicated by the lack of a "plateau" in the *M*-wave recruitment curve. Regardless, stimulation over the TA muscle belly maximally recruited the superficial region of the muscle, but not the deep, providing further evidence that stimulation over the TA muscle belly recruits superficial motor units preferentially.

4.2. Clinical implications for NMES

As the present data show, stimulation over the TA muscle belly and the CP nerve trunk activate, at least partially, different populations of motor units. More so than voluntary contractions, the efficacy of NMES-evoked contractions is limited by the early onset of fatigue (Deley et al., 2006; Theurel et al., 2007; Jubeau et al., 2008) due to the synchronous and repetitive activation of the same population of motor units (Maffiuletti, 2010). To reduce fatigue during stimulation over a muscle belly, Maffiuletti (2010) has suggested ways to recruit previously inactive motor units either by re-locating the stimulating electrodes on the muscle belly, varying the joint angle or increasing stimulation amplitude within tolerance levels. Data from the present study indicate that another way to accomplish this outcome would be to re-locate 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.

Another option would be to alternate or ‘‘interleave’’ stimulation pulses between over the muscle belly and nerve trunk. This interleaved stimulation would recruit, at least partially, different populations of motor units with every other stimulation pulse, crudely mimicking the asynchronous motor unit firing that occurs during voluntary contractions and effectively halving the firing frequencies of many of the active motor units, thereby reducing their metabolic demand. Additional options include alternating the delivery of stimulation trains, or sets of stimulation trains, between over the muscle belly and over the nerve trunk to crudely mimic the motor unit rotation observed during voluntary contractions (Bawa et al., 2006; Bawa and Murnaghan, 2009).

Consistent with previous literature (Vanderthommen et al., 2000; Farina et al., 2004; Mesin et al., 2010), the present data showed that stimulation over the muscle belly recruits superficial motor units preferentially. This spatial pattern of recruitment during stimulation over the muscle belly also means that it may not be possible to activate motor units located farthest 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 three participants even at maximal stimulator output. Further, across the group of participants, the maximal evocable *M*-wave was smaller in the deep region of the muscle compared to when the stimulation was applied over the nerve trunk. These data have implications for using NMES to generate functional contractions since preferentially activating one region of a muscle, as occurs when stimulation is applied over the muscle belly, makes the contraction less efficient due to suboptimal force transmission to the tendons (Hill, 1938; Martins et al., 1998). Therefore, when using NMES to restore movement it may be preferable to recruit motor units that are evenly distributed throughout the muscle by delivering NMES over the nerve trunk.

Changing the stimulation site may affect not only the spatial distribution of recruited motor units, but also 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 compared to stimulation over the muscle belly suggests that different types of motor units will be recruited by stimulation at each site. 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 regions (Henriksson-Larsen et al., 1983). From the present data, we 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 fibres (Gregory et al., 2001) and, as stated earlier, contains a higher density of these fibres in deeper regions of the muscle (Knight and Kamen, 2005). To target these muscle fibres most effectively, stimulation over the nerve trunk may be more appropriate. It should be noted, however, that further investigation is required to determine whether the presently observed effect of stimulation site on the spatial distribution of recruited motor units for this relatively small muscle is generalisable to other muscles. Although we would expect the effect to be more pronounced in larger muscles, we acknowledge that the effect will vary depending on the positioning of the stimulating electrodes relative to the motor points and the way in which motor axons are distributed within a particular muscle.

These data also have implications for when NMES is used to assess voluntary drive by the twitch interpolation technique (Gandevia, 2001). Specifically, our data suggest that, to ensure complete activation of the muscle in all individuals, it is most appropriate to stimulate over the nerve trunk compared to over the muscle belly. However, there are a number of limitations including stimulation discomfort and muscle co-activation associated with stimulation over a nerve trunk, which may make stimulation over the muscle belly a more appealing option (Place et al., 2010). Further, at large contraction amplitudes ($\geqslant$60% of a maximum voluntary contraction), estimations of voluntary drive to the quadriceps muscles did not differ when stimulation was applied over the muscle belly or the femoral nerve trunk (Place et al., 2010).

4.2.1. Implications for surface EMG

Although it was not the focus of the present experiments, our approach also provided some insight into how activity in superficial and deep regions of the muscle contributes to the surface EMG. This information was provided by the data from stimulating over the TA muscle belly, due to the differences in how this type of stimulation recruited motor units in the deep and superficial regions of the muscle. For example, during stimulation over the muscle belly there were no differences in the current required to achieve $M_{5\%\max}$ or $M_{50\%\max}$ between the surface and superficial recording sites. At these lower stimulation amplitudes, deep regions of the muscle were relatively inactive and, therefore, the activity in the deep regions contributed little to the surface recording and activity in the superficial regions dominated the surface recording. Interestingly, however, more current was required to achieve $M_{95\%\max}$ on the surface than the superficial recording site. We suggest that this was because, as the deep region of the muscle became more active at the higher stimulation amplitudes, some of that activity contributed to the surface recording. However, not all of the activity in the deep region of the muscle was reflected at the surface, since significantly more current was required to achieve $M_{95\%\max}$ on the deep compared to the surface recording site. This latter result is consistent with the finding that during stimulation over the TA muscle belly, peak twitch torque continued to increase after the area of the *M*-wave recorded from the surface ‘‘plateaued’’, suggesting that beyond a given stimulation level, deep regions of TA contribute little if any to the surface recording (Mesin et al., 2010). A more direct way to have tested how activity in superficial and deep regions of the muscle contributes to the surface EMG would have been to ‘‘back stimulate’’ through the deep and superficial fine-wire recording electrodes independently, thus allowing for independent activation of *only* deep or *only* superficial regions of TA, and to compare the response at the surface over a range of stimulation amplitudes.

4.3. Summary

In this study, single pulses of electrical stimulation were applied over the TA muscle belly or the CP nerve trunk over a range of stimulation amplitudes to compare the spatial distribution of motor units recruited by the two stimulation sites. Consistent with previous studies, we found that stimulation over the muscle belly recruited superficial motor units preferentially, with deeper regions of the muscle recruited as stimulation amplitude increased. In contrast, during stimulation over the nerve trunk, recruited motor units were evenly distributed between superficial and deep regions, regardless of stimulation amplitude. These results contribute to our understanding of how electrical stimulation generates muscle contractions and provides further evidence that *where* stimulation is applied markedly affects *how* contractions are produced (see Bergquist et al., 2011a, 2012). Since repetitive stimulation is used to produce contractions for rehabilitation, further investigation is required to test whether these findings hold true during repetitive stimulation.

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