Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments

Running title: Lumbar spinal stimulation

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Proposed section: Neurosystems

Number of words in the manuscript: 6,095

Number of words in the abstract: 249

Numbers of pages: 43

Number of tables: 2

Number of figures: 4

ABSTRACT

Electrical stimulation over the mastoids or thoracic spinous processes has been used to assess subcortical contribution to corticospinal excitability, but responses are difficult to evoke in the resting lower limbs or are limited to only a few muscle groups. This might be mitigated by delivering the stimuli lower on the spinal column, where the descending tracts contain a greater relative density of motoneurons projecting to lower limb muscles. We investigated activation of the corticospinal axons innervating tibialis anterior (TA) and rectus femoris (RF) by applying a single electrical stimulus over the first lumbar spinous process (LS). LS was paired with transcranial magnetic stimulation (TMS) at interstimulus intervals (ISIs) of −16 (TMS before LS) to 14 ms (LS before TMS). The relationship between muscle contraction strength (10-100% maximal) and the amplitude of single pulse TMS and LS responses were also investigated. Compared to the responses to TMS alone, responses to paired stimulation were significantly occluded in both muscles for ISIs ≥–8 ms ($p \leq 0.035$), consistent with collision of descending volleys from TMS with antidromic volleys originating from LS. This suggests that TMS and LS activate some of the same corticospinal axons. Additionally, the amplitude of TMS and LS responses increased with increasing contraction strengths with no change in onset latency, suggesting responses to LS are evoked transsynaptically and have a monosynaptic component. Taken together, these experiments provide evidence that LS is an alternative method that could be used to discern segmental changes in the corticospinal tract when targeting lower limb muscles.

INTRODUCTION

The corticospinal tract, a major descending pathway for the control of voluntary movement, includes polysynaptic (Pierrot-Deseilligny, 2002) and monosynaptic projections between the motor cortex and motoneurons of the upper and lower limb muscles (Brouwer & Ashby, 1992; Palmer & Ashby, 1992; de Noordhout *et al.*, 1999). The behaviour of the corticospinal pathway can be non-invasively assessed by transcranial magnetic stimulation (TMS). The response to TMS is measured in the electromyographic (EMG) activity of a target muscle and is referred to as the motor evoked potential (MEP). Any change in MEP size may be due to changes in the excitation or inhibition of cortical neurons or spinal motoneurons (Rossini *et al.*, 2015). To discern the contribution of spinal motoneurons to the overall response, stimulation of Ia afferents has been used previously (H-reflex; Nielsen et al. 1999). However, the H-reflex is known to be sensitive to the influence of presynaptic mechanisms (Zehr, 2002). An alternative methodological approach for assessment of motoneuron pool excitability is electrical stimulation of descending axons at a subcortical level (Ugawa *et al.*, 1991), which, unlike the H-reflex, is thought to be devoid of presynaptic influences (Nielsen & Petersen, 1994; McNeil *et al.*, 2013). For this reason, it is considered a more direct, and arguably more appropriate method for assessment of spinal motoneuron contribution to the overall corticospinal response. This is reinforced by the fact that the action potentials descending from the motor cortex are attenuated by antidromic collision of those originating from spinal stimulation, indicating activation of some of the same corticospinal axons (Ugawa *et al.*, 1991; De Noordhout *et al.*, 1992; Taylor *et al.*, 2002; Martin *et al.*, 2008). Moreover, the response to electrical stimulation of the corticospinal axons at a subcortical level with increased contraction strength is similar to that of TMS (De Noordhout *et al.*, 1992; Weavil *et al.*, 2015) and exhibits no change in onset latency (Petersen *et al.*, 2002; Martin *et al.*, 2008), suggesting a large monosynaptic component (Petersen *et al.*, 2002). Stimulation at subcortical levels has been successfully applied to

investigate the contribution of spinal motoneurons to the overall response in upper limbs, but less often in lower limbs (for review see Taylor and Gandevia, 2004). Despite the locomotive importance of the lower limbs and their common study as part of rehabilitation or training programmes (Yan *et al.*, 2005; Pollock *et al.*, 2014), there is a relative dearth of studies employing both TMS and spinal stimulation to assess segmental neural responses.

Measurement of the status of spinal motoneurons that control lower limb function is complicated by methodological challenges. For example, when stimulation is applied between the mastoids, it is difficult to evoke responses in leg muscles at rest (Ugawa *et al.*, 1995), or are limited to activation of certain lower limb muscles, e.g. tibialis anterior (TA; Claus *et al.*, 1991; De Noordhout *et al.*, 1992; Ugawa *et al.*, 1995). The stimulation over the thoracic spinous processes has been shown to evoke response in quiescent lower limb muscles, but not in all muscles and participants (Martin *et al.*, 2008). Furthermore, even if responses are evoked, they tend to be small (≤ 10% of maximal muscle response; Martin et al. 2008). Notably, stimulation of the descending tracts at the mastoid or thoracic level also stimulates motoneurons associated with control of upper limb and trunk musculature (Nathan and Smith, 1982; Nathan et al, 1996), therefore the current applied is likely shared between the motoneuron pool of multiple muscle groups (Kendall *et al.*, 2005). Excitable tissues such as muscle and upper limb nerve roots likely also become depolarised by the large current applied (Taylor, 2006), resulting in contraction of back, neck, shoulder and arm muscles (Martin *et al.*, 2008). Thus, an alternative paradigm that mitigates the aforementioned technical challenges would be advantageous for investigation of corticospinal behaviour in the lower limb muscles.

A potential solution to the methodological challenge of subcortical stimulation of the corticospinal axons when lower limb muscles are targeted could be stimulation of the lower spinal column. At the lumbar level, the descending tracts contain a greater relative density of motoneurons projecting to lower limb muscles (Sayenko *et al.*, 2015). Stimulation applied

closer to these projections will likely result in a higher current density in the lower limb motoneurons when compared to mastoid or thoracic stimulation. Indeed, Kuck *et al*. (2017) and Fernandes *et al*. (2018) have shown, via modelling techniques, that when the cathode and anode are placed over the lumbar and thoracic spinous processes, respectively, the highest density of electrical field is concentrated around the spinal cord segments associated with lower limb projections. Furthermore, these modelling studies also indicated that electric field magnitude is likely to be higher in the lateral spinal cord white matter where the lateral corticospinal tract is located. Thus, when targeting the lower limb muscles, stimuli delivered lower on the spinal tract might provide an alternative methodological paradigm to activate the descending corticospinal axons. One further consideration for assessing lower limb corticospinal excitability is that responses are commonly evoked during muscle contraction (Sidhu *et al.*, 2012; Brownstein, Ansdell, Škarabot, Frazer, *et al.*, 2018; Škarabot *et al.*, 2018). This is recommended when responses during or following locomotion are of interest (Gruet *et al.*, 2013; Kalmar, 2018; Weavil & Amann, 2018). Thus, it is important to discern how responses to lumbar stimulation behave during different levels or neural drive (i.e. contraction intensity). Similar to MEPs, if lumbar-evoked responses (LEPs) change with increasing contraction intensity (Weavil *et al.*, 2015), it would indicate that the response is primarily mediated by activation of corticospinal tract, and is an appropriate index of excitability during locomotor muscle contraction.

Therefore, in this study, we aimed to explore whether a single electrical stimulus over the first lumbar spinous process (LS) activates descending corticospinal axons innervating lower limb muscles by pairing LS with TMS of the motor cortex at appropriately timed ISIs. It was hypothesised that when the stimuli are paired at intervals shorter than the difference in latencies of each stimulus alone there will be an occlusion of the response to paired stimulation relative to the response to TMS alone. This technique has been employed previously with cervical and

thoracic stimulation to explore a similar hypothesis (Taylor *et al.*, 2002; Martin *et al.*, 2008). Additionally, the contraction strength – stimulus response curves of TMS and LS were compared. Responses were recorded in TA and rectus femoris (RF) due to the integral role of these muscles in locomotion. The former plays a role in the control of foot drop during heel strike and foot lift during the swing phase (Marsh *et al.*, 1981; Byrne *et al.*, 2007), has strong corticomotoneuronal projections (Brouwer & Ashby, 1992; Perez *et al.*, 2004) and exhibits high corticospinal drive during human gait (Schubert *et al.*, 1997). On the other hand, RF is involved in numerous activities of daily living including sit-to-stand, stair climbing and gait (Hurley *et al.*, 1998) as well as athletic activities, making it important to understand the neurophysiological behaviour of these muscles.

MATERIALS AND METHODS

Ten healthy, young volunteers (24 ± 4 years, 179 ± 8 cm, 77 ± 12 kg; 7 males, 3 females) participated in the study. All participants were free from neurological illness or musculoskeletal injury, were not taking any medications known to affect the nervous system, and had no contraindications to TMS. The study conformed to the standard of *Declaration of Helsinki*. All procedures were approved by Northumbria University Faculty of Health and Life Sciences Ethics Committee (BMS57UNNJSRD2016) and prior to any experimental protocols, all participants provided written informed consent.

Experimental design

Participants visited the laboratory on three separate occasions: a familiarisation visit, then two separate visits for the assessment of TA and RF responses. When assessing responses in the

TA, participants were sat in an isokinetic dynamometer (Cybex, Lumex Inc., USA) with hip at 60° flexion, knee and ankle at 90° flexion, and the foot strapped to the motor plate with Velcro at the level of the thalus and phalange bones. When assessing responses in the RF, participants were sat in a custom built chair with a calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) with hip and knee at 90° flexion, and the leg strapped with a non-compliant cuff ~2 cm superior to the ankle malleoli. The voltage signal originating from the dynamometer and the load cell was calibrated and converted into torque (N·m; TA setup) and force (N; RF setup) respectively, and displayed on the screen in front of the participant. All measures were performed on the right limb.

In the first part of the study, with the muscle at rest, TMS and LS were either delivered separately, or paired with different ISIs. Initially, the responses to individual TMS and LS were standardised to elicit a response that was ~10-15% of the resting maximal compound action potential (M_{max}), and the stimulus intensities required to produce these outputs were then applied during paired stimulation. Paired TMS and LS were delivered either with TMS preceding LS (ISIs from −16 to −2 ms, every 2 ms), both stimuli occurring at the same time (ISI of 0 ms), LS preceding TMS (ISIs from 2 to 14 ms, every 2 ms), or LS and TMS delivered independently of each other (Figure 1A). Therefore, there were a total of 18 different stimuli, delivered separately in 10 sets, totalling 180 stimulations. The order of each set of stimuli was randomised, with pulses within each set delivered every 5-10 seconds.

[Figure 1]

In the second part of each visit, participants performed two maximum voluntary contractions (MVCs), of which the greatest instantaneous torque/force was used to set guidelines for subsequent contractions. TMS and LS were then delivered separately at 10, 25, 50, 75 and 100% MVC (Figure 1A). The order of the type of stimulation and the contraction strength was

randomised. Five stimuli were performed at each contraction intensity for each type of stimulation, to avoid the influence of decreases in muscle function at higher contraction intensities. Stimulations were delivered once the torque (TA) and force (RF) had plateaued at the target line. At least 60 seconds rest was given between each contraction. Initially, the responses to individual TMS and LS were standardised to ~50% M_{max} during a contraction at 50% MVC, and the stimulus intensities required to produce these outputs remained constant for all contraction intensities to investigate how different muscle activity might affect the size of responses. Standardising evoked responses during contraction to a higher percentage of M_{max} than that used when evoking responses at rest was chosen to distinguish the evoked response from background EMG activity, and because the size of the response is known to be sensitive to change with contraction strength (Weavil *et al.*, 2015).

Percutaneous nerve stimulation

Percutaneous stimulation of the common peroneal nerve (40 mm cathode/anode arrangement; Digitimer, Hertfordshire, UK) and femoral nerve (3.2 cm^2 diameter, Nidd Valley Medical Ltd., Bordon, UK) was performed (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) to elicit M_{max} in the TA and RF muscles, respectively. M_{max} was elicited by gradually increasing the stimulation intensity until the EMG response plateaued. To ensure a supramaximal stimulus the intensity was further increased by 30% (mean intensity of 47 ± 14 and 249 ± 88 mA for TA and RF, respectively).

Transcranial magnetic stimulation

Single pulse TMS was delivered using a Magstim 200^2 magnetic stimulator (Magstim Co., Ltd., Whitland, UK) connected to a concave double-cone coil positioned over the left cortical hotspot for the TA and RF muscles with a posterior-to-anterior current orientation to elicit MEPs. The coil was initially positioned 1 cm lateral and posterior of the vertex (Devanne *et al.*, 1997; O'Leary *et al.*, 2015), after which it was moved in small steps medio-laterally and posterior-anteriorly around the initial position until the spot evoking the greatest MEP in the target muscle using 50-70% stimulator output was found. Once identified, this spot was marked directly on the scalp with indelible ink to ensure consistent placement throughout the trial. The intensity of stimulation was standardised to elicit a response equating to ~10-15% of the resting M_{max} (mean stimulation intensity of 54 ± 16 and 53 ± 19% for TA and RF, respectively) for the first part of the study and to ~50% M_{max} during a contraction at 50% MVC (43 ± 10% and 55 ± 10% of stimulator output for TA and RF, respectively) for the second part.

Electrical stimulation of the first lumbar spinous process

LEPs were elicited with a constant-current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Nidd Valley Medical Ltd., Bordon, UK). The cathode electrode (5 × 9 cm) was centred over the first lumbar (L_1) spinous process, with the long axis of the electrode aligned to the centre of the vertebral column (Figure 1B). The surface area of the cathode covered two spinous processes above and below the centre point (T_{11}-L_3). A cathode of large area was chosen as it produces less discomfort and greater tolerance by participants (Ugawa *et al.*, 1995; Kuhn *et al.*, 2010). The bottom of the anode (circular shape; 3.2 cm diameter) was placed in the midline of the vertebral column 5 cm above the upper edge of the cathode (Ugawa *et al.*, 1995), corresponding to the level of the eighth thoracic spinous process (T_8). Based on modelling studies, this electrode configuration was chosen as it is likely

to induce the greatest electric field magnitude between T_{10} and T_{12} spinous processes due to electric field being highest between the stimulating electrodes (Kuck *et al.*, 2017). As such, the site of greatest spinal cord activation is likely to occur between the L_1-L_5 spinal segments, corresponding to the motoneuron pools of RF and TA (Sharrad, 1964; Sayenko *et al.*, 2015). Similar to TMS, the intensity of stimulation was standardised to ~10-15% M_{max} evoked in the resting position (194 ± 93 and 168 ± 69 mA for TA and RF, respectively) and to ~50% M_{max} during a contraction at 50% MVC (216 ± 87 and 145 ± 58 mA for TA and RF, respectively). The differences in the applied current are thus likely due to different standardisation of stimulus intensities for different parts of the experiment. To ensure ventral roots were not stimulated, responses were monitored for a lack of an abrupt decrease in latency and increase in response size with voluntary contraction (Taylor, 2006). Paired LS was also performed at the target stimulus intensity at an ISI of 50 ms before the start of the main recording session, with the lack of depression of the second response excluding the possibility of stimulation of dorsal roots (Roy *et al.*, 2012; Danner *et al.*, 2016; Hofstoetter *et al.*, 2018). All participants reported they found LS to be tolerable.

Electromyography

Electromyographic (EMG) activity was recorded with a bipolar electrode arrangement (8 mm diameter, 20 mm inter-electrode distance; Kendall 1041PTS, Tyco Healthcare Group, USA) over the muscle belly of TA and RF according to SENIAM recommendations (Hermens *et al.*, 2000) with the reference electrode placed over the patella. Prior to electrode placement, the skin was thoroughly prepared including shaving, abrading with preparation gel and wiping with an alcohol swab to ensure appropriate electrode resistance (< 2 kΩ). The EMG signal was

amplified (×1000), band pass filtered (20-2000 Hz; Neurolog System, Digitimer Ltd, UK), digitised (5 kHz; CED 1401, CED, UK), acquired and analysed off line (Spike2, v8, CED, UK).

Data analysis and statistics

In the experiment assessing the interaction of LS and TMS, the data analysis was similar to that described previously by Taylor et al. (2002). Briefly, using a customised script in Spike2 (v8, CED, UK), the waveforms of individual responses to LS alone, TMS alone and paired stimulation were averaged. These are depicted in the example responses in TA (Figure 2) during selected ISIs from one individual in the top three rows. Whilst in this representative response there is evidence of facilitation (highlighted grey area) at an ISI of −14 ms, for the other ISIs shown the interaction between the stimuli makes it difficult to determine if facilitation or occlusion has occurred. Thus, the averaged response waveform to LS alone was then temporally aligned to the LS stimulus time point of the averaged response waveform to paired stimulation and graphically subtracted from the latter (paired – LEP; Figure 2, bottom row). After that, the peak-to-peak amplitude of the paired – LEP waveform was calculated and compared to the amplitude of the averaged response to TMS alone ([paired – LEP]/MEP). It has previously been suggested that the subtraction might reveal an inverted potential resulting in negative values, such as in the cases when response to LS is larger than the response to paired stimulation (Taylor *et al.*, 2002), however, this was never the case in the present data. Paired sample T-tests were used for assessing the statistical significance of the differences in the paired – LEP amplitude relative to the MEP alone amplitude. It should be noted that individuals of different height and thus different lengths of neural pathways along with reported differences in conduction velocity between individuals (Andreassen & Arendt-Nielsen, 1987; Sadoyama *et al.*, 1988) could confound the interpretation of the interaction of LS and TMS. For that reason, additional

analyses were performed to account for this potential disparity. Firstly, the difference in MEP and LEP latency was calculated estimating the time required for the first volley elicited by TMS to reach the segmental level activated by LS. This time (rounded to the nearest 2 ms) was referred to as normalised ISI of 0 ms. Subsequently, the positive and negative normalised ISI values are indicative of the first volley evoked by TMS not having arrived at or having passed the site of descending axon activation by LS, respectively (Martin *et al.*, 2008). Due to incomplete number of samples ($n < 10$), statistical analysis using paired sample T-test was not performed for this part of the analyses at normalised ISIs of –6, –4 , and 26 and 28 ms. The variability of individual responses to TMS and LS was assessed by calculating a coefficient of variation for each series of 5 evoked potentials (MEPs or LEPs) for each individual (CV = standard deviation of 5 evoked potentials ÷ mean of 5 evoked potentials × 100%). In the experiment assessing the responses with increased contraction strength, peak-to-peak amplitudes of the single pulse evoked responses were calculated, averaged and normalised to M_{max}. Background EMG activity was quantified as root mean square (RMS) in the 100-ms epoch prior to stimulus and normalised to RMS EMG activity during an MVC. A 2 × 5 repeated measures ANOVA was performed to determine whether contraction strength-response curves and background EMG activity were different. The effect of contraction strength on MEP and LEP latencies was assessed via a one-way repeated-measures ANOVA. If F-values were found to be statistically significant, analysis was continued using pairwise comparison with Bonferroni correction. All statistical analyses were performed in SPSS (v20, SPSS Inc., Chicago, IL, USA). All data are reported as means ± standard deviations. Significance was set at alpha level of 0.05. To allow for a more nuanced interpretation of the data, Cohen's d_z were calculated as an effect size measure for statistical procedures involving paired sample T-tests. Cohen's d_z was calculated as the ratio of mean difference and standard deviation of differences, which slightly differs from traditional Cohen's *d* calculation in that it is better suited for within-

subject, rather than traditional between-subject differences (Becker, 1988; Smith & Beretvas, 2009). Partial eta squared (η_p^2) were calculated as a measure of effect size for statistical procedures involving ANOVA.

[Figure 2]

RESULTS

Latencies of MEPs and LEPs at rest and across contraction intensities remained unchanged in both muscles ($p \geq 0.081$; Table 1). The response variability was greater for MEP compared to LEPs and was reduced in an active muscle compared to rest for both evoked responses (Table 2).

[Table 1]

[Table 2]

Interaction of LS and TMS

Representative traces recorded in TA from one individual assessing the interaction of LS and TMS are shown in Figure 2. Similar individual behaviour was observed across all participants as well as in the RF muscle.

In TA, the mean sample data shows that pairing the two types of stimuli resulted in occlusion ([Paired – LEP]/MEP < 1.0) of responses at ISIs between −8 and 14 ms (*P* value range = 0.001 – 0.048, d_z range = 0.5 – 1.4; Figure 3A). The paired responses were also facilitated at −14ms (*P* = 0.038, d_z = 0.6). Furthermore, six out of the ten participants also exhibited facilitation of responses ([Paired – LEP]/MEP > 1.0) at ISIs of −16 and −12 ms, respectively, but this was not

statistically significant at the group level (P = 0.195 & 0.223, d_z = 0.4 for both). For the mean sample data in TA, individual TMS responses of 17.3 ± 6.4% M_{max} and individual LS responses of 12.6 ± 5.4% M_{max} were evoked in TA.

[Figure 3]

In RF, TMS alone evoked responses of 15.3 ± 5.8% M_{max}, and LS alone evoked responses of 13.3 ± 3.3% M_{max}. The interaction of TMS and LS resulted in occlusion of responses between ISIs of −8 and 14 ms (P value range = 0.001 – 0.049, d_z range = 0.3 – 1.4; Figure 3B) and facilitation at ISIs of −16 and −14 ms (P = 0.011 & 0.031; d_z = 0.5 & 0.7). At ISIs of −12 ms, no facilitation was observed at the group level (P = 0.119, d_z = 0.5); however, on an individual level, 6 participants exhibited facilitation ([Paired – LEP]/MEP > 1.0).

The effect of timing of stimuli on interaction of LS and TMS

The responses to paired stimulation were significantly occluded relative to the response to a single TMS pulse at > 2 ms before the expected arrival of the first descending volley evoked by TMS to the segmental level of LS in both TA (P value range = 0.001 – 0.033, d_z range = 0.6 – 1.2; Figure 3C) and RF (P value range = 0.001 – 0.012, d_z range = 0.6 – 1.2; Figure 3D). The paired responses were also significantly facilitated when the first descending volley evoked by TMS was at the same level as LS in both muscles (P = 0.021 and 0.010 for TA and RF, respectively; d_z = 0.7 for both), and when LS was delivered 2 ms after the expected arrival of the first descending volley evoked by TMS in RF (P = 0.038, d_z = 0.6).

Responses with increases in contraction strength

Background muscle activity increased progressively from 10 – 100% MVC in both TA ($F_{2.4, 18.3} = 252.0$, $P < 0.001$, $\eta_p^2 = 0.97$; Figure 4A) and RF ($F_{2.1, 18.9} = 318.4$, $P < 0.001$, $\eta_p^2 = 0.97$; Figure 4B).

In TA, MEPs and LEPs were dependent on contraction strength ($F_{1.5, 13.0} = 15.1$, $P = 0.001$, $\eta_p^2 = 0.63$), such that responses peaked at 75% MVC (Figure 4C). There was also a statistically significant interaction between contraction strength and type of stimulus ($F_{4, 36} = 7.7$, $P < 0.001$, $\eta_p^2 = 0.46$) with post hoc testing showing a difference between stimuli types at 10% MVC ($p = 0.011$).

In RF, there was also a contraction type dependency of MEPs and LEPs ($F_{1.6, 7.8} = 11.9$, $P = 0.001$, $\eta_p^2 = 0.57$) insofar as the responses peaked at 50% MVC (Figure 4D). The interaction between contraction strength and type of stimulus was not significant ($F_{1.2, 5.8} = 1.9$, $P = 0.125$, $\eta_p^2 = 0.18$).

[Figure 4]

DISCUSSION

The results of these experiments show that the response to paired magnetic cortical and electrical stimulation of the lumbar spinal segments is occluded at appropriate interstimulus intervals and that responses to TMS and LS similarly increase with increases in contraction strength with no change in onset latency. This behaviour suggests that LS and TMS activate some of the same corticospinal axons and that responses to LS are evoked transsynaptically with a monosynaptic component. Thus, this stimulation technique has applicability as an alternative paradigm for investigating the contribution of spinal motoneuron excitability to the overall corticospinal response when lower limb muscles are targeted.

Evidence for stimulation of descending tracts

The occlusion observed with TMS being delivered before LS at intervals shorter than the difference in latencies of each stimulus alone corroborates previous findings when electrical stimulation was performed over the mastoids with arm and hand muscles targeted (Ugawa *et al.*, 1991; Taylor *et al.*, 2002). Similarly, when LS preceded TMS, the responses were occluded to the same degree, again confirming the findings seen when electrical stimulation was performed over the mastoids and targeting the muscles of the arm (Taylor *et al.*, 2002). This occlusion corresponded to the timing of the stimuli when LS was delivered more than 2 ms before the expected arrival of the first descending volley evoked by TMS (Martin *et al.*, 2008), which is consistent with collision of the descending cortical volleys of TMS with antidromic volley originating from LS. These findings indicate that LS activates some of the same axons as TMS, likely the pyramidal cells in the corticospinal tract (Ugawa *et al.*, 1991; Taylor *et al.*, 2002). Whilst the occluded response could have emerged due to disynaptic inhibition originating from LS-induced activation of inhibitory interneurons via cutaneous receptors of the lumbosacral region (Frigon *et al.*, 2012), this is unlikely given the facilitation that was observed at longer ISIs when TMS preceded LS (Taylor *et al.*, 2002). Facilitation corresponded to the first descending volley evoked by TMS having passed the segmental level of LS by more than 2 ms. This facilitation, which has been consistently shown for the aforementioned timing (Ugawa *et al.*, 1991; Taylor *et al.*, 2002; Martin *et al.*, 2008), is the result of the descending volley evoked by LS arriving at the motoneuron pool that is already excited by TMS descending volleys (Martin *et al.*, 2008).

In theory, it was expected that when LS was delivered prior to TMS at ISIs longer than the difference in latencies of individual stimuli, the antidromic volley would reach the cortex prior

to its excitation by the magnetic stimulus, resulting in a response similar to a single-pulse TMS. However, we found that at longer ISIs (LS preceding TMS), responses remained occluded in both muscles. This behaviour is in agreement with Taylor et al. (2002), but differ to that of Ugawa et al. (1991). However, the latter employed electrical stimulation of the cortex, whilst the former stimulated the cortex with TMS, similar to the present study, suggesting that the origin of the observed depression is cortical, possibly through inhibition via collaterals of corticospinal axons (Krnjević *et al.*, 1966; Ghosh & Porter, 1988).

There are certain factors that complicate the interpretation of the interaction between electrical stimulation of the spinal tracts and responses evoked by TMS, even if it is assumed that the pathway is purely monosynaptic (Petersen *et al.*, 2002). Firstly, magnetic and electrical stimulation differ in their mechanism of activation of neurons, such that TMS evokes multiple descending volleys, whereas LS only elicits a single descending volley (Nakamura *et al.*, 1996; Houlden *et al.*, 1999; Terao *et al.*, 2000). This makes it likely that only the first volley of TMS is affected by the collision originating from LS. Consequently, comparison of responses to paired stimulation to a single TMS response actually underestimates the occlusion as a result of collision (Martin *et al.*, 2008). Thus, despite the interaction of the stimuli being complex, our data, in conjunction with previous work in the area (Taylor *et al.*, 2002; Martin *et al.*, 2008), suggests that the single volley produced by LS can occlude the response to TMS. Secondly, the observed occlusion could be a result of descending action potentials being in a refractory state. However, this is an unlikely contributor given the observed facilitation of responses when the first descending volley evoked by TMS had passed the segmental level of LS and since occlusion occurred at ISIs far longer than the refractory period of motoneurons (> 3 ms; Day et al. 1989). Lastly, if the motoneurons are not activated monosynaptically, the paired response could be influenced by excitatory and inhibitory interneurons. However, had there been multiple synapses involved in the present study, increased excitability of motoneurons with

increased contraction strength would have likely shortened the activation time of each postsynaptic cell and thus reduced the onset latency of evoked potentials (Petersen *et al.*, 2002). This was not the case as responses to individual TMS and LS increased similarly with increased contraction strength with no change in onset latency. The increase in response amplitude with increased contraction strength is also a good indicator that the responses were evoked transsynaptically as opposed to distal to the cell bodies (Martin *et al.*, 2008). It is also worth noting that both LEPs and MEPs increased at a similar rate as shown previously (De Noordhout *et al.*, 1992; Weavil *et al.*, 2015) and peaked ≥ 75 and 50% in TA and RF, respectively, consistent with the relationship between motor unit recruitment and firing frequency of the muscles investigated (Gelli *et al.*, 2007), which determines the probability of an evoked response (Brouwer *et al.*, 1989; Bawa & Lemon, 1993; Jones & Bawa, 1999). The lack of a decrease of evoked responses during MVC disagrees with some experiments (Goodall *et al.*, 2009; Mira *et al.*, 2017), but corroborates others (Oya *et al.*, 2008; Weavil *et al.*, 2015). This discrepancy has been attributed to the dependency of responses with increased contractions strength on stimulus intensity (Oya *et al.*, 2008; Weavil *et al.*, 2015), such that the greater the stimulus intensity, the lower the probability of an evoked response with increased firing rate (Matthews, 1999). The lack of a decrease in evoked responses during MVC notwithstanding, similar behaviour of LEPs and MEPs with increased contraction strength is a good indicator that segmental responses can be assessed during a voluntary contraction which is of importance for the lower limbs, where exercise-induced alterations in corticospinal excitability are of interest (Lévénez *et al.*, 2008; Finn *et al.*, 2018).

The possibility of stimulation of other neural structures

Whilst the present data provide evidence that LS and TMS activate similar axons, i.e. the pyramidal cells in the corticospinal tract, there remains the possibility that other descending tracts might also be excited and hence be contributing to the observed effects (Ugawa *et al.*, 1991). Of particular consideration would be those tracts located in the lateral white matter, such as rubrospinal and reticulospinal tracts (Nathan and Smith, 1982; Nathan et al, 1996), as modelling studies indicate that electric field magnitude, due to LS, is likely higher at the lateral aspects of the spinal cord where these tracts are located (Fernandes *et al.*, 2018). Any potential effects from the rubrospinal tract can be discounted as this tract does not project below the cervical region in humans (Nathan & Smith, 1982). The reticulospinal tract does project down to the lumbar region, however, its contribution to the effects observed is likely small due to lower density of the axons compared to corticospinal tract (Nathan *et al.*, 1996). Thus, it appears unlikely that descending tracts other than corticospinal tract were stimulated with LS.

It should be noted that skeletal muscles such as the erector spinae surround the stimulus delivery site in the present study. Despite measures taken to ensure that only the knee-extensors and tibialis anterior muscles were voluntarily activated during testing sessions, such as strapping the torso and reducing extraneous limb movements, it is possible that participants inadvertently activated these back muscles. EMG activity of the erector spinae or other postural muscles were not recorded, but if activated, could influence the size of evoked potentials (Solopova *et al.*, 2003).

Though the aforementioned observations relating to a lack of changes in onset latency with increased contraction strength provide support for the monosynaptic nature of the pathway, the data from the present experiments does not completely exclude the influence of non-monosynaptic pathways. Indeed, a large propriospinal system has been shown to exist in humans that might influence corticospinal responses (Pierrot-Deseilligny, 2002). It is important to note that the corticospinal pathway as a whole encompasses not only cortical circuitry and

the motoneuron pool, but also any spinal interneuronal connections (Devanne *et al.*, 1997). TMS might activate inhibitory interneurons due to their lower threshold for activation in some muscles (Nielsen *et al.*, 1993), reducing the excitability at the level of the motoneuron pool leading to reduced temporal summation of the responses. Though supra-additive facilitation observed in the present experiment makes this possibility less likely, it should be noted that the lower limb muscles investigated in these experiments have been demonstrated to have di- and polysynaptic pathways (Nielsen *et al.*, 1993; Simonetta-Moreau *et al.*, 1999) and at least TA receives strong reciprocal inhibitory input (Yavuz *et al.*, 2018). Thus, further work is required to elucidate whether responses to LS are evoked purely monosynaptically, or whether they involve an interneuronal component.

When LS is performed, there is always the possibility that nerve roots are stimulated in addition to the spinal tract. Ventral roots were unlikely to have been activated in the present experiments due to the increase in the size of responses with contraction, and a lack of abrupt decrease in latency when intensity of stimulation was increased (Taylor, 2006). Similarly, the activation of dorsal roots was unlikely given the lack of depression of the second response to paired electrical stimuli at 50 ms ISI (Roy *et al.*, 2012; Danner *et al.*, 2016; Hofstoetter *et al.*, 2018). Furthermore, when dorsal roots are stimulated, the occlusion of responses to paired TMS and LS is absent, and responses to LS are not facilitated by voluntary muscle contraction (Roy *et al.*, 2014), the opposite of which was observed in the present experiments. Thus, the possibility of having activated ventral or dorsal roots with electrical stimulation is minimal.

Variability of responses

The present data show that the CVs for LEPs at rest and during contraction are lower than MEPs (see Table 2). As is well established, MEPs are inherently variable due to the fluctuating nature

of corticospinal and motoneuronal excitability (Kiers *et al.*, 1993; Ellaway *et al.*, 1998), randomness in the firing of pyramidal tract neurons and spinal motoneurons (Pitcher *et al.*, 2003) as well as desynchronization of action potentials (Magistris *et al.*, 1998). The variability of responses observed in the present study is comparable to that reported previously when similar numbers of pulses were employed (Biabani *et al.*, 2018; Brownstein, Ansdell, Škarabot, Howatson, *et al.*, 2018). A greater variability in MEPs compared to LEPs can perhaps be explained by differences in the complexity of the responses to TMS as opposed to LS as discussed above. Some of the multiple volleys evoked by TMS, particularly the later, indirect waves, can fire multiple times (Edgley *et al.*, 1997), which might contribute to the greater variability. Furthermore, greater variability of MEPs might also stem from interference signals from other cortical networks. The variability of evoked responses can be reduced by eliciting responses during a contraction (Darling *et al.*, 2006), which is shown in the present data for both MEPs and LEPs. Due to inherent variability of evoked responses a large quantity of evoked responses are recommended to ascertain a stable index of corticospinal excitability (Brownstein, Ansdell, Škarabot, Howatson, *et al.*, 2018). The present data suggests that when using LS to evoke LEPs, fewer responses might be required compared to TMS evoked MEPs. However, further work is needed to elucidate the optimal number of LS stimuli to obtain a reliable average response, and whether the inherent lower variability of LEPs relative to MEPs results in greater repeatability of responses.

Conclusion and application

Based on the occlusion of responses when the first descending volley evoked by TMS had not arrived at the segmental level, it can be concluded that electrical stimulation of the first lumbar spinous process activates some of the same corticospinal axons projecting to lower limb

muscles as transcranial magnetic stimulation of the motor cortex. These responses at rest were standardised to 10-15% M_{max} and were elicited with ease in all participants. Furthermore, responses to LS grew similarly to TMS with increasing contraction strength, suggesting transsynaptic activation. All participants found stimulations to be tolerable and whilst muscle activity of upper body muscles was not measured, the experimenters did not observe shoulder and arm movements in response to stimulation during the trials. Thus, electrical stimulation over the first lumbar spinous process can be used as an alternative method to assess corticospinal excitability at the segmental level and might be better suited when targeting lower limb muscles due to the proximity of the motoneuronal projections to the stimulating site and the ability to evoke responses in leg musculature at rest.

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Acknowledgements

The authors declare no conflict of interest, financial or otherwise. The authors would like to express gratitude to Tom Pearson of Cambridge Electronics Design, Ltd. for the design of the scripts that facilitated data analysis.

Competing interests

The authors declare no competing interests, financial or otherwise.

Author contributions

All authors conceived and designed the study; JŠ, PA and CB performed experiments; JŠ and PA analysed the data; all authors interpreted results of experiments; JŠ prepared figures; JŠ, PA and RD drafted the manuscript; all authors edited and revised manuscript and approved its final version.

Data accessibility

Raw data can be accessed upon request by contacting the corresponding author.

Abbreviations

EMG electromyography

ISI interstimulus interval

LEP lumbar evoked potential

LS electrical stimulation over the first lumbar spinous process

MEP motor evoked potential

M_{max} maximal compound action potential

MVC maximal voluntary contraction

RF rectus femoris

TA tibialis anterior

TMS transcranial magnetic stimulation

Table 1. Latencies of evoked potentials in milliseconds (mean ± SD).

	TA		RF	
	LEP	**MEP**	**LEP**	**MEP**
Rest	17.3 ± 1.4	30.7 ± 1.6	9.5 ± 1.1	22.1 ± 1.9
10% MVC	17.7 ± 1.6	30.3 ± 2.0	9.0 ± 1.0	20.8 ± 1.9
25% MVC	17.9 ± 2.0	30.3 ± 1.9	9.2 ± 1.2	20.4 ± 1.7
50% MVC	17.7 ± 1.9	29.7 ± 2.2	9.1 ± 0.9	20.4 ± 1.7
75% MVC	17.9 ± 1.9	29.9 ± 1.8	9.3 ± 0.8	20.8 ± 2.0
MVC	17.9 ± 1.9	29.8 ± 2.0	9.5 ± 1.2	20.4 ± 1.5

TA = tibialis anterior, RF = rectus femoris, MVC = maximal voluntary contraction, LEP = lumbar evoked potential, MEP = motor evoked potential. No statistical difference was noted as a function of neural drive ($p > 0.05$).

Table 2. **Variability** of evoked responses (CV%; mean ± SD).

	TA		RF	
	LEP (%)	**MEP (%)**	**LEP (%)**	**MEP (%)**
Rest	30 ± 18	48 ± 9	31 ± 22	38 ± 11
10% MVC	16 ± 11	25 ± 13	13 ± 8	20 ± 13
25% MVC	17 ± 6	25 ± 19	13 ± 9	30 ± 15
50% MVC	17 ± 8	27 ± 20	13 ± 6	24 ± 16
75% MVC	21 ± 9	30 ± 19	16 ± 10	20 ± 13
MVC	23 ± 8	23 ± 10	17 ± 4	22 ± 7

TA = tibialis anterior, RF = rectus femoris, MVC = maximal voluntary contraction, LEP = lumbar evoked potential, MEP = motor evoked potential. No statistical difference was noted as a function of neural drive ($p > 0.05$).

Figure 1. A: Experimental approach involved two parts, the first part being comprised of paired stimulation (transcranial magnetic stimulation and lumbar electrical stimulation) at rest at 16 different interstimulus inervals, and the second part consisting of single pulse magnetic and electrical stimulations at 10. 25. 50. 75 and 100% maximal voluntary contraction. B: Lumbar electrical stimulation was performed with cathode centred over L1 and anode placed over T8 spinous process. Based on modelling literature, this configuration is likely to produce the greatest electric field around the area of T10-T12 spinal segments.

Figure 2. Representative traces from a participant in tibialis anterior at different interstimulus intervals (ISIs) from a single participant. In this individual, the intensity of stimulation used across all types of stimuli produced responses to individual magnetic and electrical stimuli with amplitudes corresponding to 8.9% and 14.2% of M_{max}, respectively, and latencies of 17.2 and 31.1 ms, respectively. Evoked responses to electrical stimulation of the first lumbar spinous process alone (LEP), magnetic stimulation of the cortex alone (MEP), paired stimuli (Paired) and a subtracted response (Paired – LEP) for ISIs of −14, −8, −2, 2 and 6 ms are shown. Each trace is an average waveform of 10 responses. It is of note that the shape of the evoked response for each stimulus type is very similar. Shaded grey area is drawn for better visualisation of differences.

Figure 3. Temporal relationship of the interaction between transcranial magnetic stimulation and electrical stimulation over the first lumbar spinous process in tibialis anterior (left panel) and rectus femoris (right panel). Means and standard deviations are shown for the differences in peak-to-peak amplitudes of evoked responses between paired stimulation and electrical stimulus alone and expressed relative to the response to magnetic stimulation for 16 different interstimulus intervals ranging from −16 to 14 ms every 2 ms (A, B) and normalised interstimulus intervals when lumbar stimulation was delivered before (positive interstimulus intervals), at (0 ms) or after (negative interstimulus intervals) the first volley

evoked by transcranial magnetic stimulation was expected to arrive at the lumbar level (C, D). Horizontal dashed line represents the size of the response that would be expected if there was no physiological interaction. The black filled circles indicate the response is significantly different from the expected response ($p < 0.05$). For interstimulus intervals denoted by the grey filled circles statistical analyses was not performed due to incomplete number of samples ($n < 10$). For the normalised interstimulus interval of −6 ms in tibialis anterior (C), the responses were exceptionally large (mean ratio: 4.4; $n = 1$) and lie outside the illustrated range.

Figure 4. Root-mean-square EMG activity during 100-ms epoch prior to stimulus normalised to root-mean-square EMG activity during maximal voluntary contraction (A and B) and the amplitude of responses to motor (filled circles) and lumbar (open circles) evoked potentials at different contraction strengths (C and D).

Figure 1

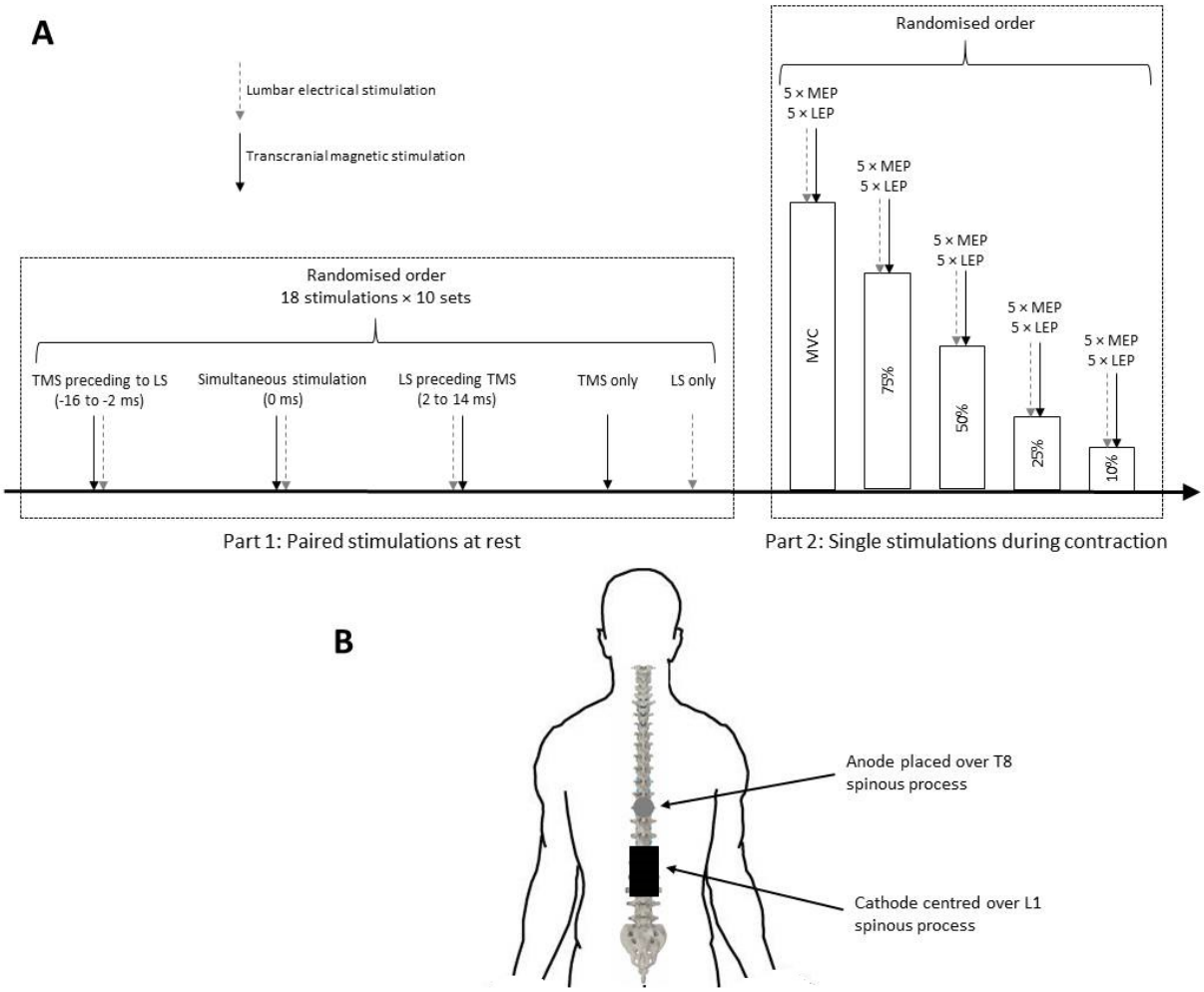

A
Lumbar electrical stimulation
Transcranial magnetic stimulation
Randomised order
18 stimulations × 10 sets
TMS preceding to LS
(-16 to -2 ms)
Simultaneous stimulation
(0 ms)
LS preceding TMS
(2 to 14 ms)
TMS only
LS only
Part 1: Paired stimulations at rest
Randomised order
5 × MEP
5 × LEP
MVC
75%
50%
25%
10%
Part 2: Single stimulations during contraction
B
Anode placed over T8
spinous process
Cathode centred over L1
spinous process

Figure 2

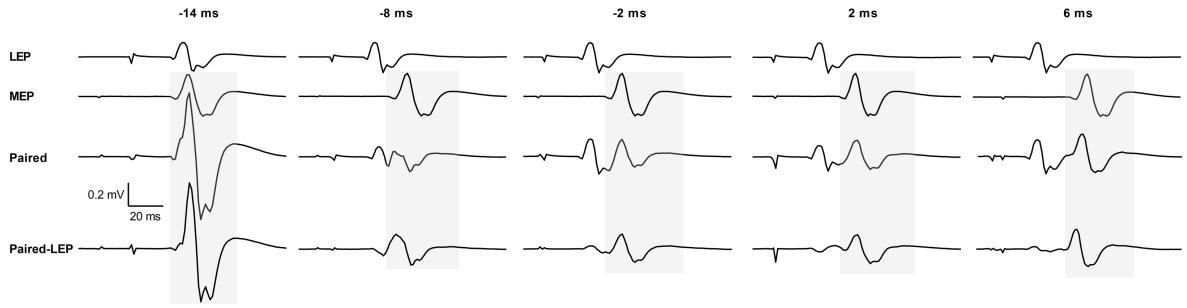


Figure 3

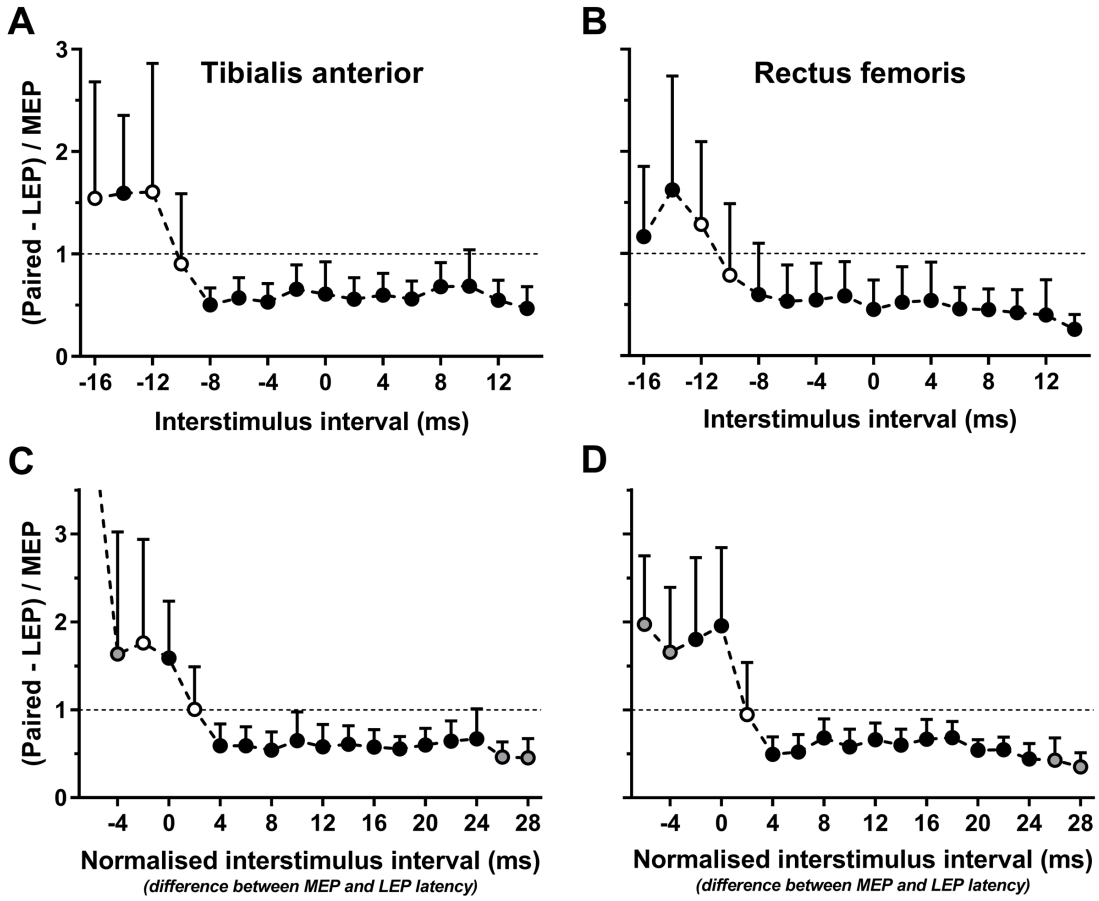
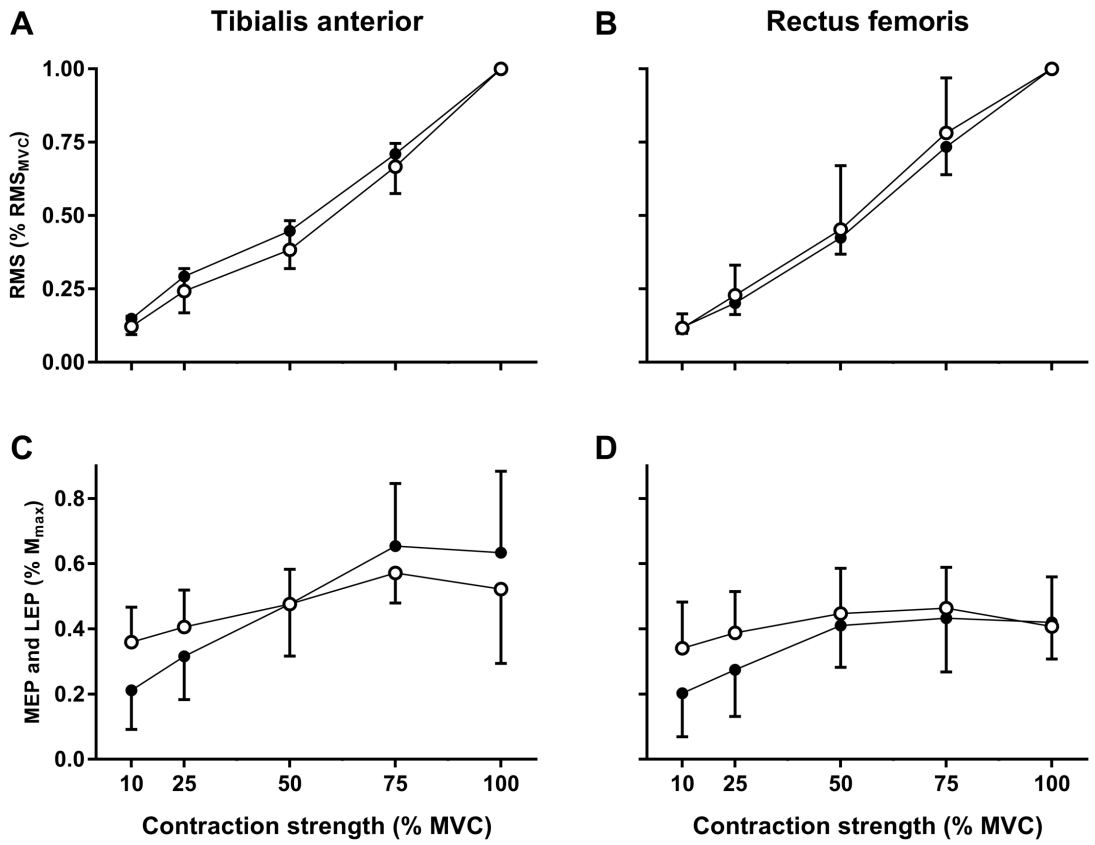

A
Tibialis anterior
(Paired - LEP) / MEP
Interstimulus interval (ms)
B
Rectus femoris
Interstimulus interval (ms)
C
(Paired - LEP) / MEP
Normalised interstimulus interval (ms)
(difference between MEP and LEP latency)
D
Normalised interstimulus interval (ms)
(difference between MEP and LEP latency)

Figure 4


A
Tibialis anterior
B
Rectus femoris
RMS (% RMSMVC)
1.00
0.75
0.50
0.25
0.00
C
D
MEP and LEP (% Mmax)
0.8
0.6
0.4
0.2
0.0
10
25
50
75
100
Contraction strength (% MVC)
Contraction strength (% MVC)