

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

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15 **Running head:** Motoneuron excitability during sustained contractions

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28 **New & Noteworthy**

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

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ABSTRACT

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50 Cervicomedullary stimulation provides a means of assessing motoneuron excitability.
51 Previous studies demonstrated that during low-intensity sustained contractions, small
52 cervicomedullary evoked potentials (CMEPs) conditioned using transcranial magnetic
53 stimulation (TMS-CMEPs) are reduced, whilst large TMS-CMEPs are less affected. Since
54 small TMS-CMEPs recruit motoneurons most active during low-intensity contractions while
55 large TMS-CMEPs recruit a high proportion of motoneurons inactive during the task, these
56 results suggest that reductions in motoneuron excitability could be dependent on repetitive
57 activation. To further test this hypothesis, this study assessed changes in small and large
58 TMS-CMEPs across low- and high-intensity contractions. Twelve participants performed a
59 sustained isometric contraction of the elbow flexor for 4.5 min at the electromyography
60 (EMG) level associated with 20% maximal voluntary contraction force (MVC; low-intensity)
61 and 70% MVC (high-intensity). Small and large TMS-CMEPs with amplitudes of ~15 and
62 ~50% M_{\max} at baseline, respectively, were delivered every minute throughout the tasks.
63 Recovery measures were taken at 1, 2.5 and 4-min post-exercise. During the low-intensity
64 trial, small TMS-CMEPs were reduced at 2-4 min ($p \leq 0.049$) by up to $-10\% M_{\max}$, while
65 large TMS-CMEPs remained unchanged ($p \geq 0.16$). During the high-intensity trial, small and
66 large TMS-CMEPs were reduced at all time-points ($p < 0.01$) by up to -14% and $-33\% M_{\max}$,
67 respectively, and remained below baseline during all recovery measures ($p \leq 0.02$). TMS-
68 CMEPs were unchanged relative to baseline during recovery following the low-intensity trial
69 ($p \geq 0.24$). These results provide novel insight into motoneuron excitability during and
70 following sustained contractions at different intensities, and suggest that contraction-induced
71 reductions in motoneuron excitability depend on repetitive activation.

72 **Keywords:** cervicomedullary evoked potentials, isometric exercise, motoneuron excitability

INTRODUCTION

73

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

84 One method of assessing motoneuron excitability is through stimulation of descending axons
85 of the corticospinal tract at the level of the cervicomedullary junction (Taylor, 2006). These
86 stimuli activate corticospinal axons which project with a large monosynaptic component
87 (Petersen et al., 2002) onto motoneurons of the upper-limbs, producing a short-latency
88 excitatory response known as the cervicomedullary motor evoked potential (CMEP) that
89 permits the quantification motoneuron excitability. Using this method, numerous studies have
90 revealed reductions in CMEPs in response to sustained isometric contractions (Butler et al.,
91 2003; Gandevia et al., 1999; Temesi et al., 2019), though others have reported an increase
92 (Hoffman et al., 2009; Lévénez et al., 2008)

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

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

113 Using this method, it has been demonstrated that during a sustained low-intensity isometric
114 contraction of the upper- (McNeil et al., 2011a) and lower-limb (Finn et al., 2018), small
115 CMEPs (McNeil et al., 2011a) and thoracic motor evoked potentials (TMEPs) (Finn et al.,
116 2018) evoked using low-intensity stimuli were substantially reduced, while large responses
117 evoked using high-intensity stimuli were less affected. Since the low-intensity stimuli likely
118 recruited the same motoneurons which were active throughout the task, while large responses
119 recruited a high proportion of inactive motoneurons, the relatively greater decline of small
120 responses suggests that reductions in excitability were greatest in motoneurons that were
121 repetitively active during the task. Studies using intramuscular combined with compound

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

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

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

153 **METHODS**

154 **Participants**

155 Using the effect size for a TMEP size \times time interaction from Finn et al. (2018), a power
156 calculation ($\alpha = 0.05$, power = 0.95) determined that a sample size of 11 participants was
157 required. To account for the possibility that large CMEPs might not be evocable in some
158 participants (McNeil et al., 2011a), 17 male participants were recruited for the study. Five
159 participants were not tested because an abrupt decrease in CMEP latency when increasing
160 stimulus intensity ($n = 1$) indicating stimulus spread to cervical roots (Taylor and Gandevia,
161 2004), an insufficient silent period duration ($n = 1$), stimulation discomfort ($n = 1$) and
162 because of an inability to sustain the required EMG level ($n = 2$). The experiment was thus
163 completed by 12 participants (mean \pm SD age: 30 ± 7 yr, stature: 177.9 ± 7.0 cm, mass: 76.5
164 ± 12.2 kg). The study received ethical approval from the local ethics committee and
165 conformed to the standards set by the *Declaration of Helsinki*, except for registration in a
166 database. Participants provided written informed consent to take part in the study.

167

168 **Experimental Design**

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

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

191

192 **Experimental procedures**

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

195 stimulation of the brachial plexus delivered at rest (described below). A brief warm-up
196 consisting of 5-7 submaximal contractions of the elbow flexors at a progressively increasing
197 intensity up to 90% of perceived maximal strength was subsequently performed. Participants
198 then performed two MVCs of the elbow flexors, with the peak value taken to calculate
199 submaximal forces. Subsequently, participants performed a 5 s isometric contraction of the
200 elbow flexors at 20% MVC for the low-intensity and 70% MVC for the high-intensity trial
201 using visual force feedback displayed on a computer monitor. The average BB smoothed
202 rectified EMG from these contractions was then calculated to derive a target EMG level for
203 the task. Whilst contracting at the target EMG level, a baseline superimposed M-wave (M_{sup})
204 was then measured using a stimulus intensity 130% of that associated with M_{max} . The TMS
205 hotspot was then determined, after which the TMS intensity was adjusted to produce a silent
206 period of at least 200 ms (described below) whilst contraction at the target EMG level.
207 Subsequently, the appropriate cervicomedullary stimulus intensity required to elicit a small
208 ($\sim 15\%$ M_{max} amplitude) and large ($\sim 50\%$ M_{max} amplitude) TMS-CMEP in the BB was
209 determined (described below). To do so, participants performed brief contractions (~ 3 s) at
210 the target EMG level and received conditioned cervicomedullary stimuli at a gradually
211 increasing intensity with each contraction. Once the appropriate stimulus intensities were
212 found, two further stimuli were delivered at the same intensity, with the average of the three
213 stimuli used as the baseline small and large TMS-CMEPs for both trials (Figure 1). One and
214 1.5 min of rest was given between contractions for baseline measures for the low- and high-
215 intensity trial, respectively. Two minutes following the final baseline measure, the sustained
216 isometric contraction began. For the low-intensity trial, this consisted of a sustained isometric
217 elbow flexion at a 20% MVC-EMG level, and for the high-intensity trial, at a 70% MVC-
218 EMG level. Both trials lasted 4.5 min. At 1, 2, 3 and 4 min of both trials, 3 cervicomedullary
219 stimuli were delivered at the intensity associated with the small TMS-CMEP and 3 at the

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

230

231 **Instrumentation**

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

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

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

256 ***Transcranial magnetic stimulation.*** Single-pulse TMS of 1 ms duration was delivered over
257 the vertex using a circular coil (13.5 cm outsider diameter) connected to a magnetic
258 stimulator (Magstim 2002, The Magstim Co., Whitland, UK). The vertex was marked as the
259 intersection of lines drawn between the preauricular points and from nasion to inion, which
260 were marked on a swim cap worn by participants. The direction of current flow in the coil
261 preferentially activated the left motor cortex. To confirm appropriate coil placement, TMS
262 was delivered at 50% maximum stimulator output (MSO) during a contraction at the target
263 EMG level, and the discernible motor evoked potential (MEP) confirmed accurate placement
264 in all participants. The stimulus intensity was subsequently increased in 10% MSO
265 increments until a silent period of 200 ms was observed whilst contracting at the target EMG
266 level (low-intensity trial, $83 \pm 13\%$; high-intensity trial, $81 \pm 14\%$ MSO; paired t-test $p =$
267 0.54).

268 **Cervicomedullary stimulation.** A high-voltage electrical current (1 ms duration, Digitimer
269 DS7R) was passed between two 30 mm diameter stimulation electrodes placed over the
270 mastoid processes (Melitracen 100) for cervicomedullary stimulations. Stimuli were
271 delivered 100 ms following the TMS conditioning stimulus (McNeil et al., 2009). The
272 stimulus intensity began at 20 mA, and was increased in 20 mA increments until a TMS-
273 CMEP of $\sim 15\%$ M_{\max} amplitude was elicited for the small TMS-CMEP (low-intensity trial,
274 79 ± 16 ; high-intensity trial, 86 ± 22 mA; paired t-test $p = 0.23$) and $\sim 50\%$ M_{\max} amplitude
275 was elicited for the large TMS-CMEP (low-intensity trial, 140 ± 27 ; high-intensity trial, 159
276 ± 43 mA; paired t-test $p = 0.36$). When increasing stimulus intensity, the CMEP was closely
277 monitored for decreases in response latency, which are indicative of nerve root stimulation
278 (Taylor and Gandevia, 2004). In all but one participant, who was subsequently excluded, no
279 such decrease was observed.

280

281 **Data analysis**

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

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

295

296 **Statistical analysis**

297 Jamovi statistical software (jamovi, version 1.0, 2019, the jamovi project; retrieved from
298 <https://www.jamovi.org>) was used for all statistical analyses. All data are presented as mean
299 \pm SD, with error bars in figures representing SD. Statistical significance was set at an α of
300 0.05. Normality of the data was assessed by the Shapiro-Wilk test, with no data requiring
301 transformation. Assumptions of sphericity were explored and controlled for all variables with
302 the Greenhouse-Geisser adjustment, where necessary. A three-way repeated measures
303 ANOVA [$2 \times 2 \times 5$; TMS-CMEP size (small TMS-CMEP/ M_{sup} and large TMS-CMEP/ M_{sup}),
304 contraction intensity (Low-intensity and High-intensity) and time (baseline, 1, 2, 3 and 4 min
305 of sustained elbow flexion)] was used to assess the effect of TMS-CMEP size and contraction
306 intensity on changes in motoneuron excitability. A separate three-way ANOVA was
307 performed to assess changes in small and large TMS-CMEP relative to baseline during the
308 recovery measures following the low- and high-intensity tasks. A two-way repeated measures
309 ANOVA (contraction intensity \times time) was used to assess changes in RPE and M_{sup} during
310 the low- and high-intensity trials. To assess changes in force and EMG_{RMS} , two one-way
311 ANOVAs were performed for the low- and high-intensity trials separately. In the event of a
312 significant interaction or main effect, analysis was continued using pairwise comparisons
313 with least significant differences. Partial eta squared (η_p^2) was calculated to estimate effect
314 sizes, with values representing small ($\eta_p^2 = 0.10$), medium ($\eta_p^2 = 0.25$) and large ($\eta_p^2 = 0.40$)
315 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.

321

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 $18.6 \pm 7.2\%$
337 maximum EMG_{RMS}, while force was $20.3 \pm 5.5\%$ MVC. The EMG_{RMS} of the BB remained
338 consistent throughout the sustained contraction and recovery measures, with no effect of time

339 ($F_{2,6,28.6} = 0.97$, $p = 0.46$, $\eta_p^2 = 0.08$; Figure 3A). Force decreased throughout the trial ($F_{1,4,14.4}$
 340 $= 4.19$, $p = 0.048$, $\eta_p^2 = 0.30$), before returning to baseline at 1 min post-exercise ($p = 0.37$;
 341 Figure 3A). For the high-intensity trial, EMG_{RMS} was $52.3 \pm 8.7\%$ maximum EMG_{RMS} , while
 342 force was $62.4 \pm 8.5\%$ MVC at baseline. Note that the force was lower than 70% MVC at
 343 baseline due to the rapid drop in force which occurred within contractions at a high EMG
 344 level during the baseline measurements. The EMG_{RMS} of the BB remained consistent
 345 throughout the sustained contraction and recovery measures, with no effect of time ($F_{3,5,38.3} =$
 346 1.11 , $p = 0.36$, $\eta_p^2 = 0.09$; Figure 3B). Force decreased throughout the trial ($F_{3,3,36.7} = 54.90$,
 347 $p < 0.01$, $\eta_p^2 = 0.83$), and was reduced at all time-points, including the recovery measures (p
 348 < 0.01).

349 At baseline, M_{max} was 16.0 ± 5.9 mV in the BB for the low-intensity trial, and 16.5 ± 5.7 mV
 350 for the high-intensity trial ($p = 0.72$). A two-way contraction intensity \times time interaction was
 351 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
 352 low-intensity trial ($p \geq 0.25$), while M_{sup} was lower than baseline at 2.5 (14.7 ± 4.7 mV; $p =$
 353 0.01) and 4 min (14.3 ± 3.8 mV; $p < 0.01$) post-exercise for the high-intensity trial.

354 For RPE, a significant two-way contraction intensity \times time interaction was found ($F_{7,77} =$
 355 5.44 , $p < 0.01$, $\eta_p^2 = 0.33$). For the low-intensity trial, RPE increased relative to baseline
 356 throughout the sustained contraction ($p \leq 0.03$) before recovering by 4 min post-exercise ($p =$
 357 0.06 ; Figure 4). For the high-intensity trial, RPE increased relative to baseline at all time-
 358 points, including the recovery measures ($p < 0.01$; Figure 4). The RPE was higher at all time-
 359 points during the high-intensity compared with low-intensity (all $p < 0.01$; Figure 4).

360

361 **TMS-CMEP**

362 The typical error for the small and large TMS-CMEPs were $0.91 \pm 0.48\%$ M_{\max} and $2.93 \pm$
363 1.12% M_{\max} , respectively. At baseline for the low-intensity trial, the small and large TMS-
364 CMEPs were $14.1 \pm 3.7\%$ M_{\max} amplitude and $54.6 \pm 8.3\%$ M_{\max} amplitude respectively. For
365 the high-intensity trial, small and large TMS-CMEPs were $14.5 \pm 3.9\%$ M_{\max} and $50.0 \pm$
366 6.5% M_{\max} respectively. No differences were found between baseline small and large TMS-
367 CMEP amplitudes for the low- and high-intensity trials ($p \geq 0.42$). A significant three-way
368 TMS-CMEP size \times contraction intensity \times time interaction was found ($F_{7,77} = 4.51$, $p < 0.01$,
369 $\eta_p^2 = 0.29$). For the low-intensity trial, the amplitude of the small TMS-CMEP was reduced
370 from 2 ($p = 0.049$), 3 ($p = 0.02$) and 4 min ($p = 0.01$) of the sustained contraction (Figure
371 5A), dropping to $5.0 \pm 3.1\%$ M_{\max} by 4 min ($d = 2.7$). In contrast, no change was found for
372 the amplitude of the large TMS-CMEP throughout the sustained low-intensity contraction (p
373 ≥ 0.16). For the high-intensity task, both the small and large TMS-CMEP amplitudes were
374 reduced at all time-points ($p < 0.01$) dropping to $1.0 \pm 0.4\%$ M_{\max} ($d = 4.6$) and $17.1 \pm 13.1\%$
375 M_{\max} by 4 min ($d = 3.2$), respectively (Figure 5B). Between trial comparisons revealed no
376 difference in the small TMS-CMEP between the low- and high-intensity trials at any time-
377 point ($p \geq 0.17$). In contrast, the large TMS-CMEP was lower during the high- versus low-
378 intensity trial at all time-points ($p < 0.01$).

379 For the recovery measures, no TMS-CMEP size \times contraction intensity \times time interaction
380 was found ($F_{3,33} = 2.1$, $p = 0.12$, $\eta_p^2 = 0.16$). However, a contraction intensity \times time
381 interaction was noted ($F_{3,33} = 5.4$, $p < 0.01$, $\eta_p^2 = 0.33$). *Post-hoc* tests revealed no difference
382 in TMS-CMEPs relative to baseline during recovery following the low-intensity trial ($p \geq$
383 0.24 ; Figure 5A). In contrast, TMS-CMEPs were reduced at all time-points during recovery
384 following the high-intensity trial ($p \leq 0.01$; Figure 5B). Between-trial comparisons showed
385 that TMS-CMEPs were lower during the high- versus low-intensity trial at 1 and 2.5 min ($p <$
386 0.01), with no difference at 4 min ($p = 0.10$).

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DISCUSSION

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

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

408 During the low-intensity contraction sustained for 4.5 min at the EMG level associated with
409 20% MVC, small TMS-CMEPs of $\sim 15\%$ M_{\max} amplitude fell below baseline after 2 min and
410 decreased to 5% M_{\max} by 4 min. Large TMS-CMEPs, in contrast, were maintained at $\sim 50\%$
411 M_{\max} throughout the sustained contraction. During the high-intensity task at the EMG level
412 associated with 70% MVC, both small and large TMS-CMEPs were reduced after just 1 min,
413 with the small TMS-CMEP dropping to 2% M_{\max} and the large TMS-CMEP to 17% M_{\max} at
414 4 min. Since cervicomedullary stimuli are thought to maintain natural recruitment order of
415 spinal motoneurons from small to large with increasing stimulus intensity, as with voluntary
416 efforts (Gandevia and Rothwell, 1987), the stimuli for the small TMS-CMEP likely activated
417 motoneurons which were active during the low-intensity task, while the large TMS-CMEP
418 activated a large proportion of inactive motoneurons. In contrast, although motor unit
419 recruitment increases in the biceps brachii up to at least 90% MVC, the majority of motor
420 units were likely activated at 70% MVC (Kukulka and Clamann, 1981). Thus, stimuli for
421 both small and large TMS-CMEPs likely activated motoneurons which were active
422 throughout the high-intensity task. Accordingly, these results indicate that repetitively active
423 motoneurons are specifically reduced in excitability compared to non-active or less active
424 motoneurons in the same pool.

425 Numerous mechanisms can influence motoneuron excitability during sustained isometric
426 contractions, including alterations in descending inputs, afferent feedback and intrinsic
427 motoneuron properties (Taylor et al., 2016). An influence of altered descending input from
428 the motor cortex can be ruled out given that responses were measured during the silent period
429 when descending motor cortical drive is interrupted (Chen et al., 1999). Regarding afferent
430 feedback, disfacilitation from Ia afferents is a possible candidate for reduced excitability
431 owing to reductions in muscle spindles discharge during isometric exercise (Macefield et al.,
432 1991). For the low-intensity task, a specific influence of motoneuron disfacilitation on the

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

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

458 larger, higher threshold motoneurons (Button et al., 2007). The precise mechanisms of late
459 adaptation are uncertain, but are thought to involve a gradual decrease in inward currents
460 (e.g. increased inactivation of Na⁺ channels implicated in action potential genesis) and/or an
461 increase in outward currents (e.g. an increase in Ca²⁺-dependent K⁺ channels that contribute
462 to after-hyperpolarisation, resulting in an increase in its magnitude and duration)
463 (Brownstone, 2006; Nordstrom et al., 2007; Powers et al., 1999). These mechanisms could in
464 turn decrease the probability of the membrane potential reaching the voltage threshold for
465 spike initiation (Powers et al., 1999), and thus impair the capacity of the continuously active
466 motoneurons to respond to synaptic input elicited following cervicomedullary stimuli.

467 While motoneuron adaptation represents an attractive hypothesis to explain the findings of
468 the present study, there are caveats to the assumption that this mechanism contributes to
469 reduced motoneuron excitability under natural conditions *in vivo* which should be considered,
470 and have been reviewed previously (Nordstrom et al., 2007). Specifically, studies assessing
471 motoneuron adaptation have primarily been performed *in vitro* and *in vivo* using reduced
472 animal preparations, when descending neuromodulatory input is absent or reduced. Inputs
473 from neuromodulators such as the monoamine serotonin, which descends from raphe nuclei
474 to form monosynaptic connections with spinal motoneurons, have a profound influence on
475 motoneuron excitability and firing behaviour (Heckman et al., 2008). As emphasised by
476 Nordstrom et al. (2007) and Gandevia (2001), descending neuromodulatory input, inducing
477 plateau potentials through persistent inward currents (PICs), can obviate the mechanisms
478 responsible for motoneuron adaptation. Indeed, Brownstone et al. (2011) demonstrated that
479 late adaptation was abolished during activation of monoaminergic pathways through brain
480 stem stimulation-induced fictive locomotion in cats. In turtle motoneurons, adaptation was
481 exhibited in the absence of PICs, but was diminished when PICs were present (Hornby et al.,
482 2002). Moreover, Button et al. (2007) found an inverse relationship between estimated PIC

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

500 In addition to the potential influence of motoneuron adaptation, a likely candidate for the
501 rapid and substantial reduction in both small and large TMS-CMEPs during the high-intensity
502 trial is intense release of serotonin from the raphe-spinal pathway, spill-over of serotonin
503 from the synapse, and subsequent activation of inhibitory extra-synaptic 5-HT₁ receptors.
504 Given the evidence derived from cats that serotonergic drive is related to exercise intensity
505 (Jacobs and Fornal, 1995), a high serotonergic drive would be expected during the high-
506 intensity task. Evidence from the turtle spinal cord has demonstrated that prolonged high
507 serotonergic drive results in spill-over of serotonin onto the axon initial segment, which

508 subsequently binds to 5-HT₁ receptors to inhibit Na⁺ channels and action potential initiation
509 (Cotel et al., 2013). Recent findings in humans showed the ingestion of paroxetine, a
510 serotonin reuptake inhibitor, exacerbated reductions in motoneuron excitability and voluntary
511 activation during sustained maximal efforts (Kavanagh et al., 2019), while the ingestion of
512 the 5-HT₁ receptor buspirone was also shown to reduce motoneuron excitability (D'Amico et
513 al., 2017). During the low-intensity task, serotonin spill-over is unlikely to have been
514 implicated in reduced motoneuron excitability for a number of reasons. Firstly, although
515 serotonin release likely increased relative to rest during the low-intensity trial,
516 neuromodulatory input was still likely to have been low given the lower motor output
517 throughout the task, as supported by the low RPE (Jacobs and Fornal, 1995). Secondly,
518 serotonin spill-over would be expected to affect diverse pools of motoneurons given the
519 diffuse nature of neuromodulatory input onto the motoneuron pool (Heckman et al., 2008)
520 and thus affect both small and large TMS-CMEPs. Thirdly, it has previously been shown that
521 paroxetine ingestion has no effect on neuromuscular function during a sustained low-intensity
522 isometric contraction (Thorstensen et al., 2020). Thus, the inhibitory effects of synaptic spill-
523 over of serotonin represents a plausible mechanism contributing to the reduced TMS-CMEPs
524 during the high-intensity trial, while it is unlikely to have played a role during the low-
525 intensity trial.

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527 **Prolonged depression of TMS-CMEPs following high-intensity contraction**

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

532 over, motoneuron recovery has a relatively slow time-course of > 1 but < 5 min (Cotel et al.,
533 2013; Perrier et al., 2018), which could explain, at least in part, the persistent reduction in
534 TMS-CMEPs during recovery. Furthermore, if spike frequency adaptation does contribute to
535 reduced motoneuron excitability during high-intensity contractions, its effects could persist
536 for several minutes post-exercise. For example, following 4 min of intermittent current
537 injection, Brownstone et al. (2011) found that cat lumbar motoneurons took 2 to 2.5 min to
538 recover. Given that spike frequency adaptation is more pronounced in higher threshold
539 motoneurons (Button et al., 2007), this could explain why reductions in CMEP persisted
540 following the high-intensity, while CMEPs quickly recovered following the low-intensity
541 task. Thus, the previously documented persistence of the mechanisms thought to contribute to
542 reduced motoneuron excitability likely explain the prolonged reductions in TMS-CMEPs
543 found in the present study.

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CONCLUSIONS

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

556 4 min recovery. The present study is the first to compare TMS-CMEPs during and following
557 low- and high-intensity sustained contractions. The contraction and stimulus intensity-
558 dependent nature of the reductions in these responses provides mechanistic insight into
559 impaired motoneuron excitability in response to isometric exercise.

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563 **Figure legends**

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

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

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

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

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

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

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

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613 **Additional information**

614 **Competing interests**

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

616 **Author contributions**

617 All work was completed at Inter-university Laboratory of Human Movement Science, UJM-
618 Saint-Etienne. C.G.B., P.A., J.S., R.S., T.L. and G.Y.M. conceived and designed the
619 experiments; C.G.B., L.E and N.R. performed the experiments; C.G.B. analysed the data;
620 C.G.B., P.A., and J.S. interpreted the results of the experiment. C.G.B. drafted the
621 manuscript; C.G.B., L.E., N.R., P.A., J.S., R.S., T.L. and G.Y.M. edited and revised the
622 manuscript. All authors approved the final manuscript and agree to be accountable for all
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630 **Data availability**

631 Data available upon request.

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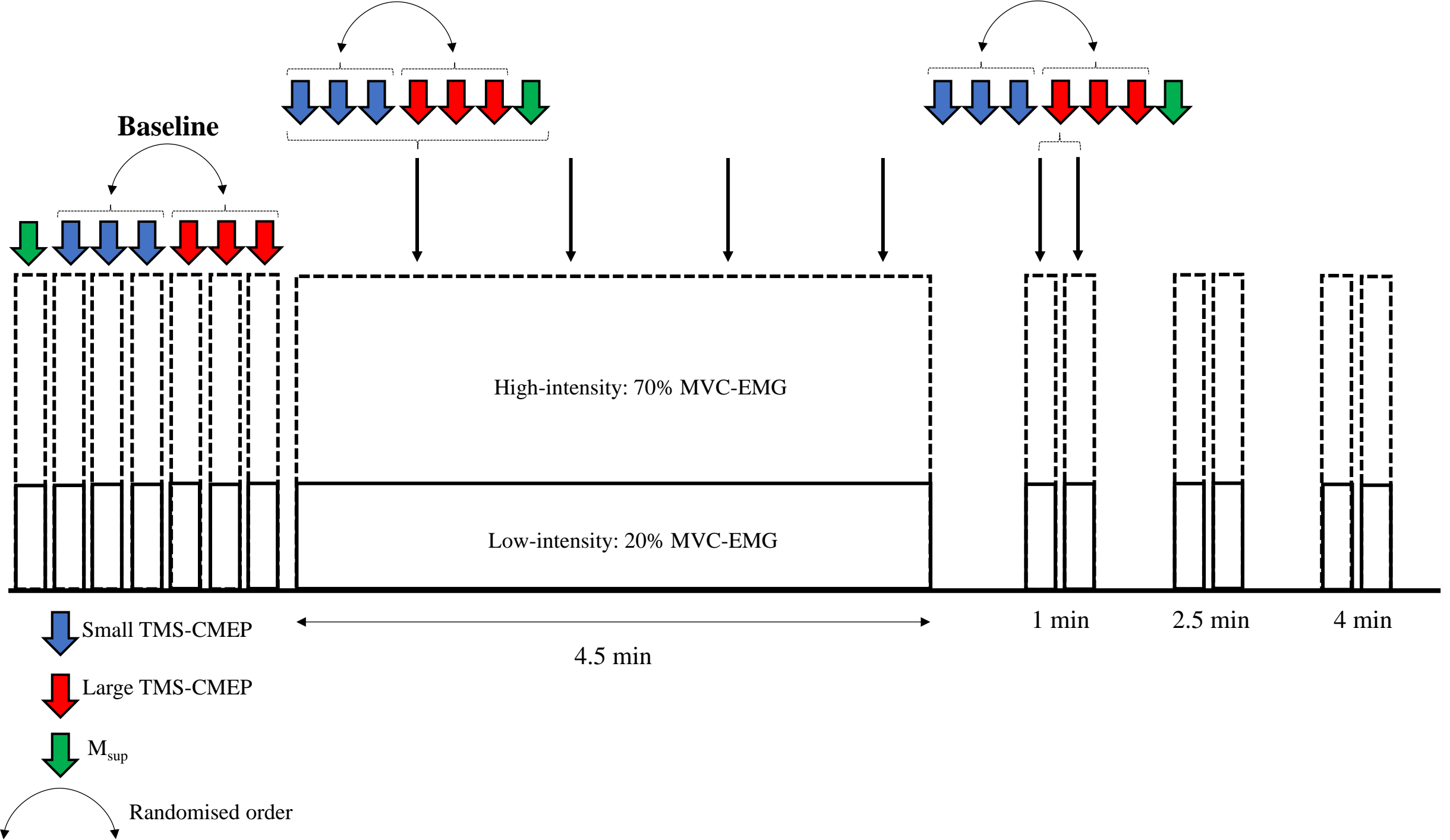
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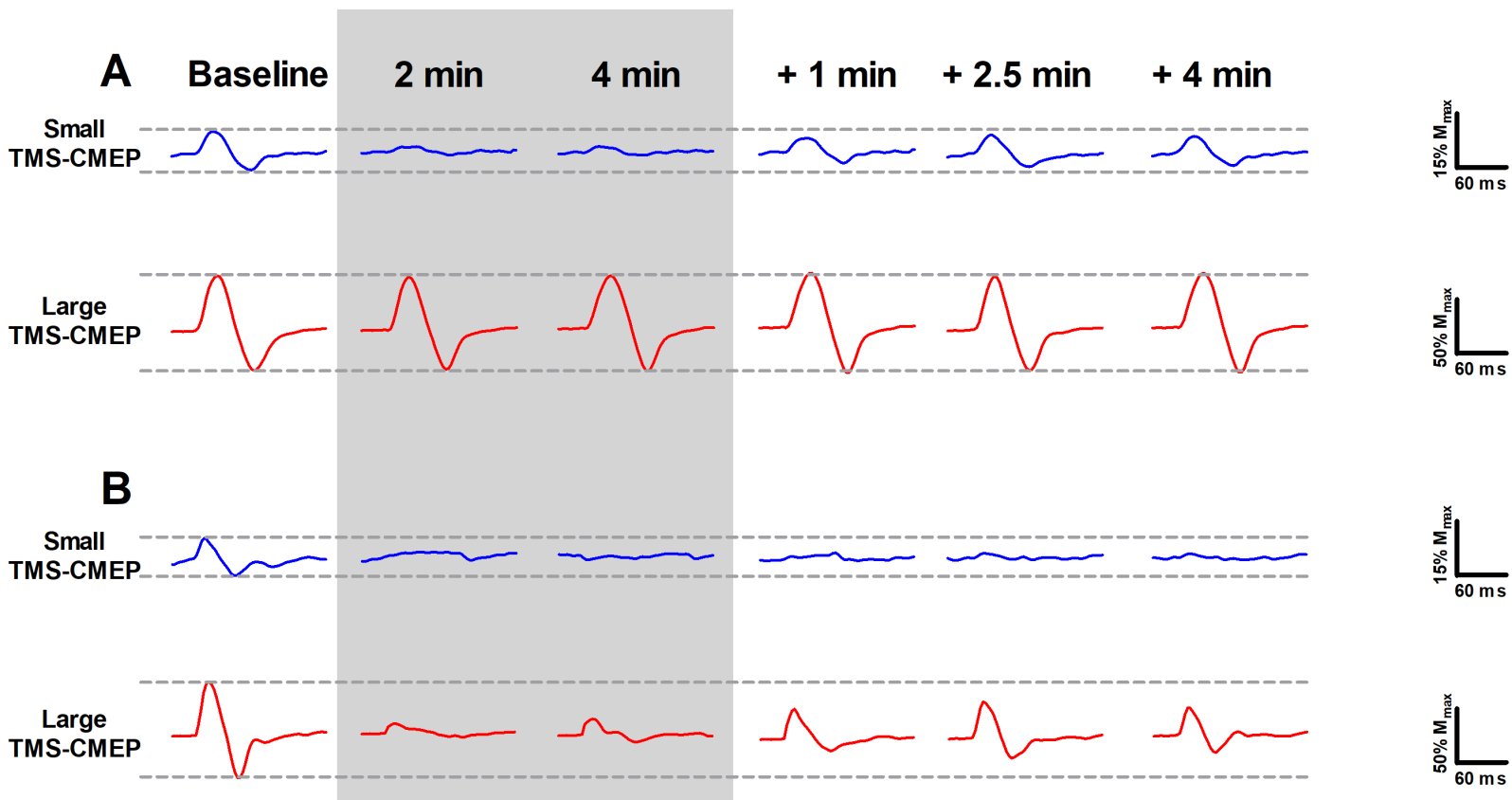
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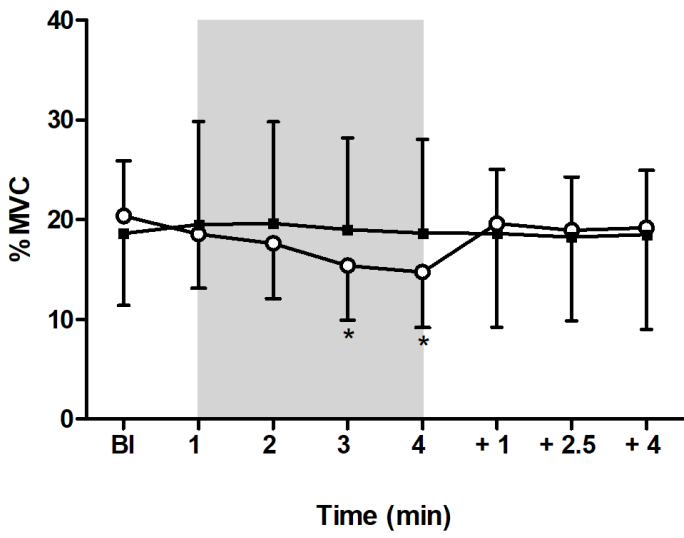
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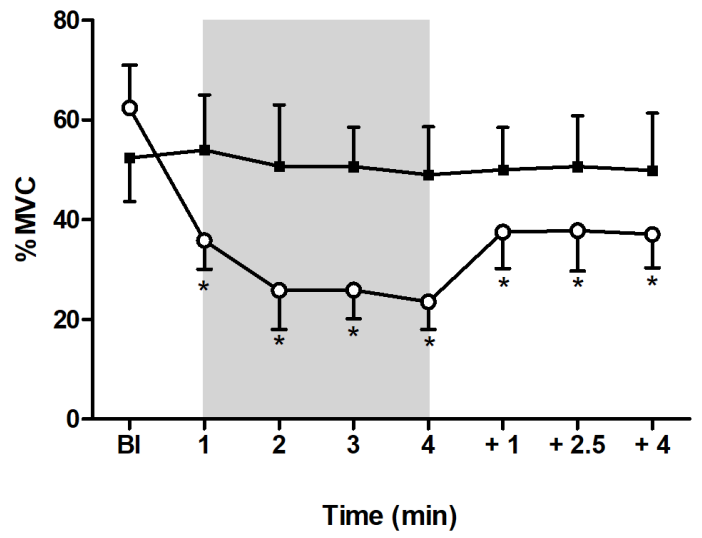


○ Force ■ EMG_{RMS}

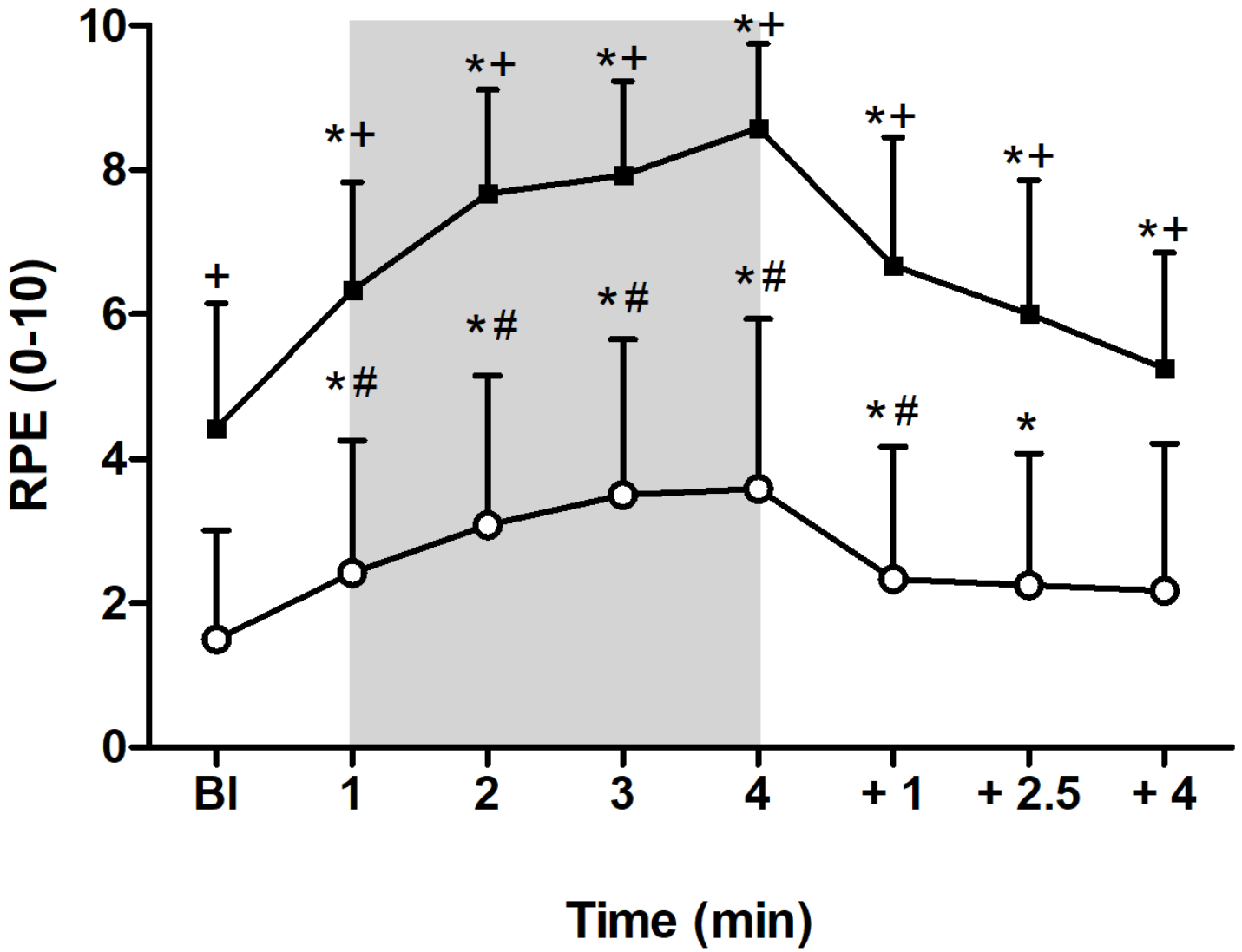
A



B

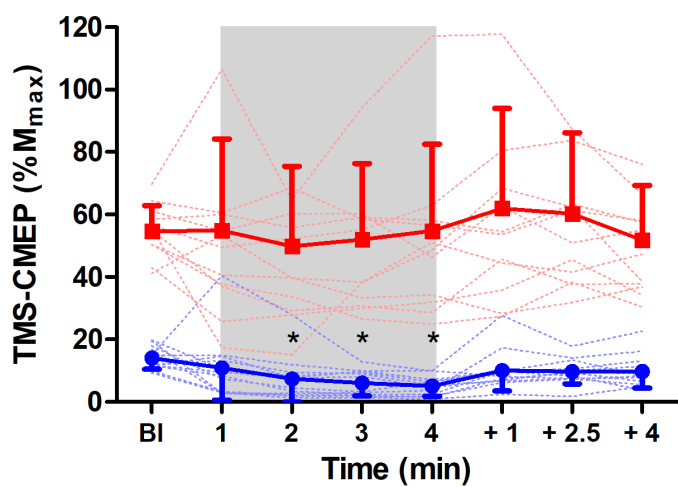


○ Low-intensity ■ High-intensity

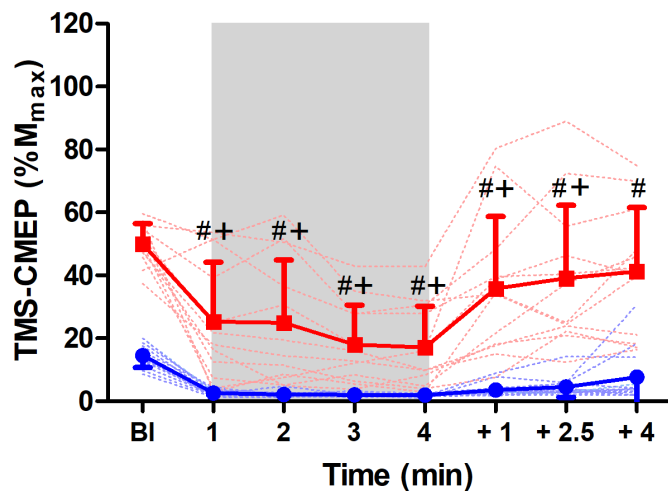


● Small TMS-CMEP ■ Large TMS-CMEP

A

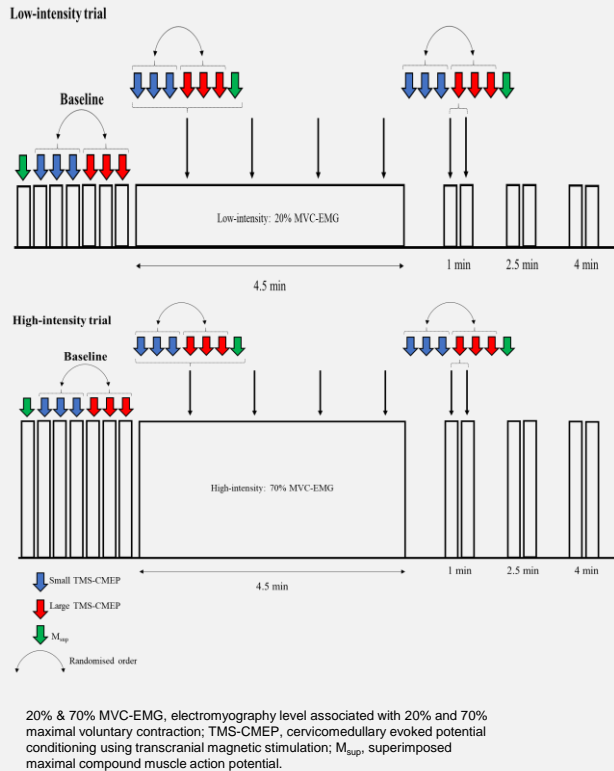


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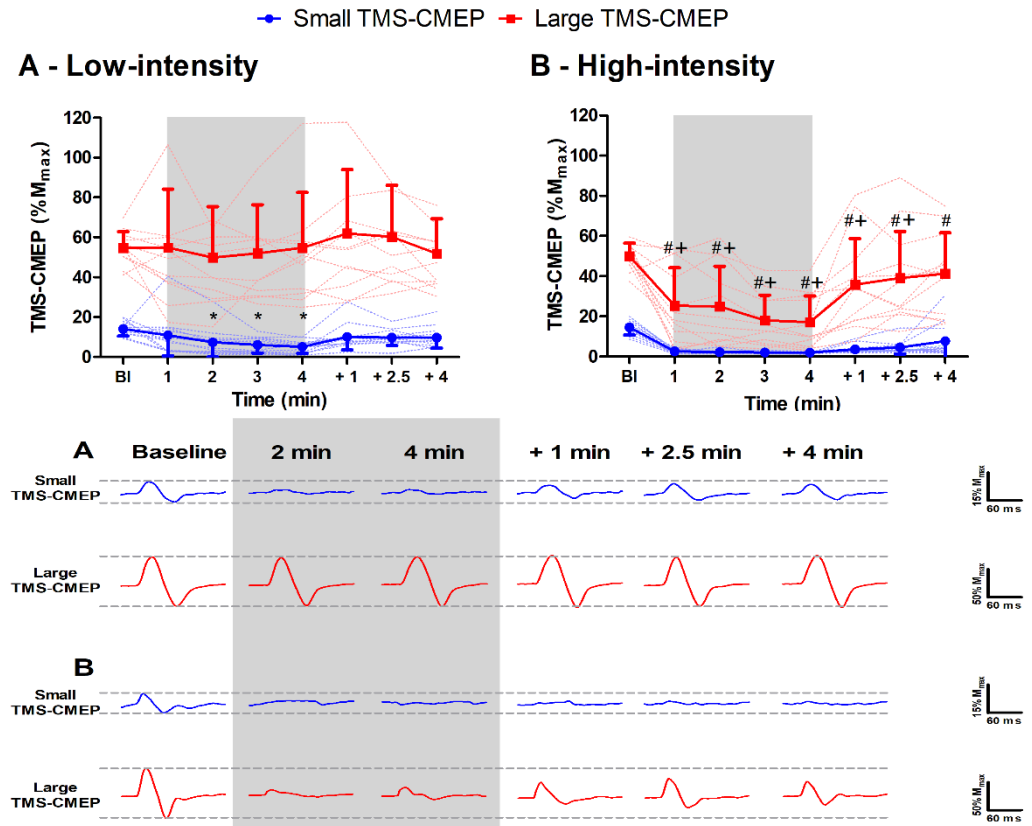


Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity

METHODS



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.