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## **Sustained maximal voluntary contractions elicit different neurophysiological responses in upper- and lower-limb muscles in men**

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**ABBREVIATIONS**

BB, *biceps brachii*; CMEP, cervicomedullary motor-evoked potential; EF, elbow flexors; EMG, electromyography; KE, knee extensors; MEP, motor-evoked potential; M<sub>MAX</sub>, maximal M wave; MVC, maximal voluntary contraction; RF, *rectus femoris*; SP<sub>SPINAL</sub>, silent period elicited by spinal stimulation; SP<sub>TMS</sub>, silent period elicited by TMS; TMEP, thoracic motor-evoked potential; TMS, transcranial magnetic stimulation.

## ABSTRACT

This study compared the effects of fatigue on corticospinal responsiveness in the upper- and lower-limb muscles of the same participants. Seven healthy males performed a 2-min maximal voluntary isometric contraction of the elbow flexors or knee extensors on four separate days. Electromyographic responses were elicited by nerve stimulation (maximal M-wave) in all sessions and by transcranial magnetic stimulation (motor-evoked potential; silent period) and spinal tract stimulation (cervicomedullary or thoracic motor-evoked potentials; silent period) in one session each per limb. During sustained maximal voluntary contractions, motor-evoked potential area normalised to M-waves increased from baseline in *biceps brachii* ( $155 \pm 55\%$ ) and *rectus femoris* ( $151 \pm 44\%$ ) (both  $p \leq 0.045$ ). At the end of maximal voluntary contractions, spinal tract motor-evoked potential area normalised to M-waves was smaller than baseline in *biceps brachii* ( $74 \pm 23\%$ ;  $p = 0.012$ ) but not *rectus femoris* ( $108 \pm 40\%$ ;  $p = 0.999$ ). The ratio of motor-evoked potential to spinal tract-evoked potential areas increased dramatically from 90 to 115 s in *biceps brachii* ( $p = 0.001$ ) but not in *rectus femoris* ( $p = 0.999$ ). Silent period durations increased similarly in both muscles ( $p \leq 0.008$ ) after transcranial and spinal stimulation. Sustained maximal contractions elicit different neurophysiological adjustments in upper- and lower-limb muscles. Specifically, motoneuronal excitability was reduced in *biceps brachii*, but not in *rectus femoris*, and this reduction required greater compensatory adjustments from the motor cortex. Therefore, changes in cortical and spinal excitability during sustained maximal exercise are likely specific to the muscle performing the task.

**Keywords:** corticospinal excitability, inhibition, maximal voluntary contraction, motoneuron, spinal stimulation, transcranial magnetic stimulation

## INTRODUCTION

Neuromuscular fatigue is an exercise-induced change in one or both of the central nervous system (e.g. motor cortex, motoneurons) and muscles that results in suboptimal force production for a given voluntary contraction or stimulation (MacIntosh and Rassier, 2002) and often manifests as a decrease in maximal voluntary contraction (MVC) force (Gandevia, 2001).

Electromyographic (EMG) responses recorded at the muscle and elicited by transcranial magnetic stimulation (TMS) [i.e. motor-evoked potentials (MEPs) that represent direct activation of corticospinal neurons in addition to activation of mono- and polysynaptic inputs to corticospinal neurons (Day et al., 1989; Di Lazzaro et al., 1998)] reflect collective motor cortical and motoneuronal excitability for a given level of muscle activation when normalised to the size of the maximal M-wave ( $M_{MAX}$ ). Electrical spinal stimulation (at either the cervicomedullary junction or over the upper thoracic spinal tract) elicits predominantly monosynaptic responses [cervicomedullary motor-evoked potentials (CMEPs) and thoracic motor-evoked potentials (TMEPs), respectively] in the upper (Petersen et al., 2002) and lower (Martin et al., 2008) limbs. Although not purely monosynaptic, at least in the upper limbs [i.e. propriospinal connections in the cervical region may influence upper-limb musculature (Gracies et al., 1991; Nielsen and Petersen, 1994)], CMEPs and TMEPs reflect the excitability of the motoneurons for a given level of muscle activation when normalised to corresponding  $M_{MAX}$  (Taylor and Gandevia, 2004). Changes in the excitability at both cortical and motoneuronal levels can be assessed when TMS and spinal stimulation are employed together. Additionally, when single-pulse TMS and spinal stimulation are delivered during voluntary contractions, the evoked potentials are followed by a period of near-silence [i.e. silent period (SP)] in the EMG signal. The period of TMS-mediated EMG suppression ( $SP_{TMS}$ ) is generated by activation of long lasting  $GABA_B$  receptors (Werhahn et al., 1999) where spinal mechanisms may be responsible for the first ~150 ms of  $SP_{TMS}$  (Yacyshyn et al., 2016) and thereafter intracortical inhibitory mechanisms (Inghilleri et al., 1993). Meanwhile, SP elicited after spinal stimulation ( $SP_{SPINAL}$ ) may be representative of motoneuronal mechanisms such as motoneuron afterhyperpolarization (Inghilleri et al., 1993; Ziemann et al., 1993) and inhibition via Renshaw cells (Ziemann et al., 1993).

Two-minute MVCs have been frequently used to assess neuromuscular fatigue in both the upper [e.g. (Butler et al., 2003; Taylor et al., 1996; Yacyshyn et al., 2018)] and lower [e.g. (Goodall et al., 2009; Kennedy et al., 2016)] limbs. A fundamental change observed over the course of the 2-min MVCs in all of these studies is the large decrease in force, a finding that

we also recently observed using the same experimental model in both the elbow flexors (EF; -58% pre- to immediately post-exercise) and knee extensors (KE; -70% pre- to immediately post-exercise) in the same participants (Vernillo et al., 2018). In this study, the decrease in both MVC force and voluntary activation was greater in EF than KE. Normalised MEP area and duration of  $SP_{TMS}$  were also observed to increase in both EF and KE from pre- to immediately post-exercise (Vernillo et al., 2018).

Several studies have investigated corticospinal and spinal excitability changes in the upper limbs during a 2-min MVC [e.g. (Butler et al., 2003; Taylor et al., 1996)]. For example, an early TMS study demonstrated that both *biceps brachii* (BB) MEP area and  $SP_{TMS}$  increased during a 2-min MVC (Taylor et al., 1996). Butler et al. (2003) observed smaller BB CMEPs during the final 40 s of a 2-min MVC compared to pre-exercise. Finally, McNeil et al. (2009) compared the responses of TMS and spinal stimulation during TMS-elicited silent periods during a 2-min EF MVC and observed decreases in CMEP and MEP areas to less than 10% of control values. This result indicates that when excitability is assessed in the absence of descending drive, almost all inhibition occurs at the spinal level. Of the studies employing 2-min MVCs to assess lower-limb fatigue, to date only Kennedy et al. (2016) has examined changes in spinal and corticospinal excitability. The authors observed that while *vastus lateralis* MEP area increased by 37% during the 2-min MVC, *vastus lateralis* TMEP area was unchanged.  $SP_{TMS}$  and  $SP_{SPINAL}$  also increased by 18 and 32%, respectively.

Although considerable research has been performed in isolated upper- and lower-limb muscles, much of the aforementioned research has focused on neurophysiological responses to the exercise model itself, rather than on limb-specific physiological differences. It cannot be assumed that the conclusions of these studies also apply when the same participants perform the same fatiguing model using both EF and KE. Thus, it still remains to be established whether cortical and spinal excitability and inhibition change similarly during sustained maximal exercise in the upper and lower limbs in the same participants. Given the functional differences of proximal upper- and lower-limb muscles during any form of physical activity, it is important to elucidate the susceptibility to fatigue and the mechanisms responsible within the same individual. Therefore, the aim of this study was to investigate whether the magnitude and etiology of EMG measures elicited by TMS and spinal stimulation are similar between upper and lower limbs in the same participants during a 2-min MVC.

## **EXPERIMENTAL PROCEDURES**

## Participants

Twelve healthy males participated in this study (age:  $31 \pm 9$  years; height:  $179 \pm 7$  cm; body mass:  $75 \pm 9$  kg). One participant was left-hand dominant and reported being comfortable performing upper-limb tasks (see *Experimental protocol*) with his right arm. All other participants were right-limb dominant for both limbs. Because literature has thus far reported inconsistent effects of limb dominance in regards to fatigability [e.g. (Severijns et al., 2015; Teo et al., 2012; Williams et al., 2002)] or parameters elicited by TMS [e.g. (Dharmadasa et al., 2019; Livingston et al., 2010; Teo et al., 2012)], the results were unlikely to be unaffected by one left-handed participant. Exclusion criteria for participation were injury to either the upper or lower limb during the previous six months, history of heart disease or hypertension and contraindications to TMS (Rossi et al., 2011). Participants were instructed to avoid the consumption of caffeine on the day of the experiment and avoid performing any strenuous exercise during the 48 h prior to testing. All participants were informed of the experimental protocol and all associated risks prior to giving written informed consent. This study conformed to the standards set by the Declaration of Helsinki, except for registration in a database, and was approved by the local ethics committee (University of Calgary Conjoint Health Research Ethics Board, #REB14-1625).

## Experimental protocol

Results from some of the data collected from this protocol have previously been published (Vernillo et al., 2018; 2019). Each participant completed one familiarization session and two experimental sessions (sessions 1 and 3 below). A subset of 8 participants completed two additional sessions (sessions 2 and 4 below) with spinal stimulation; however due to difficulties in consistently eliciting CMEPs and TMEPs in one participant, only the results for 7 participants are presented (age:  $33 \pm 11$  years; height:  $179 \pm 7$  cm; body mass:  $74 \pm 9$  kg). The two or four sessions were counterbalanced and pseudorandomised. During the familiarization session, participants performed maximal and submaximal voluntary isometric contractions of EF and KE with and without TMS, spinal stimulation and peripheral nerve stimulation. The experimental sessions (see Fig. 1A) were (1) a 2-min EF MVC with TMS and peripheral nerve stimulation, (2) a 2-min EF MVC with spinal and peripheral nerve stimulation, (3) a 2-min KE MVC with TMS and peripheral nerve stimulation and (4) a 2-min KE MVC with spinal and peripheral nerve stimulation. All tests were separated by 3-7 days and each participant performed all tests at the same approximate time of day.

### **Neuromuscular testing protocol**

In sessions 1 and 3, participants performed sustained 2-min isometric MVCs where TMS followed by peripheral nerve stimulation 2-3 s later (i.e. to ensure that the participant had returned to maximal force after any disruption caused by the first stimulus) were manually delivered at 5, 30, 60, 90 and 115 s. Thus, there were 5 MEPs and 5  $M_{MAX}$  collected during each 2-min MVC. In sessions 2 and 4, the protocol was the same except TMS was replaced by spinal stimulation, which meant that 5 TMEPs and 5  $M_{MAX}$  were collected. During all voluntary isometric contractions, visual feedback of the force produced was displayed on a computer screen directly in front of the participants who also received constant standardised encouragement during the 2-min MVCs.

### **Force and electromyographic recordings**

Muscle forces were obtained from voluntary and evoked isometric contractions. All measurements were taken from the participants' right limbs. EF force was assessed by calibrated force transducer (2712 - 200 daN, Sensy, Jumet, Belgium). Participants were seated upright in a chair with right arm in a custom-built dynamometer. Both shoulder and elbow joints were at 90°, with the forearm in a supinated position. A non-compliant strap secured the wrist to the dynamometer.

KE force was measured by a calibrated force transducer with amplifier (LC101-2K, Omegadyne, Sunbury, OH) attached by a non-compliant strap to the right leg immediately proximal to the malleoli of the ankle joint. Participants were seated upright in a custom-built chair with both right knee and hips at 90° of flexion and secured by chest and hip straps. The force transducer was fixed to the chair such that force was measured in direct line to the applied force.

Electromyographic activity of the right EF (BB), elbow extensors (long head of *triceps brachii*), KE (*rectus femoris*, RF) and hamstrings (*biceps femoris*) was recorded with pairs of self-adhesive surface (10-mm recording diameter) electrodes (Meditrace 100, Covidien, Mansfield, MA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the medial epicondyle of the humerus or patella. Placement of EMG electrodes for BB and *triceps brachii* followed SENIAM recommendations (Hermens et al., 1999). The placement for RF and *biceps femoris* was between the most distal motor point and the distal tendon as per Botter et al. (2011). A low impedance (<5 k $\Omega$ ) between electrodes was obtained by shaving and gently abrading the skin and then cleaning it with isopropyl alcohol. Signals were analogue-to-digially converted at a sampling rate of 2000 Hz by PowerLab system

(16/35, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 500) with band pass filter (5-500 Hz) and analyzed offline using Labchart 8 software (ADInstruments).

### **Peripheral stimulation**

For EF, single electrical stimuli of 200- $\mu$ s duration were delivered to the brachial plexus via constant-current stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode (Meditrace 100) was securely taped in the supraclavicular fossa and the anode (Durastick Plus, DJO Global, Vista, CA) was placed over the acromion. For KE, single electrical stimuli were delivered via constant-current stimulator (Session 3: 1-ms duration via DS7A, Digitimer; Session 4: 200- $\mu$ s duration via DS7AH, Digitimer) to the right femoral nerve. Stimuli to the femoral nerve were delivered via a cathode securely taped into the femoral triangle (Meditrace 100) and a 50  $\times$  90 mm rectangular anode (Durastick Plus, DJO Global) in the gluteal fold.

Single stimuli were delivered incrementally in the relaxed muscle until M-wave amplitude of BB or RF plateaued. A stimulus intensity of 130% of the minimum stimulus intensity to elicit M-waves of maximal amplitude ( $M_{MAX}$ ) was employed to ensure supramaximality. Stimulus intensity was determined at the start of each session. In EF, the supramaximal stimulus intensity was  $138 \pm 65$  mA for brachial plexus stimulation for session 1 ( $n = 12$ ). For the 7 participants who participated in both EF sessions, the supramaximal stimulus intensities were  $146 \pm 82$  mA (session 1) and  $153 \pm 95$  mA (session 2). In KE, the supramaximal stimulus intensity was  $84 \pm 36$  mA for session 3 ( $n = 12$ ). For the 7 participants who participated in both KE sessions the supramaximal stimulus intensities were  $70 \pm 28$  mA (session 3) and  $158 \pm 50$  mA (session 4).

### **Transcranial magnetic stimulation**

Single TMS pulses (100- $\mu$ s rise time; 1-ms duration) were delivered to elicit MEPs during voluntary contractions of EF and KE. The left motor cortex was stimulated by a magnetic stimulator (Magstim 200<sup>2</sup>, The Magstim Company Ltd, Whitland, UK) with a 110-mm concave double-cone coil (maximum output of 1.4 T) to induce a postero-anterior current. Participants wore a lycra swim cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. During EF, every centimeter was demarcated from 3 to 5 cm to the left of the vertex and from the vertex to 2 cm posterior. During KE, every centimeter was demarcated from the vertex to 2 cm posterior to the vertex

along the nasal-inion line and 1 cm laterally over the left motor cortex. At each of these points, a stimulus was delivered at 50% maximal stimulator output during voluntary contractions at 20% MVC. The optimal coil position was the site where the largest BB and RF MEPs were elicited in EF and KE, respectively. These sites were drawn on the swim cap and employed throughout the session.

During brief voluntary isometric contractions at 50% MVC, stimulus intensity was increased until MEP amplitudes were 50% of  $M_{MAX}$  amplitude in both BB and RF. The stimulus intensity was verified from the mean amplitude of 4 MEPs. Mean stimulus intensities were  $46 \pm 20\%$  and  $49 \pm 13\%$  in sessions 1 (EF) and 3 (KE), respectively ( $n = 12$ ), and  $45 \pm 17\%$  and  $51 \pm 14\%$  in EF and KE, respectively ( $n = 7$ ). Participants were also instructed to re-contrast to the pre-stimulus force level as quickly as possible after TMS delivery (Mathis et al., 1998).

### **Spinal stimulation**

Single electrical stimuli of 500- $\mu$ s duration were delivered via constant-current stimulator (DS7A, Digitimer) between the mastoid processes to elicit cervicomedullary motor-evoked potentials (CMEPs) in EF or to the thoracic spinal cord to elicit thoracic motor-evoked potentials (TMEPs) in KE. In EF, stimulating electrodes (Meditrace 100) were secured over the groove 1-2 cm medial and superior to the tips of the two mastoid processes with the cathode on the left side and anode on the right side (Gandevia et al., 1999; Taylor and Gandevia, 2004). In KE, the stimulating electrodes (Meditrace 100) were secured between the T3 and T4 vertebrae for the cathode and in a thoracic vertebral space 5-10 cm superior, but below the C7 vertebra, for the anode (Kennedy et al., 2016). Spinal stimulation was delivered at the thoracic level since motoneurons in the spine are believed to be monosynaptic (Nielsen and Petersen, 1994) and this method has been demonstrated to elicit TMEPs (Kennedy et al., 2016). The stimulus intensity was determined during brief voluntary isometric contractions at 50% MVC and the stimulus intensity was increased until the amplitude of BB CMEPs and RF TMEPs, normalised to the corresponding  $M_{MAX}$ , matched MEP amplitudes, also normalised to the corresponding  $M_{MAX}$ , from the respective muscles from sessions 1 and 3. The stimulus intensity was verified from the mean amplitude of 4 CMEPs or TMEPs. Mean stimulus intensities were  $151 \pm 44$  mA and  $578 \pm 125$  mA in EF and KE, respectively. The difference between CMEP and MEP amplitudes during the brief voluntary isometric contractions at 50% MVC was  $0.1 \pm 0.1\%$  of  $M_{MAX}$  ( $p = 0.994$ ) in EF and in KE the difference between TMEP and MEP amplitudes was  $0.1 \pm 0.5\%$  of  $M_{MAX}$  ( $p = 0.992$ ). Spinal stimulation was always

delivered once the participant had contracted to the appropriate force level and the force had stabilised during voluntary contractions. Participants were instructed to re-contract to the pre-stimulus force level as quickly as possible after the spinal stimulus was delivered.

## **Data analysis**

### ***Force***

The force was calculated as the mean of the 500-ms window before each TMS pulse (sessions 1 and 3) and spinal stimulus (sessions 2 and 4) during the 2-min MVC. Results of a more detailed analysis for the force change in both EF and KE have been previously reported (Vernillo et al., 2018).

### ***Peripheral stimulation***

The  $M_{MAX}$  area was calculated for each M-wave from the initial deflection to the crossing of the horizontal axis for the second time (Martin et al., 2006a).

### ***TMS***

Areas of MEPs were measured from the initial deflection to the crossing of the horizontal axis for the second time (Martin et al., 2006a) and normalised to  $M_{MAX}$  area elicited 2-3 s later. The duration of  $SP_{TMS}$  was determined visually and defined as the duration from the TMS pulse to the return of continuous voluntary EMG (Taylor et al., 1996).

### ***Spinal stimulation***

Areas of CMEPs and TMEPs were measured from the initial deflection to the crossing of the horizontal axis for the second time (Martin et al., 2006a) and normalised to  $M_{MAX}$  area elicited 2-3 s later. MEP areas (from sessions 1 and 3) were also normalised to areas of CMEPs and TMEPs (from sessions 2 and 4, respectively) corresponding to the same muscle and same time-point in order to assess changes in cortical excitability. The duration of  $SP_{SPINAL}$  was determined visually as the duration from the spinal stimulus to the return of continuous voluntary EMG (Taylor et al., 1996).

## **Statistical analysis**

Results are presented in the text as means  $\pm$  SD and in figures as means  $\pm$  SEM. All data during the sustained 2-min MVC contractions were normalised as a percentage of the values at the 5-s time-point of the 2-min MVC except the MEP/CMEP and MEP/TMEP ratios at each time-point. A normal distribution for all variables was verified by the Shapiro-Wilk test. Generalised estimating equations (GEE) were employed to account for the unbalanced participant numbers ( $n = 12$  for TMS sessions and  $n = 7$  for spinal stimulation sessions)

(Liang and Zeger, 1986). This was done to verify that there was not a difference in the results for TMS measures between the entire group of participants and the sub-group that also performed sessions 2 and 4 with spinal stimulation. The results indicate that there was not a stimulation technique (spinal stimulation *versus* TMS) effect for force ( $\chi^2(1) = 0.282$ ,  $p = 0.596$ ),  $M_{MAX}$  ( $\chi^2(1) = 1.082$ ,  $p = 0.298$ ), MEP ( $\chi^2(1) = 0.266$ ,  $p = 0.606$ ) or  $SP_{TMS}$  ( $\chi^2(1) = 0.450$ ,  $p = 0.502$ ) whether 7 or 12 participants were considered (values at 5 s of the 2-min MVC are presented in Table 1). Therefore, the results section only presents data from 7 participants for all parameters. Then GEE was used to obtain unbiased estimates and to take into account the correlation between repeated measurements in force and EMG parameters during the 2-min MVCs and the small sample size (Liang and Zeger, 1986). When significant main effects of GEE were observed, Bonferroni's test was used for *post-hoc* analysis. As a measure of effect size, Cohen's  $d$  was calculated and values of 0.2, 0.6 and 0.8 were the boundaries for being considered small, medium and large, respectively (Cohen, 1988). Statistical analyses were performed using IBM<sup>TM</sup> SPSS<sup>TM</sup> Statistics (version 25, IBM Corp., Somers, New York, NY). Statistical significance was set at  $\alpha < 0.05$ .

## RESULTS

Maximal voluntary force, the duration of  $SP_{SPINAL}$  and  $SP_{TMS}$ , and areas of  $M_{MAX}$ , MEP, CMEP and TMEP at 5 s during the 2-min MVC are presented in Table 1.

### Force

The decrease in MVC force from 5 to 115 s was not influenced by stimulation technique (i.e. TMS and spinal stimulation) for either EF (Session 1:  $-67 \pm 8\%$ ; Session 2:  $-65 \pm 8\%$ ) or KE (Session 3:  $-75 \pm 3\%$ ; Session 4:  $-75 \pm 4\%$ ) ( $\chi^2(1) = 0.767$ ,  $p = 0.381$ ). There was a significant muscle effect, indicating a greater force decrease in KE than EF ( $\chi^2(1) = 17.259$ ,  $p < 0.001$ ).

### M-waves

The changes in  $M_{MAX}$  area are presented in Fig. 2A. There were no muscle ( $\chi^2(1) = 1.102$ ,  $p = 0.294$ ) or stimulation technique ( $\chi^2(1) = 1.741$ ,  $p = 0.187$ ) effects. There was a significant time effect ( $\chi^2(4) = 31.171$ ,  $p < 0.001$ ) and *post-hoc* analysis indicates that  $M_{MAX}$  area increased from 5 to 30 s (mean increase 22-47%;  $p < 0.001$ ,  $d = 2.55$ ) and was stable thereafter. There was no technique (spinal stimulation or TMS)  $\times$  muscle (BB *versus* RF)  $\times$  time interaction ( $\chi^2(4) = 5.072$ ,  $p = 0.280$ ).

### Evoked potentials

The changes in MEP and CMEP or TMEP areas are presented in Fig. 2B and 2C, respectively. There was no muscle effect ( $\chi^2(1) = 0.266$ ,  $p = 0.606$ ) for areas of evoked potentials elicited by TMS and spinal stimulation. However, there were both time ( $\chi^2(4) = 69.072$ ,  $p < 0.001$ ) and stimulation technique ( $\chi^2(1) = 4.098$ ,  $p = 0.043$ ) effects, the latter indicating that MEP areas were on average larger than CMEP and TMEP areas across the 2-min MVC. There was also a technique  $\times$  muscle  $\times$  time interaction ( $\chi^2(4) = 10.466$ ,  $p = 0.033$ ). Specifically, BB MEP increased in size at the end of the 2-min MVC and was larger than at 5 s only at 115 s (+55% from 5 s;  $p = 0.045$ ,  $d = 1.52$ ). RF MEP also increased in size during the 2-min MVC and was significantly larger than at 5 s from 60 s ( $p \leq 0.008$  for all points). CMEP area was unchanged from 5-90 s and then decreased at 115 s (-26% from 5 s;  $p = 0.012$ ,  $d = 1.74$ ) while TMEP area was significantly larger at 30 s than at 5 s (+54% from 5 s;  $p = 0.002$ ,  $d = 1.97$ ), 90 s (+13% from 5 s;  $p < 0.001$ ,  $d = 1.23$ ) and 115 s (+8% from 5 s;  $p < 0.001$ ,  $d = 1.21$ ). The change in RF MEP area was greater than the change in TMEP area at 90 s (+67 *versus* +13% from 5 s;  $p < 0.001$ ,  $d = 1.52$ ) and 115 s (+51 *versus* +8% from 5 s;  $p = 0.036$ ,  $d = 1.11$ ) while the change in BB MEP area was greater than the change in CMEP area only at 115 s (+55 *versus* -36% from 5 s;  $p < 0.001$ ,  $d = 2.07$ ).

### MEP/CMEP and MEP/TMEP

The changes in the ratios of MEP/CMEP and MEP/TMEP are presented in Fig. 3. There was a time effect ( $\chi^2(4) = 47.260$ ,  $p < 0.001$ ) and muscle  $\times$  time interaction ( $\chi^2(4) = 14.204$ ,  $p = 0.007$ ) but no muscle effect ( $\chi^2(1) = 0.932$ ,  $p = 0.334$ ). MEP/CMEP was stable from 5-90 s and then increased at 115 s ( $p = 0.001$ ,  $d = 1.35$ ) while MEP/TMEP was stable from 5-60 s before becoming significantly greater than 5 s at 90 s ( $p = 0.001$ ,  $d = 1.28$ ).

### Silent periods

The changes in the duration of SP<sub>SPINAL</sub> and SP<sub>TMS</sub> are presented in Fig. 4. There were no muscle ( $\chi^2(1) = 0.532$ ,  $p = 0.470$ ) or stimulation technique ( $\chi^2(1) = 2.356$ ,  $p = 0.125$ ) effects; however, there was a time effect ( $\chi^2(4) = 93.795$ ,  $p < 0.001$ ) of increasing SP duration. There was also a technique  $\times$  muscle  $\times$  time interaction ( $\chi^2(4) = 15.799$ ,  $p = 0.003$ ). Specifically, SP<sub>TMS</sub> increased from 5 s and was significantly longer by 30 s in RF (+23% from 5 s;  $p = 0.002$ ,  $d = 2.26$ ) and 60 s in BB (+54% from 5 s;  $p = 0.001$ ,  $d = 1.99$ ). SP<sub>SPINAL</sub> also increased and was significantly longer than at 5 s from 30-115 s in both BB and RF ( $p \leq 0.002$  for all

points). The increase in RF  $SP_{SPINAL}$  duration was also greater than the increase in RF  $SP_{TMS}$  duration at 30 (+49 *versus* +23% from 5 s;  $p = 0.004$ ,  $d = 1.59$ ), 60 (+73 *versus* +32% from 5 s;  $p < 0.001$ ,  $d = 1.77$ ) and 115 s (+74 *versus* +39% from 5 s;  $p = 0.047$ ,  $d = 1.12$ ) while in BB there was no difference in the increase in SP between techniques.

## DISCUSSION

This study examined for the first time cortical and motoneuronal responsiveness in the upper and lower limbs of the same participants during a sustained 2-min maximal isometric contraction. The purpose was to examine whether corticospinal responses during the same fatiguing model were similar (or different) in upper- and lower-limb muscles, in order to provide a test of generality, reducing the amount of error from natural variance between studies that assessed corticospinal excitability during sustained MVCs while fatiguing either EF or KE. The results demonstrate that i) corticospinal excitability ( $MEP/M_{MAX}$ ) increased to the same extent in BB and RF, ii) motoneuronal excitability ( $CMEP/M_{MAX}$ ) decreased for BB at the end of the 2-min MVC while RF motoneuronal excitability ( $TMEP/M_{MAX}$ ) was not different from the beginning at any point, iii)  $MEP/CMEP$  increased dramatically at the end of the 2-min MVC while  $MEP/TMEP$  was unchanged at the end of the 2-min MVC compared to baseline and iv) SP durations following TMS and spinal stimulation increased in both BB and RF. Taken together, the current results suggest that excitability of cortical neurons increases more in BB, potentially to counteract the greater decrease in spinal excitability in this muscle group.

### Upper limbs

The changes during the 2-min EF MVC in this study are in agreement with previously-reported changes from the beginning to end of 2-min MVCs [e.g. (Butler et al., 2003; Taylor et al., 1996)]. Again, comparisons between studies are complicated since previous studies used control contractions prior to the 2-min MVC as baseline values whereas the current study used 5 s of the 2-min MVC as a baseline for comparisons to 30, 60, 90 and 115 s. The use of 5 s as the reference for comparison was necessary because pre-exercise control contractions in the TMS conditions (sessions 1 and 3) employed a different TMS intensity for the assessment of voluntary activation pre and post 2-min MVC [see Vernillo et al. (2018) for further details] and performance of further pre-exercise MVCs were not performed to mitigate the risk of premature muscle fatigue.

Of the studies that have employed 2-min MVCs, (Butler et al., 2003; McNeil et al., 2009; Taylor et al., 1996; Taylor et al., 1999; Yacyshyn et al., 2018) only McNeil et al. (2009) has reported a change (i.e. increase in  $SP_{TMS}$  duration from control MVCs at 9 s) between pre-exercise control contractions and the first measurements (i.e. within the first 15 s of the 2-min MVC) for any of  $M_{MAX}$ , MEP, CMEP and  $SP_{TMS}$ ). The 55% increase in normalised BB MEP area to the end of the 2-min EF MVC in the present study is comparable to the 56% increase reported by Taylor et al. (1996) and greater than the ~40% increase in Taylor et al. (1999). For BB  $SP_{TMS}$  duration, Taylor et al. (1996) reported an increase of ~20-25% from pre-exercise for the period from 30 s and Taylor et al. (1999) an increase of 38% to its peak duration after approximately 60 s. McNeil et al. (2009) also reported a 41% increase in BB  $SP_{TMS}$  duration from pre-exercise values to 117 s. We similarly observed an increase in BB  $SP_{TMS}$  duration (+25% at 30 s, +54% at 60 s and +45% at 115 s) and a plateau from 60 s.

When using spinal stimulation, we observed a decrease of 32% in normalised BB CMEP area at the end of the 2-min MVC, comparable to the 22% decrease Butler et al. (2003) reported. As with  $SP_{TMS}$ ,  $SP_{SPINAL}$  increased during the 2-min MVC (+62% at 115 s) in the present study; however, the increase above baseline values became significant earlier for  $SP_{SPINAL}$  (at 30 s *versus* 60 s for  $SP_{TMS}$ ). This increase in  $SP_{SPINAL}$  duration is greater than the 38% increase Taylor et al. (1996) observed in *brachioradialis*, a minor EF muscle, in a small subset of 3 participants.

### **Lower limbs**

This study reinforces the findings of Kennedy et al. (2016) in the *vastus lateralis* where there was an increase in MEP area and  $SP_{TMS}$  and  $SP_{SPINAL}$  duration from the beginning to the end of the 2-min KE MVC without a change in TMEP area. The increases in RF MEP area (+51%),  $SP_{TMS}$  (+39%) and  $SP_{SPINAL}$  (+74%) from 5 to 115 s in the present study were greater than those observed (+38%, +18% and +32%, respectively) by Kennedy et al. (2016) from pre-exercise control contractions to the end of the 2-min MVC. However, it is important to note that Kennedy et al. (2016) compared changes from pre-exercise control contractions, as opposed to changes from 5 s of the 2-min MVC in the present study, impeding direct comparisons. Unlike in Kennedy et al. (2016), a larger normalised RF TMEP area at 30 s than at 5, 90 and 115 s was observed in the present study. This result suggests that initially spinal excitability increased before returning to baseline levels in RF. However, Kennedy et al. (2016) did not observe any change in *vastus lateralis* TMEP area during the 2-min MVC. No

other studies are known to have investigated these parameters during a 2-min MVC in lower-limb muscles.

### **Upper versus lower limbs**

Motor-evoked potential area normalised to  $M_{MAX}$  increased over the course of the 2-min MVC in both BB and RF (Fig. 2B). Similarly,  $SP_{SPINAL}$  and  $SP_{TMS}$  increased comparably for both BB and RF (Fig. 4). However, several differences between BB and RF were observed.

The key difference was in the timeline and direction of changes in normalised CMEP and TMEP areas. While TMEP area initially increased (154% of baseline at 30 s) before returning to baseline values (108% of baseline at 115 s), CMEP area was not different from baseline until decreasing to 74% of baseline values at 115 s (Fig. 2C). Decreased spinal excitability during ongoing voluntary descending drive has been suggested to be due to one or both of changes to intrinsic motoneuronal properties and changes to reflex pathways, including disfacilitation occurring via a reduction in muscle-spindle discharge (McNeil et al., 2009). The maintenance of BB MEP normalised to  $M_{MAX}$  (Fig. 2B) and the dramatic increase in the MEP/CMEP ratio at 115 s (Fig. 3) suggests an increase in cortical excitability that was able to maintain corticospinal excitability in the face of reduced motoneuronal excitability. Conversely, both cortical and motoneuronal excitability were maintained at the end of the 2-min KE MVC, as demonstrated by the lack of significant change in either RF MEP or TMEP areas from 90 to 115 s (Fig. 2). However, the change in RF MEP (i.e. response elicited by stimulation of the motor cortex) from baseline was significantly greater than the change in TMEP (i.e. response elicited from stimulation of the thoracic spine) from baseline at both 90 and 115 s. Because RF MEP area increased more than RF TMEP area, it may be that excitatory output from the motor cortex to RF increased during the last ~30 s of the 2-min MVC to compensate for the return of motoneuronal excitability to baseline following an increase during the first half of the contraction (significant at 30 s; Fig. 2B). Meanwhile, the change in BB MEP was only greater than the change in CMEP at 115 s. These findings are further reflected by the changes in both MEP/CMEP and MEP/TMEP (Fig. 3) although there was no difference between these ratios at 115 s ( $P = 0.051$ ). Further investigation is warranted to examine the possibility of differences at the cortical level. Collectively, these results suggest that at least one of intrinsic motoneuronal properties or the capacity for reflex pathways to influence motoneuronal excitability is more greatly affected in EF than in KE and that in both muscle groups, the motor cortex attempts to compensate for changes at the motoneuronal level.

Despite greater changes in corticospinal excitability in the EF at the end of the 2-min MVC (decrease in BB CMEP and increase in MEP/CMEP), the decrease in maximal force was greater in KE. This may be due to greater central fatigue (as demonstrated by a greater decrease in voluntary activation) previously observed by our group in KE than EF (Vernillo et al., 2018). A number of other possibilities could explain why motoneuronal excitability decreased in *biceps brachii* but not *rectus femoris* at the end of the 2-min MVC. First, it has previously been observed that activity-dependent changes in intrinsic motoneuronal properties differ between muscles [e.g. (Gandevia et al., 1999; Giesebrecht et al., 2010)]. Therefore, it is essential that the physiological differences in strength and type (direct monosynaptic *versus* indirect disynaptic) of corticospinal connections and projections to different muscle groups are acknowledged. This is particularly important for comparisons of upper versus lower limbs and extensor versus flexor muscle groups (Brouwer and Ashby, 1990; de Noordhout et al., 1999) in addition to consideration of mechanisms that have received little consideration in humans such as reticulospinal inputs (Fisher et al., 2012). It is also unclear how the effect of stimulating at different levels of the spinal cord (i.e. transmastoid level to elicit CMEPs and thoracic level to elicit TMEPs) influences and interacts with the strength and type of corticospinal connections. Finally, linking back to the greater central fatigue we previously observed for KE than EF (Vernillo et al., 2018), fatigue is also likely to differentially alter excitatory and inhibitory pathways to distinct muscles and/or muscle groups [e.g. feedback from type III and IV muscle afferents as investigated by Martin et al. (2006b)]. Regardless of the mechanism(s), previous and present findings indicate it is inappropriate to directly compare or extrapolate results or findings from one muscle (or muscle group) to others, even within an individual.

### **Limitations**

Determination of the intensity of TMS and spinal stimulation was performed during voluntary isometric contractions at 50% as per Kennedy et al. (2016) in order to reduce the development of fatigue prior to the 2-min MVC. However, the difference between determining optimal stimulus intensities at 50% MVC and performing the fatiguing intervention at 100% MVC led to less precise matching of MEPs and CMEPs or TMEPs at 100% MVC. Evaluations performed prior to the 2-min MVC and during recovery from the 2-min MVC are presented elsewhere (Vernillo et al., 2018). Furthermore, although additional pre-exercise MVCs could have been performed, as previously mentioned, they were not due to concern that this would have contributed to premature muscle fatigue. Another limitation is that transcranial and

spinal stimuli were delivered during different experimental sessions. Despite standardization of the testing procedures and comparable force changes between sessions of the same limb, properties of the corticospinal tract could have initially been different or changed differently between days and thus influenced the results. Similarly, stimulating at different levels of the spinal cord (i.e. mastoid process to elicit CMEPs and thoracic spine to elicit TMEPs) may have introduced variability; however, evidence supports motoneurons being predominantly monosynaptic in nature (Nielsen and Petersen, 1994), allowing use of stimulus methods as in previous EF (Butler et al., 2003) and KE (Kennedy et al., 2016) studies.

The current study corroborates previous findings during a sustained MVC in either upper (EF) or lower (KE) limbs individually, specifically that MEP and  $M_{MAX}$  areas and  $SP_{TMS}$  duration increase in both limbs. It also corroborates a decrease in motoneuronal excitability in BB and no motoneuronal excitability change in KE muscles after 2 min. This study is also the first to demonstrate that changes at the motoneuronal level, and potentially also at the cortical level, during sustained maximal isometric exercise differ between the muscles of the upper (BB) and lower (RF) limbs in the same participants. Specifically, normalised RF TMEP area was maintained at the end of the 2-min MVC whereas normalised BB CMEP area was 26% lower than baseline. Also, the increase in SP elicited by spinal stimulation was greater than the increase in TMS-elicited SP in RF at the end of the 2-min MVC whereas there were comparable increases in SPs elicited by both methods in BB. These findings demonstrate differences in the spinal modulation of maximal voluntary drive to the upper and lower limbs during sustained maximal exercise. Furthermore, despite EF having been widely used in research to assess global neuromuscular responses, the present results suggest that EF should not be used as a representative of muscle functioning in the human body, especially because of the importance of lower-limb muscles in ambulatory activities of daily-living and rehabilitation. This may have application, for example, in assessing relative injury risk, whereby greater fatigability for a relative task may indicate a greater injury risk.

### **Conflict of interest**

The authors have no conflicts of interest to declare.

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## REFERENCES

Botter A, Oprandi G, Lanfranco F, Allasia S, Maffiuletti NA, Minetto MA. (2011) Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning. *Eur J Appl Physiol* 111:2461-2471.

Brouwer B, Ashby P. (1990) Corticospinal projections to upper and lower limb spinal motoneurons in man. *Electroencephalogr Clin Neurophysiol* 76:509-519.

Butler JE, Taylor JL, Gandevia SC. (2003) Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J Neurosci* 23:10224-10230.

Cohen J. (1988) *Statistical power analysis for the behavioral sciences*. Mahwah, NJ: Lawrence Erlbaum.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD. (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *J Physiol* 412:449-473.

de Noordhout AM, Rapisarda G, Bogacz D, Gerard P, De Pasqua V, Pennisi G, Delwaide PJ. (1999) Corticomotoneuronal synaptic connections in normal man: an electrophysiological study. *Brain* 122 1327-1340.

Dharmadasa T, Matamala JM, Howells J, Simon NG, Vucic S, Kiernan MC. (2019) The effect of coil type and limb dominance in the assessment of lower-limb motor cortex excitability using TMS. *Neurosci Lett* 699:84-90.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, et al. (1998) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroencephalogr Clin Neurophysiol* 109:397-401.

Fisher KM, Zaaimi B, Baker SN. (2012) Reticular formation responses to magnetic brain stimulation of primary motor cortex. *J Physiol* 590:4045-4060.

Gandevia SC. (2001) Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81:1725-1789.

Gandevia SC, Petersen N, Butler JE, Taylor JL. (1999) Impaired response of human motoneurons to corticospinal stimulation after voluntary exercise. *J Physiol* 521:749-759.

Giesebrecht S, Martin PG, Gandevia SC, Taylor JL. (2010) Facilitation and inhibition of tibialis anterior responses to corticospinal stimulation after maximal voluntary contractions. *J Neurophysiol* 103:1350-1356.

Goodall S, Romer LM, Ross EZ. (2009) Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp Physiol* 94:995-1004.

Gracies JM, Meunier S, Pierrot-Deseilligny E, Simonetta M. (1991) Pattern of propriospinal-like excitation to different species of human upper limb motoneurons. *J Physiol* 434:151-167.

Hermens HJ, Freriks B, Merletti R, Hägg GG, Stegeman D, Blok J, Rau G, Disselhorst - Klug C. (1999) SENIAM 8: European recommendations for surface electromyography. Enschede, Netherlands: Roessingh Research and Development.

Inghilleri M, Berardelli A, Cruccu G, Manfredi M. (1993) Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol* 466:521-534.

Kennedy DS, McNeil CJ, Gandevia SC, Taylor JL. (2016) Effects of fatigue on corticospinal excitability of the human knee extensors. *Exp Physiol* 101:1552-1564.

Liang K-Y, Zeger SL. (1986) Longitudinal data analysis using generalized linear models. *Biometrika* 73:13-22.

Livingston SC, Goodkin HP, Ingersoll CD. (2010) The influence of gender, hand dominance, and upper extremity length on motor evoked potentials. *J Clin Monit Comput* 24:427-436.

MacIntosh BR, Rassier DE. (2002) What is fatigue? *Can J Appl Physiol* 27:42-55.

Martin PG, Butler JE, Gandevia SC, Taylor JL. (2008) Noninvasive stimulation of human corticospinal axons innervating leg muscles. *J Neurophysiol* 100:1080-1086.

Martin PG, Gandevia SC, Taylor JL. (2006a) Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *J Neurophysiol* 95:3512-3518.

Martin PG, Smith JL, Butler JE, Gandevia SC, Taylor JL. (2006b) Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans. *J Neurosci* 26:4796-4802.

Mathis J, de Quervain D, Hess CW. (1998) Dependence of the transcranially induced silent period on the 'instruction set' and the individual reaction time. *Electroencephalogr Clin Neurophysiol* 109:426-435.

McNeil CJ, Martin PG, Gandevia SC, Taylor JL. (2009) The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. *J Physiol* 587:5601-5612.

Nielsen J, Petersen N. (1994) Is presynaptic inhibition distributed to corticospinal fibres in man? *J Physiol* 477:47-58.

Petersen NT, Taylor JL, Gandevia SC. (2002) The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *J Physiol* 544:277-284.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. (2011) Screening questionnaire before TMS: an update. *Clin Neurophysiol* 122:1686.

Severijns D, Lamers I, Kerkhofs L, Feys P. (2015) Hand grip fatigability in persons with multiple sclerosis according to hand dominance and disease progression. *J Rehabil Med* 47:154-160.

Taylor JL, Butler JE, Allen GM, Gandevia SC. (1996) Changes in motor cortical excitability during human muscle fatigue. *J Physiol* 490:519-528.

Taylor JL, Butler JE, Gandevia SC. (1999) Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. *Exp Brain Res* 127:108-115.

Taylor JL, Gandevia SC. (2004) Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol* 96:1496-1503.

Teo WP, Rodrigues JP, Mastaglia FL, Thickbroom GW. (2012) Changes in corticomotor excitability and inhibition after exercise are influenced by hand dominance and motor demand. *Neuroscience* 210:110-117.

Vernillo G, Temesi J, Martin M, Millet GY. (2018) Mechanisms of fatigue and recovery in upper versus lower limbs in men. *Med Sci Sports Exerc* 50:334-343.

Vernillo G, Temesi J, Martin M, Millet GY. (2019) Do aerobic characteristics explain isometric exercise-induced neuromuscular fatigue and recovery in upper and lower limbs? *J Sports Sci* 37:387-395.

Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. (1999) Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* 517:591-597.

Williams DM, Sharma S, Bilodeau M. (2002) Neuromuscular fatigue of elbow flexor muscles of dominant and non-dominant arms in healthy humans. *J Electromyogr Kinesiol* 12:287-294.

Yacyshyn AF, Nettleton J, McNeil CJ. (2018) The effects of sex and motoneuron pool on central fatigue. *Med Sci Sports Exerc* 50:1061-1069.

Yacyshyn AF, Woo EJ, Price MC, McNeil CJ. (2016) Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. *Exp Brain Res* 234:3457-3463.

Ziemann U, Netz J, Szelenyi A, Homberg V. (1993) Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neurosci Lett* 156:167-171.

**Table 1.** Force and electromyographic parameters at 5 s of the 2-min MVCs across the four experimental sessions.

	Mean <i>n</i> = 12	SD	Mean <i>n</i> = 7	SD
TMS sessions (1 and 3)				
MVC (N)				
EF	282	57	276	65
KE	545	95	562	78
$M_{MAX}$ (mV·s)				
BB	0.078	0.018	0.084	0.018
RF	0.049	0.011	0.046	0.011
MEP (% $M_{MAX}$ )				
BB	69	21	74	19
RF	61	16	64	18
$SP_{TMS}$ (ms)				
BB	165	95	165	97
RF	160	86	165	89
Spinal stimulation sessions (2 and 4)				
MVC (N)				
EF			275	50
KE			554	73
$M_{MAX}$ (mV·s)				
BB			0.088	0.019
RF			0.042	0.018
CMEP or TMEP (% $M_{MAX}$ )				
BB			66	22
RF			54	19
$SP_{SPINAL}$ (ms)				
BB			59	7
RF			58	10

BB: *biceps brachii*; CMEP: cervicomedullary motor-evoked potential area; EF: elbow flexor; KE: knee extensor; MEP: motor-evoked potential area;  $M_{MAX}$ : maximal M-wave area; MVC: maximal voluntary contraction; RF: *rectus femoris*;  $SP_{SPINAL}$ : silent period elicited by spinal stimulation;  $SP_{TMS}$ : silent period elicited by transcranial magnetic stimulation; TMEP: thoracic motor-evoked potential area; TMS: transcranial magnetic stimulation.

## FIGURE CAPTIONS

**FIG. 1.** (A) The 2-min maximal voluntary contraction (MVC) fatigue protocol performed during four separate sessions [(1) 2-min elbow-flexor (EF) MVC with transcranial magnetic stimulation (TMS) and peripheral nerve stimulation, (2) 2-min EF MVC with spinal and peripheral nerve stimulation, (3) 2-min knee-extensor (KE) MVC with TMS and peripheral nerve stimulation and (4) 2-min KE MVC with spinal and peripheral nerve stimulation]. Stimuli were delivered at 5, 30, 60, 90 and 115 s of the 2-min MVC. (B) Electromyography from a representative participant with TMS in *biceps brachii* (BB) and *rectus femoris* (RF). The shaded areas indicate the motor-evoked potential (MEP) elicited in BB and RF. (C) Electromyography from the same participant with spinal stimulation in BB and RF. The shaded areas indicate the cervicomedullary motor-evoked potential (CMEP) elicited in BB and thoracic motor-evoked potential (TMEP) elicited in RF.

**FIG. 2.** Changes in (A) maximal M-wave ( $M_{MAX}$ ) area (B) motor-evoked potential (MEP) area normalised to  $M_{MAX}$  and (C) cervicomedullary motor-evoked potential (CMEP) and thoracic motor-evoked potential (TMEP) areas normalised to  $M_{MAX}$  ( $CMEP/M_{MAX}$  and  $TMEP/M_{MAX}$ , respectively) during the 2-min maximal voluntary contraction (MVC) for knee extensors (*rectus femoris*, RF) and elbow flexors (*biceps brachii*, BB) [for all panels  $n = 7$ ]. Values are means (A) or means  $\pm$  SEM (B, C) and were normalised as a percentage of the evaluation at 5 s of the 2-min MVC. Only technique (transcranial magnetic stimulation or spinal stimulation)  $\times$  muscle  $\times$  time interactions are presented. For differences between time-points within the same muscle \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  where dashed lines are for BB and solid lines are for RF. For differences in BB between  $\Delta$ MEP and  $\Delta$ CMEP at a specific time-point &,  $p < 0.05$ . For differences in RF between  $\Delta$ MEP and  $\Delta$ TMEP at a specific time-point @,  $p < 0.05$ .

**FIG. 3.** Changes in the ratios of motor-evoked potential to cervicomedullary motor-evoked potential areas (MEP/CMEP) and motor-evoked potential to thoracic motor-evoked potential areas (MEP/TMEP) during the 2-min maximal voluntary contraction (MVC) for knee extensors (*rectus femoris*, RF) and elbow flexors (*biceps brachii*, BB) [ $n = 7$ ]. Values are means  $\pm$  SEM. For differences between time-points within the same muscle \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  where the dashed line is for BB and the solid line is for RF.

**FIG. 4.** Changes in duration of (A) silent period elicited by transcranial magnetic stimulation ( $SP_{TMS}$ ) and (B) silent period elicited by spinal stimulation ( $SP_{SPINAL}$ ) during the 2-min maximal voluntary contraction (MVC) for elbow flexors (*biceps brachii*, BB) and knee extensors (*rectus femoris*, RF) [for all panels  $n = 7$ ]. Values are means  $\pm$  SEM and were normalised as a percentage of the evaluation at 5 s of the 2-min MVC. Only technique (transcranial magnetic stimulation or spinal stimulation)  $\times$  muscle  $\times$  time interactions are presented. For differences between time-points within the same muscle \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  where dashed lines are for BB and solid lines are for RF. For differences in RF between  $\Delta SP_{TMS}$  and  $\Delta SP_{SPINAL}$  at a specific time-point @,  $p < 0.05$ . Grey shaded areas indicate a difference in  $\Delta SP_{TMS}$  or  $\Delta SP_{SPINAL}$  between BB and RF at a specific time-point.