Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity

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New & Noteworthy

This study measured motoneuron excitability using cervicomedullary evoked potentials conditioned using transcranial magnetic stimulation (TMS-CMEP) of both small and large amplitudes during sustained low- and high-intensity contractions of the elbow flexors. During the low-intensity task, only the small TMS-CMEP was reduced. During the high-intensity task, both small and large TMS-CMEPs were substantially reduced. These results indicate that repetitively active motoneurons are specifically reduced in excitability compared to less active motoneurons in the same pool.

ABSTRACT

Cervicomedullary stimulation provides a means of assessing motoneuron excitability. Previous studies demonstrated that during low-intensity sustained contractions, small cervicomedullary evoked potentials (CMEPs) conditioned using transcranial magnetic stimulation (TMS-CMEPs) are reduced, whilst large TMS-CMEPs are less affected. Since small TMS-CMEPs recruit motoneurons most active during low-intensity contractions while large TMS-CMEPs recruit a high proportion of motoneurons inactive during the task, these results suggest that reductions in motoneuron excitability could be dependent on repetitive activation. To further test this hypothesis, this study assessed changes in small and large TMS-CMEPs across low- and high-intensity contractions. Twelve participants performed a sustained isometric contraction of the elbow flexor for 4.5 min at the electromyography (EMG) level associated with 20% maximal voluntary contraction force (MVC; low-intensity) and 70% MVC (high-intensity). Small and large TMS-CMEPs with amplitudes of ~15 and ~50% M_{max} at baseline, respectively, were delivered every minute throughout the tasks. Recovery measures were taken at 1, 2.5 and 4-min post-exercise. During the low-intensity trial, small TMS-CMEPs were reduced at 2-4 min ($p \leq 0.049$) by up to −10% M_{max}, while large TMS-CMEPs remained unchanged ($p \geq 0.16$). During the high-intensity trial, small and large TMS-CMEPs were reduced at all time-points ($p < 0.01$) by up to −14% and −33% M_{max}, respectively, and remained below baseline during all recovery measures ($p \leq 0.02$). TMS-CMEPs were unchanged relative to baseline during recovery following the low-intensity trial ($p \geq 0.24$). These results provide novel insight into motoneuron excitability during and following sustained contractions at different intensities, and suggest that contraction-induced reductions in motoneuron excitability depend on repetitive activation.

Keywords: cervicomedullary evoked potentials, isometric exercise, motoneuron excitability

INTRODUCTION

Motoneurons represent the final common pathway of the motor system, through which descending commands from higher brain areas are transmitted to evoke mechanical responses in skeletal muscle (Heckman and Enoka, 2012; Sherrington, 1952). During high-intensity (Temesi et al., 2019) or prolonged contractions (Finn et al., 2018; McNeil et al., 2011a), the efficacy of descending command can be impaired owing to reductions in motoneuron excitability, rendering motoneurons less responsive to synaptic input. Reductions in motoneuron excitability have implications for exercise tolerance due to the requirement for a greater synaptic input in order to maintain motoneuron activation (Héroux et al., 2016; Johnson et al., 2004), and a decrease in muscle activation and force if reduced excitability cannot be overcome (Weavil and Amann, 2018).

One method of assessing motoneuron excitability is through stimulation of descending axons of the corticospinal tract at the level of the cervicomedullary junction (Taylor, 2006). These stimuli activate corticospinal axons which project with a large monosynaptic component (Petersen et al., 2002) onto motoneurons of the upper-limbs, producing a short-latency excitatory response known as the cervicomedullary motor evoked potential (CMEP) that permits the quantification motoneuron excitability. Using this method, numerous studies have revealed reductions in CMEPs in response to sustained isometric contractions (Butler et al., 2003; Gandevia et al., 1999; Temesi et al., 2019), though others have reported an increase (Hoffman et al., 2009; Lévénez et al., 2008)

The capacity of motoneurons to fire in response to synaptic input depends not only on intrinsic motoneuron properties, but also on the sum of the multiple inputs received by the motoneurons, all of which can be altered during strenuous exercise (Macefield et al., 1991; Martin et al., 2006b; Zytnicki et al., 1990). In particular, descending excitatory input from the

corticospinal tract has a profound influence on motoneuron excitability, inducing an increase in excitability up to contraction intensities of ~50% MVC (Martin et al., 2006a). This is an important consideration when measuring CMEPs in response to sustained contractions given that descending drive generally increases to compensate for downstream impairments (Hoffman et al., 2009; Lévénez et al., 2008). As such, measurement of the CMEP in the presence of ongoing descending drive is influenced by both alterations in the level of corticospinal input and motoneuron excitability, and it is not possible to discriminate between these mechanisms when interpreting changes in the CMEP. To mitigate this issue, an experimental technique was developed in which a transcranial magnetic stimulation (TMS) conditioning stimulus is delivered over the motor cortex to temporarily interrupt descending drive (a phenomenon termed the silent period), and a cervicomedullary stimulation is delivered during the silent period (McNeil et al., 2009). This technique provides an opportunity to assess motoneuron excitability without the confounding influence of altered descending drive, and with minimal disruption to the exercise task. Under these conditions, alterations in motoneuron excitability can reflect intrinsic perturbations and/or changes in afferent feedback.

Using this method, it has been demonstrated that during a sustained low-intensity isometric contraction of the upper- (McNeil et al., 2011a) and lower-limb (Finn et al., 2018), small CMEPs (McNeil et al., 2011a) and thoracic motor evoked potentials (TMEPs) (Finn et al., 2018) evoked using low-intensity stimuli were substantially reduced, while large responses evoked using high-intensity stimuli were less affected. Since the low-intensity stimuli likely recruited the same motoneurons which were active throughout the task, while large responses recruited a high proportion of inactive motoneurons, the relatively greater decline of small responses suggests that reductions in excitability were greatest in motoneurons that were repetitively active during the task. Studies using intramuscular combined with compound

EMG found that when the firing rate of a target motor unit was held constant during an isometric contraction, the compound EMG signal increased (Héroux et al., 2016; Johnson et al., 2004). These results indicate that a greater level of excitatory drive was required to maintain motoneuron firing rate, likely owing to reduced excitability in the repetitively active target motor unit. Finally, using high-density EMG, Farina et al. (2009) found an increase in the recruitment threshold of only the most active motor units during repeated ramp contractions, possibly due to a reduction in their excitability. Accordingly, converging evidence points towards repetitive activation-induced alterations in the intrinsic properties of motoneurons, with properties such as spike-frequency adaptation, increased recruitment thresholds, prolonged after-hyperpolarisation and altered persistent inward currents commonly proposed to contribute to reduced motoneuron excitability (Farina et al., 2009; Héroux et al., 2016; Johnson et al., 2004).

While a number of studies have indicated that reductions in motoneuron excitability are dependent on repetitive activation (Farina et al., 2009; Finn et al., 2018; Héroux et al., 2016; Johnson et al., 2004; McNeil et al., 2011a), these studies have only employed low-intensity isometric contractions. Comparing alterations in small and large CMEPs across low and high contraction intensities would bring new insight into the influence of repetitive activation on motoneuron excitability. Furthermore, studies assessing motoneuron excitability using conditioned CMEPs or TMEPs in response to high-intensity contractions have done so using relatively small responses (~10-20% M_{max}) (Brownstein et al., 2020; McNeil et al., 2011b; McNeil et al., 2009; Sidhu et al., 2018), likely reflecting the excitability of lower threshold motoneurons. Activating a greater proportion of the motoneuron pool using high-intensity stimuli could give a more comprehensive understanding of the effects of high-intensity contractions on motoneuron excitability. Finally, an assessment of conditioned CMEPs during the post-exercise recovery period is warranted to compare the effects of low- and

high-intensity exercise on recovery of motoneuron excitability, which is currently unknown. Accordingly, the aim of the present study was to compare changes in small and large conditioned CMEPs during low- and high-intensity isometric contractions. It was hypothesised that small CMEPs would be reduced by low-intensity contractions and large CMEPs would be less affected, while both small and large CMEPs would be reduced during high-intensity contractions.

METHODS

Participants

Using the effect size for a TMEP size × time interaction from Finn et al. (2018), a power calculation (alpha = 0.05, power = 0.95) determined that a sample size of 11 participants was required. To account for the possibility that large CMEPs might not be evocable in some participants (McNeil et al., 2011a), 17 male participants were recruited for the study. Five participants were not tested because an abrupt decrease in CMEP latency when increasing stimulus intensity (n = 1) indicating stimulus spread to cervical roots (Taylor and Gandevia, 2004), an insufficient silent period duration (n = 1), stimulation discomfort (n = 1) and because of an inability to sustain the required EMG level (n = 2). The experiment was thus completed by 12 participants (mean ± SD age: 30 ± 7 yr, stature: 177.9 ± 7.0 cm, mass: 76.5 ± 12.2 kg). The study received ethical approval from the local ethics committee and conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. Participants provided written informed consent to take part in the study.

Experimental Design

All participants in the study were well familiarised with performing isometric exercise, and receiving spinal electrical stimulations. Participants visited the laboratory on two separate

occasions for two experimental trials. The experimental protocol is depicted in Figure 1. The trials consisted of a sustained isometric contraction of the elbow flexors for 4.5 min at a constant EMG level. One experimental trial consisted of a low-intensity contraction at the EMG level associated with 20% MVC (Low-intensity), with the other trial consisting of a high-intensity contraction at the EMG level associated with 70% MVC (High-intensity). For the purposes of the present study, it was deemed appropriate to time-match the low- and high-intensity tasks to assess the effect of contraction intensity on motoneuron excitability independently of differences in contraction duration. A 4.5 min contraction was chosen as pilot testing revealed that the high-intensity task could be sustained for at least this duration in all of the pilot participants, whilst also being of sufficient duration to permit the assessment of the kinetics of change in motoneuron excitability throughout the task. Previous studies have also shown that reduction in motoneuron excitability plateaus within 4 mins of a sustained contraction (Finn et al., 2018; McNeil et al., 2011a) , thus prolonging contractions would have been unnecessary to test the hypothesis. Furthermore, although some degree of motor unit substitution likely occurs during sustained EMG tasks, a sustained EMG level was deemed appropriate for the design of the present study to ensure a constant muscle activity (and thus similar level of motoneuron output) in order to test the hypothesis that active motoneurons would exhibit the greatest reductions in excitability. For both trials, small and large CMEPs conditioned with TMS were measured at baseline, every minute throughout the sustained contraction, and after 1, 2.5 and 4 min of post-exercise recovery.

Experimental procedures

The low- and high-intensity trials were performed in a randomised order. The experiments began with the determination of M_{max} amplitude of the biceps brachii (BB) using electrical

stimulation of the brachial plexus delivered at rest (described below). A brief warm-up consisting of 5-7 submaximal contractions of the elbow flexors at a progressively increasing intensity up to 90% of perceived maximal strength was subsequently performed. Participants then performed two MVCs of the elbow flexors, with the peak value taken to calculate submaximal forces. Subsequently, participants performed a 5 s isometric contraction of the elbow flexors at 20% MVC for the low-intensity and 70% MVC for the high-intensity trial using visual force feedback displayed on a computer monitor. The average BB smoothed rectified EMG from these contractions was then calculated to derive a target EMG level for the task. Whilst contracting at the target EMG level, a baseline superimposed M-wave (M_{sup}) was then measured using a stimulus intensity 130% of that associated with M_{max}. The TMS hotspot was then determined, after which the TMS intensity was adjusted to produce a silent period of at least 200 ms (described below) whilst contraction at the target EMG level. Subsequently, the appropriate cervicomedullary stimulus intensity required to elicit a small (~15% M_{max} amplitude) and large (~50% M_{max} amplitude) TMS-CMEP in the BB was determined (described below). To do so, participants performed brief contractions (~3 s) at the target EMG level and received conditioned cervicomedullary stimuli at a gradually increasing intensity with each contraction. Once the appropriate stimulus intensities were found, two further stimuli were delivered at the same intensity, with the average of the three stimuli used as the baseline small and large TMS-CMEPs for both trials (Figure 1). One and 1.5 min of rest was given between contractions for baseline measures for the low- and high-intensity trial, respectively. Two minutes following the final baseline measure, the sustained isometric contraction began. For the low-intensity trial, this consisted of a sustained isometric elbow flexion at a 20% MVC-EMG level, and for the high-intensity trial, at a 70% MVC-EMG level. Both trials lasted 4.5 min. At 1, 2, 3 and 4 min of both trials, 3 cervicomedullary stimuli were delivered at the intensity associated with the small TMS-CMEP and 3 at the

intensity associated with the large TMS-CMEP (Figure 1). One M_{sup} was delivered after these measurements. About 4 s separated each stimulation, with measurement period lasted ~25 s. Recovery measurements were taken 1, 2.5 and 4 min after completion of the task. During recovery measurements, a brief rest period of ~5 s was given between sets of small and large TMS-CMEP stimuli. The order of the small and large TMS-CMEP stimuli was randomised during both the sustained contraction and recovery measures. Following the first baseline contraction at the target EMG level, prior to each group of stimuli being delivered throughout the sustained task, and following each group of stimuli delivered during the recovery period, participants were asked their rate of perceived effort (RPE) in the elbow flexors on a scale from 0 to 10 (Borg, 1990).

Instrumentation

Force and EMG recordings. Participants were seated with their right arm firmly secured to an isometric dynamometer. The right arm was supinated, with a strap across the wrist and the dynamometer. The seat height was adjusted to ensure that the elbow and shoulder were maintained at 90º flexion. The participants' back was rested against the backrest of the chair, and a consistent posture was maintained throughout the trials. Electrical activity from the belly of the BB and brachioradialis (BR) were recorded with self-adhesive surface electrodes (Melitracen 100; Covidien, Mansfield, MA) using a bipolar electrode configuration, with electrodes placed 30 mm apart. A reference electrode was placed on the medial epicondyle. Prior to electrode placement, the skin was shaved, abraded and cleaned with isopropyl alcohol to limit impedance. Signals were amplified (× 1000) via an octal bio-amplifier (ML138; ADInstruments, Bella Vista, Australia), band-pass filtered (5-500 Hz), and analogue-to-digital converted at a sampling rate of 2 kHz by a PowerLab Sytem (16/30;

ADInstruments). To assist participants in visualising and maintaining the EMG levels, the BB rectified EMG was smoothed with a 200 ms time constant.

Brachial plexus stimulation. For the assessment of M_{max} and M_{sup}, motor nerve stimulation was delivered to the brachial plexus at Erb's point. Single rectangular electrical pulses with 1 ms duration and 400 V maximal output voltage were delivered via a constant-current stimulation (DS7R; Digitimer, Welwyn Garden City, UK) using a 30 mm diameter surface cathode at Erb's point and anode placed over the acromion (Melitracen 100). Electrical stimuli were first administered at 20 mA and were then increased in 20 mA increments until M_{max} was elicited. The resulting stimulation intensity was then increased by 30% to account for activity-induced changes in axonal excitability (low-intensity trial, 129 ± 33; high-intensity trial 114 ± 27 mA; paired t-test p = 0.22). This intensity was used for M_{sup} measurements throughout the sustained contractions and during recovery.

Transcranial magnetic stimulation. Single-pulse TMS of 1 ms duration was delivered over the vertex using a circular coil (13.5 cm outsider diameter) connected to a magnetic stimulator (Magstim 2002, The Magstim Co., Whitland, UK). The vertex was marked as the intersection of lines drawn between the preauricular points and from nasion to inion, which were marked on a swim cap worn by participants. The direction of current flow in the coil preferentially activated the left motor cortex. To confirm appropriate coil placement, TMS was delivered at 50% maximum stimulator output (MSO) during a contraction at the target EMG level, and the discernible motor evoked potential (MEP) confirmed accurate placement in all participants. The stimulus intensity was subsequently increased in 10% MSO increments until a silent period of 200 ms was observed whilst contracting at the target EMG level (low-intensity trial, 83 ± 13%; high-intensity trial, 81 ± 14% MSO; paired t-test p = 0.54).

Cervicomedullary stimulation. A high-voltage electrical current (1 ms duration, Digitimer DS7R) was passed between two 30 mm diameter stimulation electrodes placed over the mastoid processes (Melitracen 100) for cervicomedullary stimulations. Stimuli were delivered 100 ms following the TMS conditioning stimulus (McNeil et al., 2009). The stimulus intensity began at 20 mA, and was increased in 20 mA increments until a TMS-CMEP of ~15% M_{max} amplitude was elicited for the small TMS-CMEP (low-intensity trial, 79 ± 16; high-intensity trial, 86 ± 22 mA; paired t-test $p = 0.23$) and ~50% M_{max} amplitude was elicited for the large TMS-CMEP (low-intensity trial, 140 ± 27; high-intensity trial, 159 ± 43 mA; paired t-test $p = 0.36$). When increasing stimulus intensity, the CMEP was closely monitored for decreases in response latency, which are indicative of nerve root stimulation (Taylor and Gandevia, 2004). In all but one participant, who was subsequently excluded, no such decrease was observed.

Data analysis

To quantify the small and large TMS-CMEPs, the average amplitude of the 3 measures taken at each time-point was calculated. The small and large responses were expressed relative to the M_{sup} measured after each respective set of measurements. Mean force and EMG root mean square (EMG_{RMS}) were calculated over a 200 ms epoch prior to each TMS conditioning stimulus. These were subsequently averaged across the 6 measurements (i.e. 3 for the small and 3 for the large TMS-CMEPs) at each time-point to measure force and EMG_{RMS} throughout the task. The EMG_{RMS} was expressed relative to the maximum EMG_{RMS}, obtained over a 500 ms epoch during the plateau in force during the MVC, which derived the peak value. The mean force was expressed relative to MVC. When determining the appropriate TMS intensity, the duration of the silent period was calculated by visual inspection of the raw

EMG trace from the point of stimulation until the resumption of pre-stimulus EMG for at least 100 ms (Škarabot et al., 2019). During off-line analysis, LabChart (ADInstruments) software was used to determine all measures.

Statistical analysis

Jamovi statistical software (jamovi, version 1.0, 2019, the jamovi project; retrieved from https://www.jamovi.org) was used for all statistical analyses. All data are presented as mean ± SD, with error bars in figures representing SD. Statistical significance was set at an α of 0.05. Normality of the data was assessed by the Shapiro-Wilk test, with no data requiring transformation. Assumptions of sphericity were explored and controlled for all variables with the Greenhouse-Geisser adjustment, where necessary. A three-way repeated measures ANOVA [2 × 2 × 5; TMS-CMEP size (small TMS-CMEP/M_{sup} and large TMS-CMEP/M_{sup}), contraction intensity (Low-intensity and High-intensity) and time (baseline, 1, 2, 3 and 4 min of sustained elbow flexion)] was used to assess the effect of TMS-CMEP size and contraction intensity on changes in motoneuron excitability. A separate three-way ANOVA was performed to assess changes in small and large TMS-CMEP relative to baseline during the recovery measures following the low- and high-intensity tasks. A two-way repeated measures ANOVA (contraction intensity × time) was used to assess changes in RPE and M_{sup} during the low- and high-intensity trials. To assess changes in force and EMG_{RMS}, two one-way ANOVAs were performed for the low- and high-intensity trials separately. In the event of a significant interaction or main effect, analysis was continued using pairwise comparisons with least significant differences. Partial eta squared (η_p^2) was calculated to estimate effect sizes, with values representing small ($\eta_p^2 = 0.10$), medium ($\eta_p^2 = 0.25$) and large ($\eta_p^2 = 0.40$) effects. Cohen's *d* effect size was calculated for focused within-trial pairwise comparisons

316 between the TMS-CMEP at baseline and 4 min, and were interpreted as small (≥ 0.2),
317 moderate (≥ 0.6) and large (≥ 1.2). To assess the measurement error associated with
318 measuring three TMS-CMEPs per time-point, the within-subject typical error was calculated
319 at each time point. The average typical error for all time points and for all participants and
320 both trials was then calculated for the small and large TMS-CMEPs separately.

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322

323 **RESULTS**

324 The target EMG was successfully maintained for both the low- and high-intensity trials,
325 during which force was progressively decreased. The amplitudes of the TMS-CMEPs were
326 well-matched between the low- and high-intensity trials. In the BB, the change in the TMS-
327 CMEP during a sustained 4.5 min contraction was TMS-CMEP size and contraction intensity
328 dependent. During the sustained low-intensity contraction, only the small TMS-CMEP was
329 reduced, with no change in the large TMS-CMEP. During the high-intensity contraction, both
330 the small and large TMS-CMEPs were reduced. The large TMS-CMEP was thus reduced to a
331 greater extent during the high-intensity compared with the low-intensity contraction. Figure 2
332 displays representative traces for the small and large TMS-CMEPs from the low- and high-
333 intensity trials.

334

335 **EMG_{RMS}, force, M_{sup} and RPE**

336 During the brief baseline contractions for the low-intensity trial, EMG_{RMS} was 18.6 ± 7.2%
337 maximum EMG_{RMS}, while force was 20.3 ± 5.5% MVC. The EMG_{RMS} of the BB remained
338 consistent throughout the sustained contraction and recovery measures, with no effect of time

($F_{2.6,28.6} = 0.97$, $p = 0.46$, $\eta_p^2 = 0.08$; Figure 3A). Force decreased throughout the trial ($F_{1.4,14.4} = 4.19$, $p = 0.048$, $\eta_p^2 = 0.30$), before returning to baseline at 1 min post-exercise ($p = 0.37$; Figure 3A). For the high-intensity trial, EMG_{RMS} was 52.3 ± 8.7% maximum EMG_{RMS}, while force was 62.4 ± 8.5% MVC at baseline. Note that the force was lower than 70% MVC at baseline due to the rapid drop in force which occurred within contractions at a high EMG level during the baseline measurements. The EMG_{RMS} of the BB remained consistent throughout the sustained contraction and recovery measures, with no effect of time ($F_{3.5,38.3} = 1.11$, $p = 0.36$, $\eta_p^2 = 0.09$; Figure 3B). Force decreased throughout the trial ($F_{3.3,36.7} = 54.90$, $p < 0.01$, $\eta_p^2 = 0.83$), and was reduced at all time-points, including the recovery measures ($p < 0.01$).

At baseline, M_{max} was 16.0 ± 5.9 mV in the BB for the low-intensity trial, and 16.5 ± 5.7 mV for the high-intensity trial ($p = 0.72$). A two-way contraction intensity × time interaction was found for M_{sup} ($F_{3.3,32.5} = 3.3$, $p = 0.03$, $\eta_p^2 = 0.25$). No change was found for M_{sup} during the low-intensity trial ($p \geq 0.25$), while M_{sup} was lower than baseline at 2.5 (14.7 ± 4.7 mV; $p = 0.01$) and 4 min (14.3 ± 3.8 mV; $p < 0.01$) post-exercise for the high-intensity trial.

For RPE, a significant two-way contraction intensity × time interaction was found ($F_{7,77} = 5.44$, $p < 0.01$, $\eta_p^2 = 0.33$). For the low-intensity trial, RPE increased relative to baseline throughout the sustained contraction ($p \leq 0.03$) before recovering by 4 min post-exercise ($p = 0.06$; Figure 4). For the high-intensity trial, RPE increased relative to baseline at all time-points, including the recovery measures ($p < 0.01$; Figure 4). The RPE was higher at all time-points during the high-intensity compared with low-intensity (all $p < 0.01$; Figure 4).

TMS-CMEP

The typical error for the small and large TMS-CMEPs were 0.91 ± 0.48% M_{max} and 2.93 ± 1.12 % M_{max}, respectively. At baseline for the low-intensity trial, the small and large TMS-CMEPs were 14.1 ± 3.7% M_{max} amplitude and 54.6 ± 8.3% M_{max} amplitude respectively. For the high-intensity trial, small and large TMS-CMEPs were 14.5 ± 3.9% M_{max} and 50.0 ± 6.5% M_{max} respectively. No differences were found between baseline small and large TMS-CMEP amplitudes for the low- and high-intensity trials ($p \geq 0.42$). A significant three-way TMS-CMEP size × contraction intensity × time interaction was found ($F_{7,77} = 4.51$, $p < 0.01$, $\eta_p^2 = 0.29$). For the low-intensity trial, the amplitude of the small TMS-CMEP was reduced from 2 ($p = 0.049$), 3 ($p = 0.02$) and 4 min ($p = 0.01$) of the sustained contraction (Figure 5A), dropping to 5.0 ± 3.1% Mmax by 4 min ($d = 2.7$). In contrast, no change was found for the amplitude of the large TMS-CMEP throughout the sustained low-intensity contraction ($p \geq 0.16$). For the high-intensity task, both the small and large TMS-CMEP amplitudes were reduced at all time-points ($p < 0.01$) dropping to 1.0 ± 0.4% M_{max} ($d = 4.6$) and 17.1 ± 13.1% M_{max} by 4 min ($d = 3.2$), respectively (Figure 5B). Between trial comparisons revealed no difference in the small TMS-CMEP between the low- and high-intensity trials at any time-point ($p \geq 0.17$). In contrast, the large TMS-CMEP was lower during the high- versus low-intensity trial at all time-points ($p < 0.01$).

For the recovery measures, no TMS-CMEP size × contraction intensity × time interaction was found ($F_{3,33} = 2.1$, $p = 0.12$, $\eta_p^2 = 0.16$). However, a contraction intensity × time interaction was noted ($F_{3,33} = 5.4$, $p < 0.01$, $\eta_p^2 = 0.33$). *Post-hoc* tests revealed no difference in TMS-CMEPs relative to baseline during recovery following the low-intensity trial ($p \geq 0.24$; Figure 5A). In contrast, TMS-CMEPs were reduced at all time-points during recovery following the high-intensity trial ($p \leq 0.01$; Figure 5B). Between-trial comparisons showed that TMS-CMEPs were lower during the high- versus low-intensity trial at 1 and 2.5 min ($p < 0.01$), with no difference at 4 min ($p = 0.10$).

DISCUSSION

The aim of the present study was to assess changes in small and large TMS-conditioned CMEPs during sustain isometric contractions at low and high intensities. A key a novel finding from the present study was that, in line with the hypothesis and corroborating previous findings (Finn et al., 2018; McNeil et al., 2011a), only small TMS-CMEP amplitudes were reduced during low-intensity contraction, whereas both small and large TMS-CMEPs were reduced during the high-intensity contraction. Furthermore, following the high-intensity contraction, TMS-CMEPs remained below baseline following 4 min of recovery. These results indicate that reductions in motoneuron excitability during sustained contractions are task-intensity dependent, and provide insight into motoneuron excitability during recovery following low- and high-intensity contractions.

Reductions in motoneuron excitability during sustained isometric contractions are task-intensity dependent

During the low-intensity contraction sustained for 4.5 min at the EMG level associated with 20% MVC, small TMS-CMEPs of ~15% M_{max} amplitude fell below baseline after 2 min and decreased to 5% M_{max} by 4 min. Large TMS-CMEPs, in contrast, were maintained at ~50% M_{max} throughout the sustained contraction. During the high-intensity task at the EMG level associated with 70% MVC, both small and large TMS-CMEPs were reduced after just 1 min, with the small TMS-CMEP dropping to 2% M_{max} and the large TMS-CMEP to 17% M_{max} at 4 min. Since cervicomedullary stimuli are thought to maintain natural recruitment order of spinal motoneurons from small to large with increasing stimulus intensity, as with voluntary efforts (Gandevia and Rothwell, 1987), the stimuli for the small TMS-CMEP likely activated motoneurons which were active during the low-intensity task, while the large TMS-CMEP activated a large proportion of inactive motoneurons. In contrast, although motor unit recruitment increases in the biceps brachii up to at least 90% MVC, the majority of motor units were likely activated at 70% MVC (Kukulka and Clamann, 1981). Thus, stimuli for both small and large TMS-CMEPs likely activated motoneurons which were active throughout the high-intensity task. Accordingly, these results indicate that repetitively active motoneurons are specifically reduced in excitability compared to non-active or less active motoneurons in the same pool.

Numerous mechanisms can influence motoneuron excitability during sustained isometric contractions, including alterations in descending inputs, afferent feedback and intrinsic motoneuron properties (Taylor et al., 2016). An influence of altered descending input from the motor cortex can be ruled out given that responses were measured during the silent period when descending motor cortical drive is interrupted (Chen et al., 1999). Regarding afferent feedback, disfacilitation from Ia afferents is a possible candidate for reduced excitability owing to reductions in muscle spindles discharge during isometric exercise (Macefield et al., 1991). For the low-intensity task, a specific influence of motoneuron disfacilitation on the

small TMS-CMEP cannot be ruled out given that Ia afferent input is strongest in lower threshold motoneurons (Heckman and Binder, 1988). A reduction in this input could thus have an accentuated influence on the small compared with large TMS-CMEP given that the former likely reflects the excitability of lower threshold motoneurons, while the latter includes both low and high threshold motoneurons. Reduced Ia input might be of particular importance during the silent period given that the firing of muscle spindles is responsible for the EMG bursts which occurs when the force drops and the muscle lengthens, thus providing strong facilitatory input to motoneurons (Butler et al., 2012). For the high-intensity task, an influence of disfacilitation is less likely given that previous work has demonstrated that the application of tendon vibration, which excites muscle spindles (Roll et al., 1989), had no impact on the reduction in TMS-CMEPs during a sustained MVC of the elbow flexors (McNeil et al., 2011b). Finally, a role for inhibitory group III/IV afferent feedback is unlikely since these afferents facilitate, rather than inhibit CMEPs measured in the elbow flexors (Martin et al., 2006b; Martin et al., 2008). Thus, while an influence of motoneuron disfacilitation during the low-intensity task is possible, altered afferent feedback is unlikely to be responsible for the reduced TMS-CMEPs found during the high-intensity task.

Given that TMS-CMEPs were only reduced when they activated the same motoneurons which were likely active throughout the task, the results point towards repetitive activation-induced alterations in the intrinsic properties of motoneurons, rendering them less responsive to synaptic input. One of the leading hypotheses to explain repetitive activation-induced reductions in motoneuron excitability is late spike frequency adaptation (Bigland-Ritchie et al., 1986; Finn et al., 2018; Héroux et al., 2016; Johnson et al., 2004; McNeil et al., 2011a). This phenomenon is characterised by a gradual decline in motoneuron firing rate in the presence of a constant depolarising input elicited throughout intra or extracellular current injections (Kernell and Monster, 1982; Spielmann et al., 1993), and is more pronounced in

larger, higher threshold motoneurons (Button et al., 2007). The precise mechanisms of late adaptation are uncertain, but are thought to involve a gradual decrease in inward currents (e.g. increased inactivation of Na^+ channels implicated in action potential genesis) and/or an increase in outward currents (e.g. an increase in Ca^{2+}-dependent K^+ channels that contribute to after-hyperpolarisation, resulting in an increase in its magnitude and duration) (Brownstone, 2006; Nordstrom et al., 2007; Powers et al., 1999). These mechanisms could in turn decrease the probability of the membrane potential reaching the voltage threshold for spike initiation (Powers et al., 1999), and thus impair the capacity of the continuously active motoneurons to respond to synaptic input elicited following cervicomedullary stimuli.

While motoneuron adaptation represents an attractive hypothesis to explain the findings of the present study, there are caveats to the assumption that this mechanism contributes to reduced motoneuron excitability under natural conditions *in vivo* which should be considered, and have been reviewed previously (Nordstrom et al., 2007). Specifically, studies assessing motoneuron adaptation have primarily been performed *in vitro* and *in vivo* using reduced animal preparations, when descending neuromodulatory input is absent or reduced. Inputs from neuromodulators such as the monoamine serotonin, which descends from raphe nuclei to form monosynaptic connections with spinal motoneurons, have a profound influence on motoneuron excitability and firing behaviour (Heckman et al., 2008). As emphasised by Nordstrom et al. (2007) and Gandevia (2001), descending neuromodulatory input, inducing plateau potentials through persistent inward currents (PICs), can obviate the mechanisms responsible for motoneuron adaptation. Indeed, Brownstone et al. (2011) demonstrated that late adaptation was abolished during activation of monoaminergic pathways through brain stem stimulation-induced fictive locomotion in cats. In turtle motoneurons, adaptation was exhibited in the absence of PICs, but was diminished when PICs were present (Hornby et al., 2002). Moreover, Button et al. (2007) found an inverse relationship between estimated PIC

amplitude and late adaptation in anaesthetized rats. Taking the apparent negating influence of PICs on motoneuron adaptation into account, one possibility is that PICs were progressively reduced during the sustained contractions, thereby increasing the susceptibility of motoneurons to late adaptation. Indeed, indirect evidence in humans indicate that PICs could be reduced in response to isometric exercise (Kirk et al., 2019; Mendes and Kalmar, 2015). While the mechanisms responsible for any sustained contraction-induced reduction in PICs are unknown, an increase in the level of disynaptic reciprocal inhibition, which is a potent inhibitor of PICs (Hyngstrom et al., 2007), represents one possibility given that antagonist activity is known to increase during sustained isometric contractions (Lévénez et al., 2008). However, this mechanism seems unlikely since reciprocal inhibition has been shown to decrease, rather than increase during co-activation (Nielsen and Kagamihara, 1992), and previous work has shown no effect of co-activation on PICs (Foley and Kalmar, 2019). Another possibility is an impairment in the efficacy of PIC channels with repetitive activation, with evidence in rats suggesting that PIC channels could be inactivated during prolonged constant current input (Button et al., 2007). However, there remains limited evidence on the effects of sustained exercise on PICs, and this suggestion thus remains speculative.

In addition to the potential influence of motoneuron adaptation, a likely candidate for the rapid and substantial reduction in both small and large TMS-CMEPs during the high-intensity trial is intense release of serotonin from the raphe-spinal pathway, spill-over of serotonin from the synapse, and subsequent activation of inhibitory extra-synaptic 5-HT_1 receptors. Given the evidence derived from cats that serotonergic drive is related to exercise intensity (Jacobs and Fornal, 1995), a high serotonergic drive would be expected during the high-intensity task. Evidence from the turtle spinal cord has demonstrated that prolonged high serotonergic drive results in spill-over of serotonin onto the axon initial segment, which

subsequently binds to 5-HT_1 receptors to inhibit Na^+ channels and action potential initiation (Cotel et al., 2013). Recent findings in humans showed the ingestion of paroxetine, a serotonin reuptake inhibitor, exacerbated reductions in motoneuron excitability and voluntary activation during sustained maximal efforts (Kavanagh et al., 2019), while the ingestion of the 5-HT_1 receptor buspirone was also shown to reduce motoneuron excitability (D'Amico et al., 2017). During the low-intensity task, serotonin spill-over is unlikely to have been implicated in reduced motoneuron excitability for a number of reasons. Firstly, although serotonin release likely increased relative to rest during the low-intensity trial, neuromodulatory input was still likely to have been low given the lower motor output throughout the task, as supported by the low RPE (Jacobs and Fornal, 1995). Secondly, serotonin spill-over would be expected to affect diverse pools of motoneurons given the diffuse nature of neuromodulatory input onto the motoneuron pool (Heckman et al., 2008) and thus affect both small and large TMS-CMEPs. Thirdly, it has previously been shown that paroxetine ingestion has no effect on neuromuscular function during a sustained low-intensity isometric contraction (Thorstensen et al., 2020). Thus, the inhibitory effects of synaptic spill-over of serotonin represents a plausible mechanism contributing to the reduced TMS-CMEPs during the high-intensity trial, while it is unlikely to have played a role during the low-intensity trial.

Prolonged depression of TMS-CMEPs following high-intensity contraction

During recovery measurements following the high-intensity trial, the small TMS-CMEP remained below baseline up to 4 min post-exercise, while the large TMS-CMEP remained below baseline at all time-points during recovery. Thus, following high-intensity contraction, motoneuron excitability remains depressed for a prolonged period. Following serotonin spill-

over, motoneuron recovery has a relatively slow time-course of > 1 but < 5 min (Cotel et al., 2013; Perrier et al., 2018), which could explain, at least in part, the persistent reduction in TMS-CMEPs during recovery. Furthermore, if spike frequency adaptation does contribute to reduced motoneuron excitability during high-intensity contractions, its effects could persist for several minutes post-exercise. For example, following 4 min of intermittent current injection, Brownstone et al. (2011) found that cat lumbar motoneurons took 2 to 2.5 min to recover. Given that spike frequency adaptation is more pronounced in higher threshold motoneurons (Button et al., 2007), this could explain why reductions in CMEP persisted following the high-intensity, while CMEPs quickly recovered following the low-intensity task. Thus, the previously documented persistence of the mechanisms thought to contribute to reduced motoneuron excitability likely explain the prolonged reductions in TMS-CMEPs found in the present study.

CONCLUSIONS

The present study found that during low-intensity exercise, small, but not large TMS-CMEPs are reduced, whereas both small and large TMS-CMEPs are reduced during high-intensity exercise. Thus, TMS-CMEPs were only reduced when they activated the same motoneurons likely active throughout the task. These results are indicative of repetitive activation-induced reductions in motoneuron excitability. Likely candidate mechanisms underpinning the reduced motoneuron excitability during the low-intensity task include motoneuron disfacilitation and adaptation, while adaptation and serotonin spill-over could have contributed to the rapid and substantial decline in TMS-CMEPs during the high-intensity task. Furthermore, an original finding from the present study was the prolonged reduction in TMS-CMEPs following high-intensity exercise, which maintained below baseline following

4 min recovery. The present study is the first to compare TMS-CMEPs during and following low- and high-intensity sustained contractions. The contraction and stimulus intensity-dependent nature of the reductions in these responses provides mechanistic insight into impaired motoneuron excitability in response to isometric exercise.

Figure legends

Figure 1. Protocol schematic. At baseline, 3 cervicomedullary stimuli were delivered to elicit small cervicomedullary evoked potentials conditioned using transcranial magnetic stimulation (TMS-CMEPs, ~15% of maximum compound muscle action potential, M_{max}) and large TMS-CMEPs (~50% M_{max}). Stimuli were delivered during a low-intensity contraction at the EMG level associated with 20% maximum voluntary contraction (MVC), and a high-intensity contraction at the EMG level associated with 70% MVC. Participants then performed a 4.5 min sustained contraction at these respective EMG levels for the low- and high-intensity trials. Three small and 3 large TMS-CMEPs were delivered every minute, in addition to one superimposed M-wave (M_{sup}). Recovery measures were taken at 1, 2.5 and 4 min post-exercise. The order of the 3 small and large TMS-CMEPs was randomised.

Figure 2. Raw traces from a single participant across the experiment. Panel A displays small (blue traces) and large (red traces) cervicomedullary motor evoked potentials (CMEPs) conditioned by transcranial magnetic stimulation (TMS-CMEP) during a sustained low-intensity contraction (grey shaded area) of 4.5 min duration. Panel B displays small and large TMS-CMEPs during a sustained high-intensity contraction of the same duration. Recovery measures were taken at 1 min, 2.5 min and 4 min. Dashed horizontal lines indicate the

amplitude of the baseline responses. Note the 2:1 scaling ratio for large and small responses used to improve clarity.

Figure 3. Changes in force [% maximum voluntary contraction (%MVC)] and root mean square (RMS) electromyography (EMG) (%maximum EMG_{RMS}) of the biceps brachii during a sustained elbow flexion contraction performed at a low-intensity (Panel A) and high-intensity (Panel B; $n = 12$). Data were analysed via a one-way repeated measures ANOVA to assess the change in force and EMG over time. Grey shaded area represents the sustained contraction, error bars represent standard deviation. * $p < 0.05$, significant difference from baseline force. Bl, baseline.

Figure 4. Rate of perceived effort (RPE) measured during a sustained elbow flexion contraction performed at a low-intensity and high-intensity. Grey shaded area represents the sustained contraction, error bars represent standard deviation. Data were analysed via a two-way repeated measures ANOVA (time × contraction intensity) to assess the change in RPE over time during the low- and high-intensity trial ($n = 12$). * $p < 0.05$, significant difference from baseline RPE. + $p < 0.05$, significant difference from low-intensity RPE at corresponding time-point. Bl, baseline.

Figure 5. Amplitudes of small and large cervicomedullary evoked potentials conditioned using transcranial magnetic stimulation (TMS-CMEP) during a sustained elbow flexion contraction performed at a low-intensity (Panel A) and high-intensity (Panel B). Individual data for the small TMS-CMEPs are shown through blue dashed line, with large TMS-CMEP shown through red dashed lines. Data were analysed via a three-way repeated measures ANOVA (TMS-CMEP size × contraction intensity × time) to assess changes in small and large TMS-CMEPs over time during the low- and high-intensity trials ($n = 12$). Grey shaded area represents the sustained contraction, error bars represent standard deviation. * $p < 0.05$

significant differences from baseline for small TMS-CMEP; # $p < 0.05$ significant differences from baseline for small and large TMS-CMEP; + $p < 0.05$ significant differences between large TMS-CMEP during the low- and high-intensity at corresponding time-point. Bl, baseline.

Additional information

Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

All work was completed at Inter-university Laboratory of Human Movement Science, UJM-Saint-Etienne. C.G.B., P.A., J.S., R.S., T.L. and G.Y.M. conceived and designed the experiments; C.G.B., L.E and N.R. performed the experiments; C.G.B. analysed the data; C.G.B., P.A., and J.S. interpreted the results of the experiment. C.G.B. drafted the manuscript; C.G.B., L.E., N.R., P.A., J.S., R.S., T.L. and G.Y.M. edited and revised the manuscript. All authors approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Data availability

Data available upon request.

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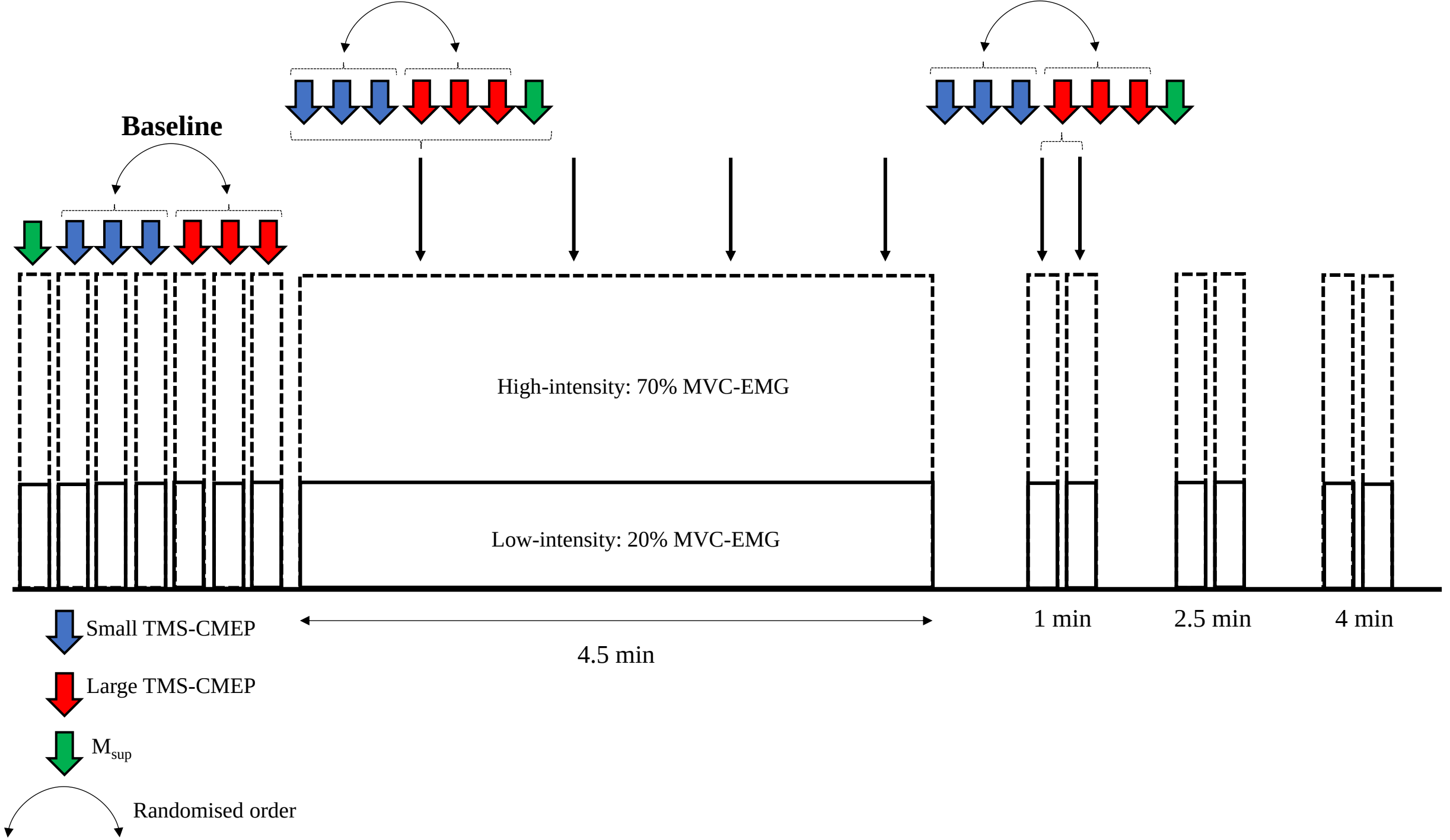
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Low-intensity: 20% MVC-EMG
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Large TMS-CMEP
M_{sup}
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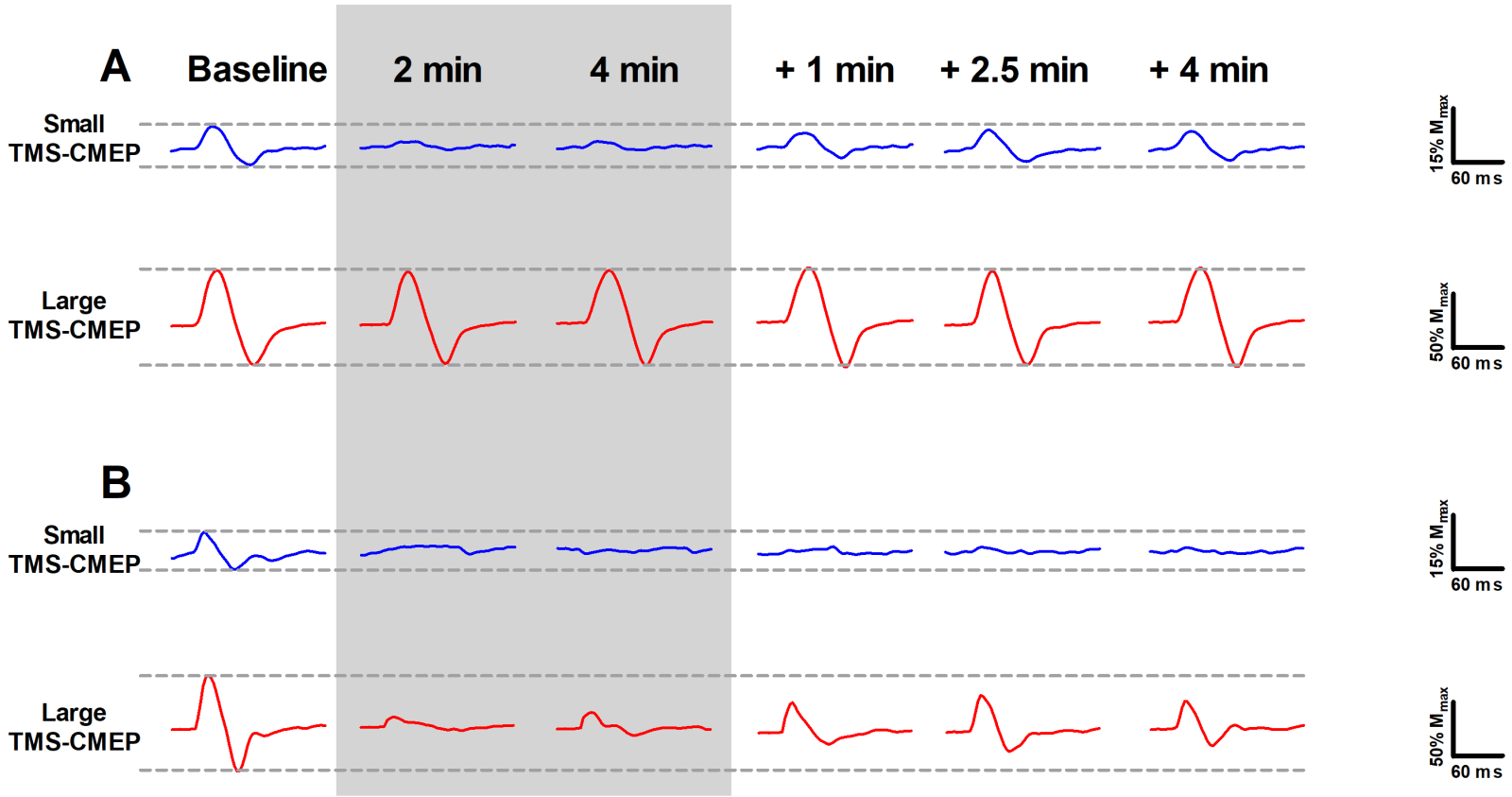
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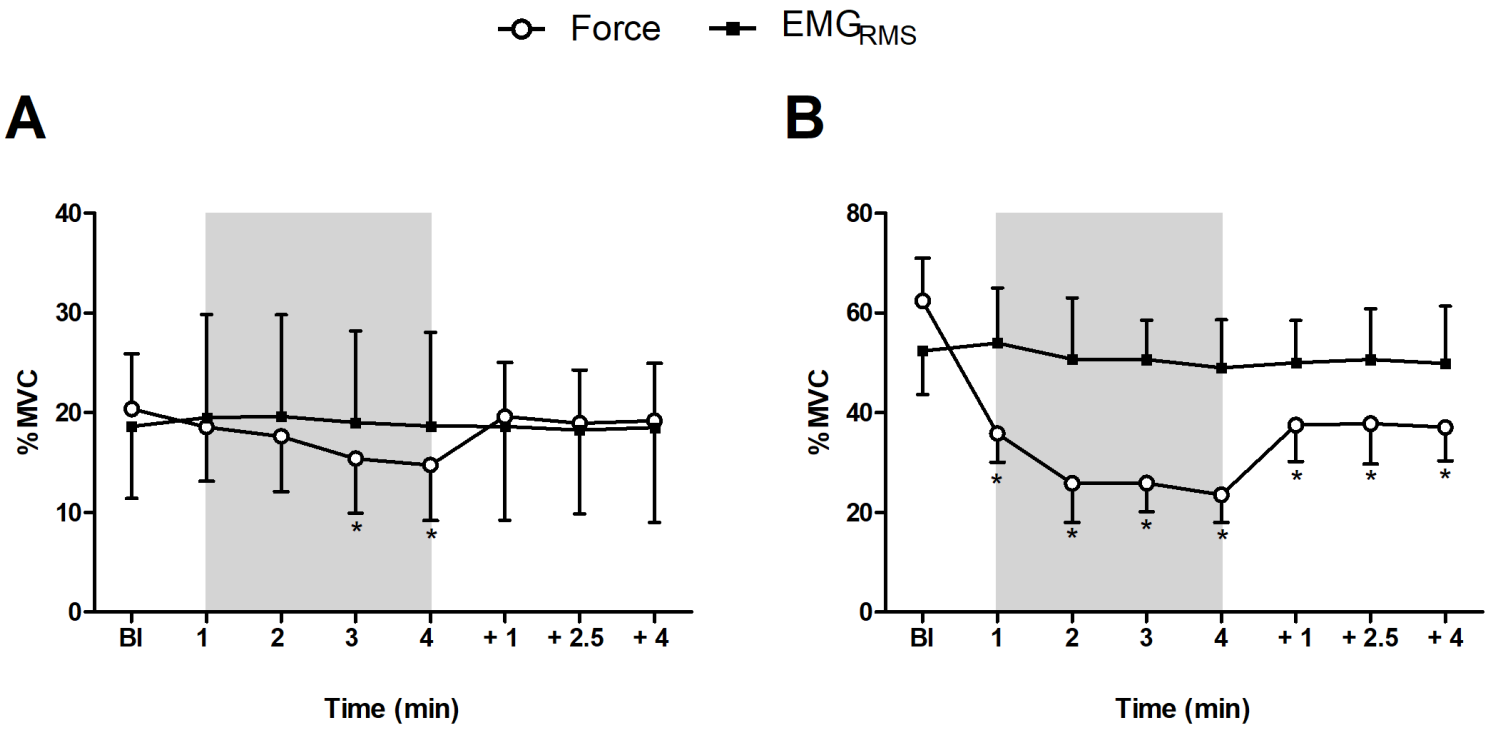
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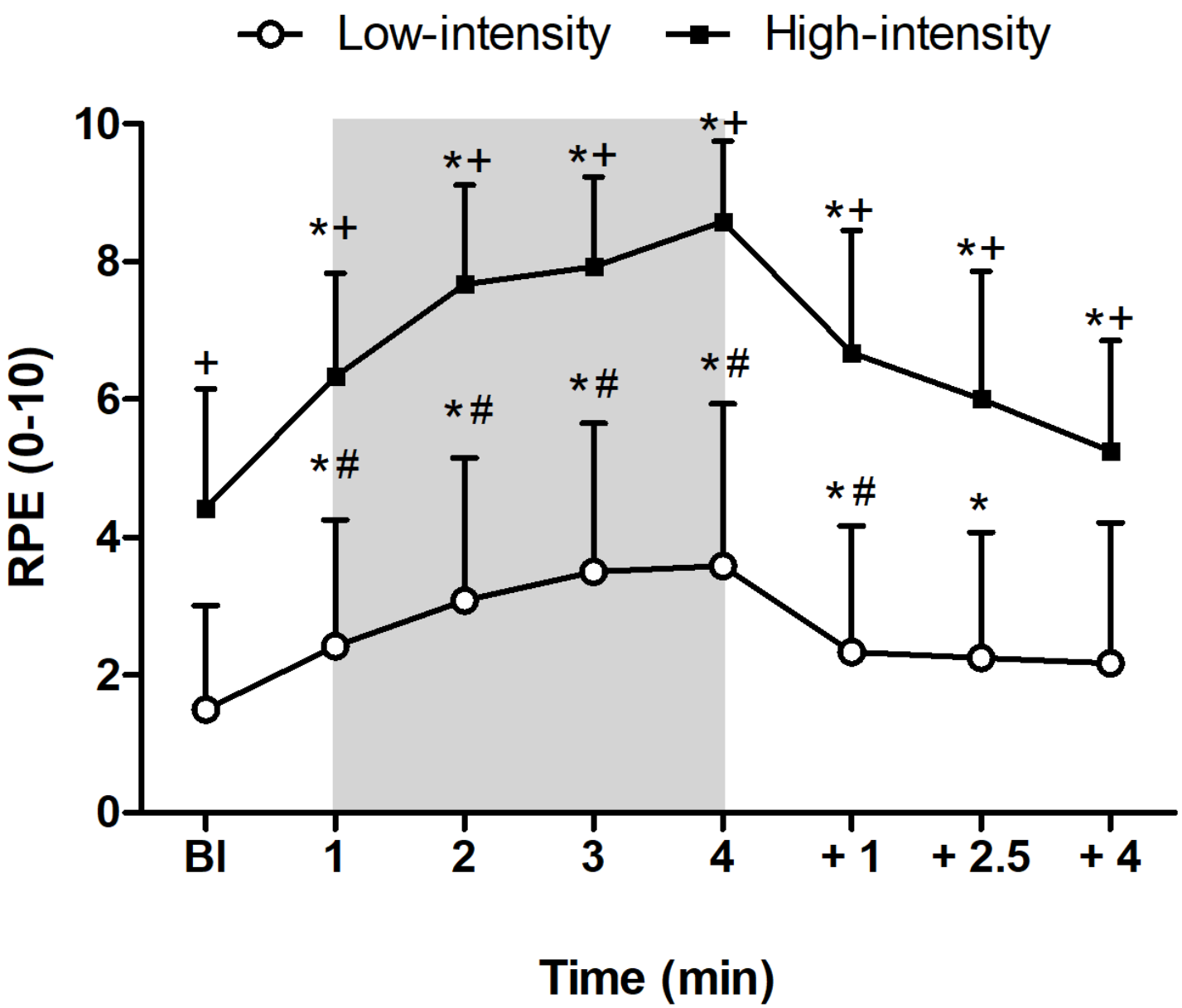
Low-intensity
High-intensity
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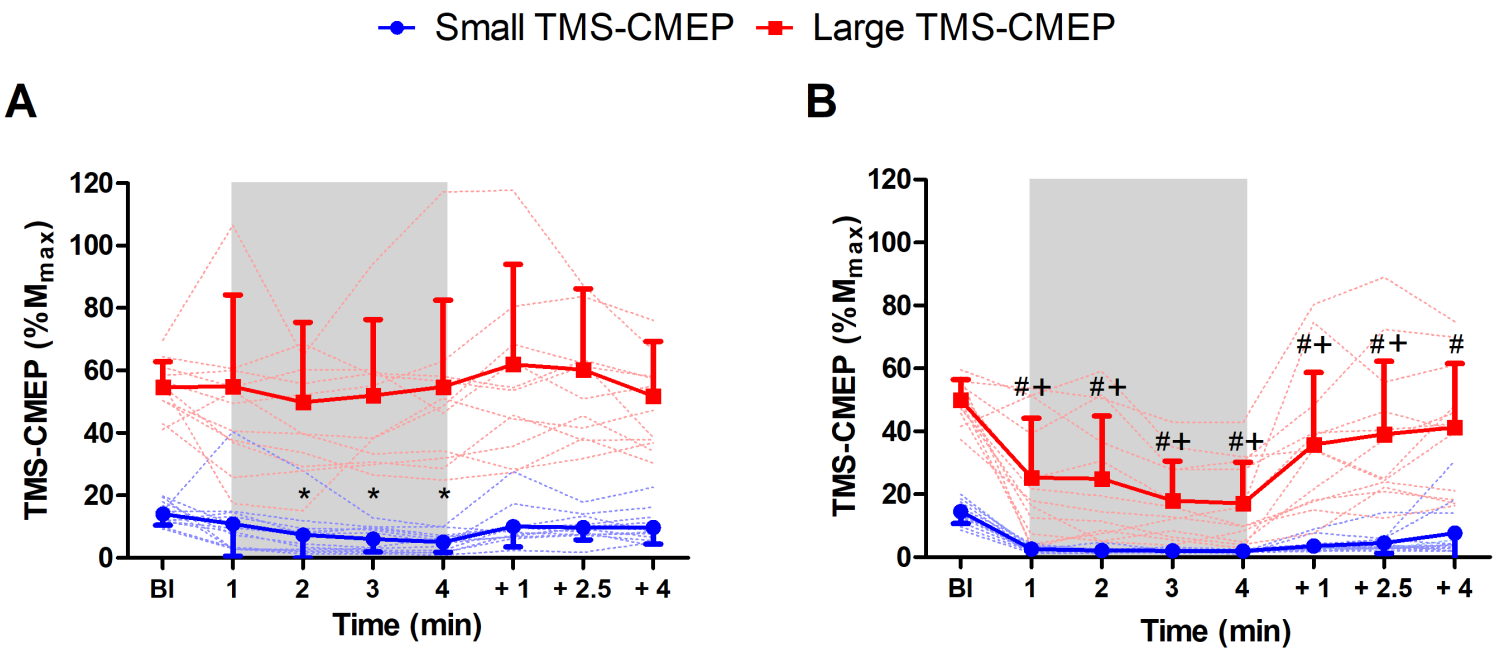
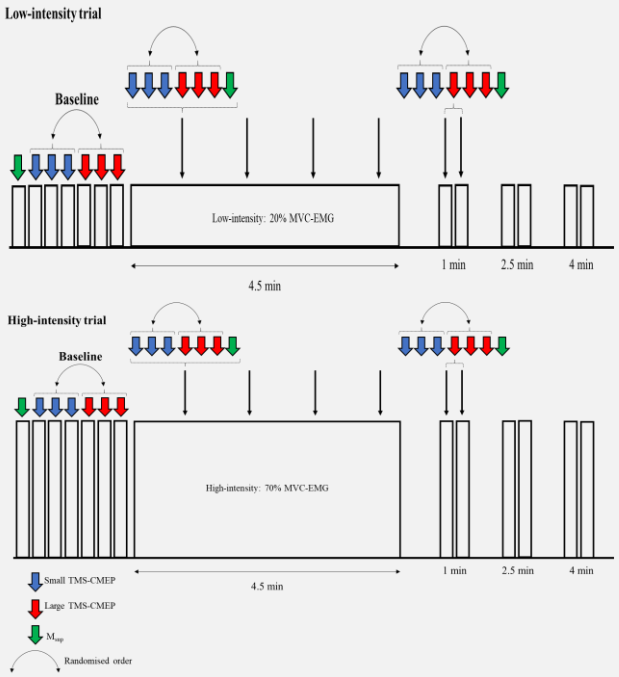

Small TMS-CMEP
Large TMS-CMEP
A
B
TMS-CMEP (%Mmax)
120
100
80
60
40
20
0
Bl
1
2
3
4
+ 1
+ 2.5
+ 4
Time (min)
*
*
*
#+
#+
#+
#+
#+
#+
#

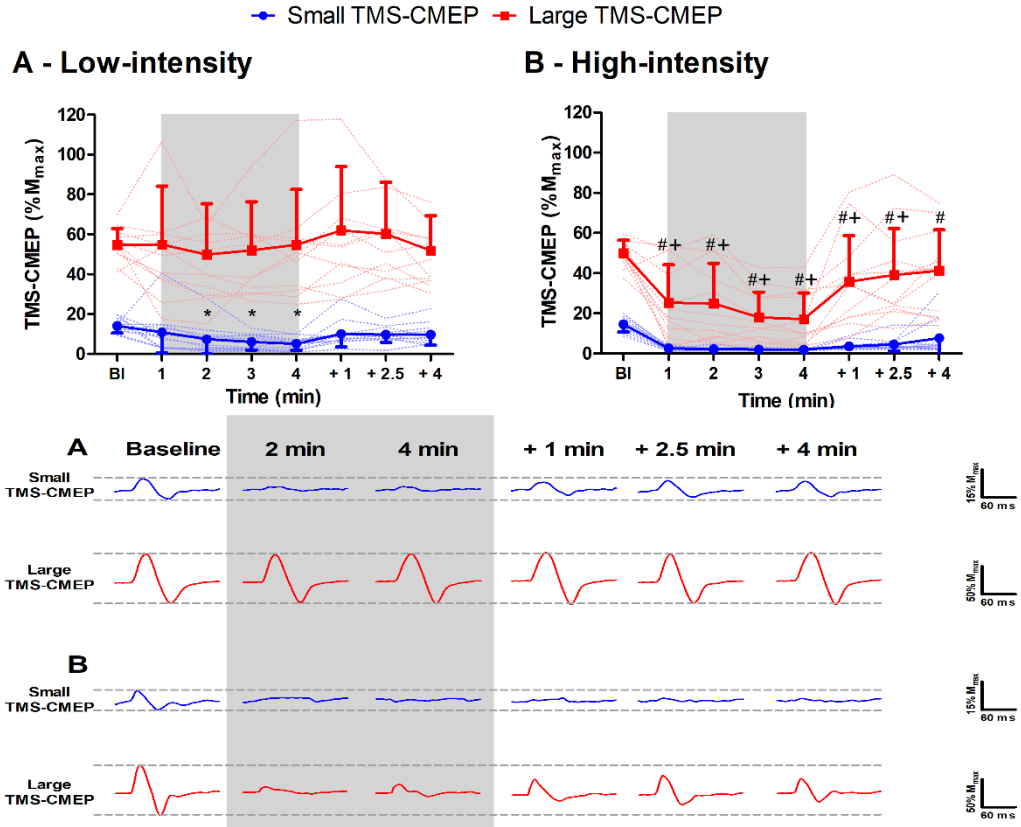
Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity

METHODS



20% & 70% MVC-EMG, electromyography level associated with 20% and 70% maximal voluntary contraction; TMS-CMEP, cervicomedullary evoked potential conditioning using transcranial magnetic stimulation; M_{sup}, superimposed maximal compound muscle action potential.

OUTCOME



CONCLUSION: TMS-CMEPs were only reduced when they activated those motoneurons likely recruited throughout the task. These results are indicative of repetitive activation-induced reductions in motoneuron excitability.