Northumbria Research Link

1 **Reductions in motoneuron excitability during sustained isometric**

2 **contractions are dependent on stimulus and contraction intensity**

3 Callum G Brownstein[1], Loïc Espeit[1], Nicolas Royer[1], Paul Ansdell[2], Jakob Škarabot[3], Robin

4 Souron[4], Thomas Lapole[1], Guillaume Y Millet[1,5]

5 [1]Univ Lyon, UJM-Saint-Etienne, Inter-university Laboratory of Human Movement Science,

6 EA 7424, F-42023, Saint-Etienne, France

7 [2]Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences,

8 Northumbria University, Newcastle upon Tyne, United Kingdom

9 [3]School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire,

10 United Kingdom

11 [4]Laboratory of Impact of Physical Activity on Health (IAPS), UR n°201723207F, University

12 of Toulon, France

13 [5]Institut Universitaire de France (IUF)

14

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

16 **Address for correspondence:**

17 Dr. Callum BROWNSTEIN
18 Laboratoire Interuniversitaire de Biologie de la Motricité
19 Bâtiment IRMIS
20 10 rue de la Marandière
21 42270 Saint Priest en Jarez
22 France
23 04 77 42 18 83
24 Email: callum.brownstein@univ-st-etienne.fr

25

26

27

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.

36

37

38

39

40

41

42

43

44

45

46

47

48

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≤0.049) by up to −10% M_{max}, while large TMS-CMEPs remained unchanged (p≥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≤0.02). TMS-CMEPs were unchanged relative to baseline during recovery following the low-intensity trial (p≥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

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 (~10-20% 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 × 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 ± 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

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

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 90º 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 (× 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 ± 13%; high-intensity trial, 81 ± 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 ~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 ~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   ± 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 × 2 × 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 × 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 × 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 × 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**

The typical error for the small and large TMS-CMEPs were $0.91 \pm 0.48\%$ M_{max} and $2.93 \pm$ 1.12% M_{max}, respectively. At baseline for the low-intensity trial, the small and large TMS-CMEPs were $14.1 \pm 3.7\%$ M_{max} amplitude and $54.6 \pm 8.3\%$ M_{max} amplitude respectively. For the high-intensity trial, small and large TMS-CMEPs were $14.5 \pm 3.9\%$ M_{max} and $50.0 \pm$ 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 $\times$ contraction intensity $\times$ 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 $5.0 \pm 3.1\%$ 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 $1.0 \pm 0.4\%$ M_{max} ($d = 4.6$) and $17.1 \pm 13.1\%$ 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 $\times$ contraction intensity $\times$ time interaction was found ($F_{3,33} = 2.1$, $p = 0.12$, $\eta_p^2 = 0.16$). However, a contraction intensity $\times$ 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 ($p = 0.10$).

387

388

389

390

391

392

393

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.

405

Reductions in motoneuron excitability during sustained isometric contractions are task-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 ~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 ~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 Ca^{2+}-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 (Hyngstrom 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 $5-HT_1$ 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-HT$_1$ 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-HT$_1$ 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.

526

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.

544

545   **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.

560

561

562

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 × 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 × contraction intensity × 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.

608

609

610

611

612

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

623 aspects of the work in ensuring that questions related to the accuracy or integrity of any part

624 of the work are appropriately investigated and resolved. All persons designated as authors

625 qualify for authorship, and all those who qualify for authorship are listed.

630 **Data availability**

631 Data available upon request.

632

633

634

635 **References**

636 Bigland-Ritchie, B.R., Dawson, N.J., Johansson, R.S., Lippold, O.C., 1986. Reflex origin for the slowing
637 of motoneurone firing rates in fatigue of human voluntary contractions. The Journal of physiology
638 379, 451-459.
639 Borg, G., 1990. Psychophysical scaling with applications in physical work and the perception of
640 exertion. Scandinavian journal of work, environment & health 16 Suppl 1, 55-58.
641 Brownstein, C.G., Souron, R., Royer, N., Singh, B., Lapole, T., Millet, G.Y., 2020. Disparate kinetics of
642 change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing
643 isometric knee extension. Journal of applied physiology (Bethesda, Md. : 1985) 128(1), 159-167.
644 Brownstone, R.M., 2006. Beginning at the end: repetitive firing properties in the final common
645 pathway. Progress in neurobiology 78(3-5), 156-172.
646 Brownstone, R.M., Krawitz, S., Jordan, L.M., 2011. Reversal of the late phase of spike frequency
647 adaptation in cat spinal motoneurons during fictive locomotion. J Neurophysiol 105(3), 1045-1050.
648 Butler, J.E., Petersen, N.C., Herbert, R.D., Gandevia, S.C., Taylor, J.L., 2012. Origin of the low-level
649 EMG during the silent period following transcranial magnetic stimulation. Clinical neurophysiology :
650 official journal of the International Federation of Clinical Neurophysiology 123(7), 1409-1414.
651 Butler, J.E., Taylor, J.L., Gandevia, S.C., 2003. Responses of human motoneurons to corticospinal
652 stimulation during maximal voluntary contractions and ischemia. The Journal of neuroscience : the
653 official journal of the Society for Neuroscience 23(32), 10224-10230.
654 Button, D.C., Kalmar, J.M., Gardiner, K., Cahill, F., Gardiner, P.F., 2007. Spike frequency adaptation of
655 rat hindlimb motoneurons. Journal of applied physiology (Bethesda, Md. : 1985) 102(3), 1041-1050.
656 Chen, R., Lozano, A.M., Ashby, P., 1999. Mechanism of the silent period following transcranial
657 magnetic stimulation. Evidence from epidural recordings. Experimental brain research 128(4), 539-
658 542.

659   Cotel, F., Exley, R., Cragg, S.J., Perrier, J.F., 2013. Serotonin spillover onto the axon initial segment of
660   motoneurons induces central fatigue by inhibiting action potential initiation. Proceedings of the
661   National Academy of Sciences of the United States of America 110(12), 4774-4779.
662   D'Amico, J.M., Butler, A.A., Héroux, M.E., Cotel, F., Perrier, J.M., Butler, J.E., Gandevia, S.C., Taylor,
663   J.L., 2017. Human motoneurone excitability is depressed by activation of serotonin 1A receptors
664   with buspirone. The Journal of physiology 595(5), 1763-1773.
665   Farina, D., Holobar, A., Gazzoni, M., Zazula, D., Merletti, R., Enoka, R.M., 2009. Adjustments differ
666   among low-threshold motor units during intermittent, isometric contractions. J Neurophysiol 101(1),
667   350-359.
668   Finn, H.T., Rouffet, D.M., Kennedy, D.S., Green, S., Taylor, J.L., 2018. Motoneuron excitability of the
669   quadriceps decreases during a fatiguing submaximal isometric contraction. Journal of Applied
670   Physiology 124(4), 970-979.
671   Foley, R.C.A., Kalmar, J.M., 2019. Estimates of persistent inward current in human motor neurons
672   during postural sway. J Neurophysiol 122(5), 2095-2110.
673   Gandevia, S.C., 2001. Spinal and supraspinal factors in human muscle fatigue. Physiological reviews
674   81(4), 1725-1789.
675   Gandevia, S.C., Petersen, N., Butler, J.E., Taylor, J.L., 1999. Impaired response of human
676   motoneurones to corticospinal stimulation after voluntary exercise. The Journal of physiology 521 Pt
677   3(Pt 3), 749-759.
678   Gandevia, S.C., Rothwell, J.C., 1987. Knowledge of motor commands and the recruitment of human
679   motoneurons. Brain : a journal of neurology 110 (Pt 5), 1117-1130.
680   Heckman, C.J., Binder, M.D., 1988. Analysis of effective synaptic currents generated by homonymous
681   Ia afferent fibers in motoneurons of the cat. J Neurophysiol 60(6), 1946-1966.
682   Heckman, C.J., Enoka, R.M., 2012. Motor unit. Comprehensive Physiology 2(4), 2629-2682.
683   Heckman, C.J., Johnson, M., Mottram, C., Schuster, J., 2008. Persistent inward currents in spinal
684   motoneurons and their influence on human motoneuron firing patterns. The Neuroscientist : a
685   review journal bringing neurobiology, neurology and psychiatry 14(3), 264-275.
686   Héroux, M.E., Butler, A.A., Gandevia, S.C., Taylor, J.L., Butler, J.E., 2016. Time course of human
687   motoneuron recovery after sustained low-level voluntary activity. Journal of Neurophysiology
688   115(2), 803-812.
689   Hoffman, B.W., Oya, T., Carroll, T.J., Cresswell, A.G., 2009. Increases in corticospinal responsiveness
690   during a sustained submaximal plantar flexion. Journal of applied physiology (Bethesda, Md. : 1985)
691   107(1), 112-120.
692   Hornby, T.G., McDonagh, J.C., Reinking, R.M., Stuart, D.G., 2002. Effects of Excitatory Modulation on
693   Intrinsic Properties of Turtle Motoneurons. Journal of Neurophysiology 88(1), 86-97.
694   Hyngstrom, A.S., Johnson, M.D., Miller, J.F., Heckman, C.J., 2007. Intrinsic electrical properties of
695   spinal motoneurons vary with joint angle. Nature neuroscience 10(3), 363-369.
696   Jacobs, B.L., Fornal, C.A., 1995. Activation of 5-HT neuronal activity during motor behavior. Seminars
697   in Neuroscience 7(6), 401-408.
698   Johnson, K.V., Edwards, S.C., Van Tongeren, C., Bawa, P., 2004. Properties of human motor units
699   after prolonged activity at a constant firing rate. Experimental brain research 154(4), 479-487.
700   Kavanagh, J.J., McFarland, A.J., Taylor, J.L., 2019. Enhanced availability of serotonin increases
701   activation of unfatigued muscle but exacerbates central fatigue during prolonged sustained
702   contractions. The Journal of physiology 597(1), 319-332.
703   Kernell, D., Monster, A.W., 1982. Time course and properties of late adaptation in spinal
704   motoneurones of the cat. Experimental brain research 46(2), 191-196.
705   Kukulka, C.G., Clamann, H.P., 1981. Comparison of the recruitment and discharge properties of
706   motor units in human brachial biceps and adductor pollicis during isometric contractions. Brain
707   research 219(1), 45-55.

708 Lévénez, M., Garland, S.J., Klass, M., Duchateau, J., 2008. Cortical and spinal modulation of
709 antagonist coactivation during a submaximal fatiguing contraction in humans. J Neurophysiol 99(2),
710 554-563.
711 Macefield, G., Hagbarth, K.E., Gorman, R., Gandevia, S.C., Burke, D., 1991. Decline in spindle support
712 to alpha-motoneurones during sustained voluntary contractions. The Journal of physiology 440, 497-
713 512.
714 Martin, P.G., Gandevia, S.C., Taylor, J.L., 2006a. Output of human motoneuron pools to corticospinal
715 inputs during voluntary contractions. J Neurophysiol 95(6), 3512-3518.
716 Martin, P.G., Smith, J.L., Butler, J.E., Gandevia, S.C., Taylor, J.L., 2006b. Fatigue-sensitive afferents
717 inhibit extensor but not flexor motoneurons in humans. The Journal of neuroscience : the official
718 journal of the Society for Neuroscience 26(18), 4796-4802.
719 Martin, P.G., Weerakkody, N., Gandevia, S.C., Taylor, J.L., 2008. Group III and IV muscle afferents
720 differentially affect the motor cortex and motoneurones in humans. The Journal of physiology
721 586(5), 1277-1289.
722 McNeil, C.J., Giesebrecht, S., Gandevia, S.C., Taylor, J.L., 2011a. Behaviour of the motoneurone pool
723 in a fatiguing submaximal contraction. The Journal of physiology 589(Pt 14), 3533-3544.
724 McNeil, C.J., Giesebrecht, S., Khan, S.I., Gandevia, S.C., Taylor, J.L., 2011b. The reduction in human
725 motoneurone responsiveness during muscle fatigue is not prevented by increased muscle spindle
726 discharge. The Journal of physiology 589(Pt 15), 3731-3738.
727 McNeil, C.J., Martin, P.G., Gandevia, S.C., Taylor, J.L., 2009. The response to paired motor cortical
728 stimuli is abolished at a spinal level during human muscle fatigue. The Journal of physiology 587(Pt
729 23), 5601-5612.
730 Nielsen, J., Kagamihara, Y., 1992. The regulation of disynaptic reciprocal Ia inhibition during co-
731 contraction of antagonistic muscles in man. The Journal of physiology 456, 373-391.
732 Nordstrom, M.A., Gorman, R.B., Laouris, Y., Spielmann, J.M., Stuart, D.G., 2007. Does motoneuron
733 adaptation contribute to muscle fatigue? Muscle & nerve 35(2), 135-158.
734 Perrier, J.-F., Rasmussen, H.B., Jørgensen, L.K., Berg, R.W., 2018. Intense Activity of the Raphe Spinal
735 Pathway Depresses Motor Activity via a Serotonin Dependent Mechanism. Frontiers in Neural
736 Circuits 11(111).
737 Petersen, N.T., Taylor, J.L., Gandevia, S.C., 2002. The effect of electrical stimulation of the
738 corticospinal tract on motor units of the human biceps brachii. The Journal of physiology 544(Pt 1),
739 277-284.
740 Powers, R.K., Sawczuk, A., Musick, J.R., Binder, M.D., 1999. Multiple mechanisms of spike-frequency
741 adaptation in motoneurones. Journal of physiology, Paris 93(1-2), 101-114.
742 Roll, J.P., Vedel, J.P., Ribot, E., 1989. Alteration of proprioceptive messages induced by tendon
743 vibration in man: a microneurographic study. Experimental brain research 76(1), 213-222.
744 Sherrington, C., 1952. The integrative action of the nervous system. CUP Archive.
745 Sidhu, S.K., Weavil, J.C., Thurston, T.S., Rosenberger, D., Jessop, J.E., Wang, E., Richardson, R.S.,
746 McNeil, C.J., Amann, M., 2018. Fatigue-related group III/IV muscle afferent feedback facilitates
747 intracortical inhibition during locomotor exercise. The Journal of physiology 596(19), 4789-4801.
748 Škarabot, J., Mesquita, R.N.O., Brownstein, C.G., Ansdell, P., 2019. Myths and Methodologies: How
749 loud is the story told by the transcranial magnetic stimulation-evoked silent period? Experimental
750 physiology 104(5), 635-642.
751 Spielmann, J.M., Laouris, Y., Nordstrom, M.A., Robinson, G.A., Reinking, R.M., Stuart, D.G., 1993.
752 Adaptation of cat motoneurons to sustained and intermittent extracellular activation. The Journal of
753 physiology 464, 75-120.
754 Taylor, J.L., 2006. Stimulation at the cervicomedullary junction in human subjects. Journal of
755 electromyography and kinesiology : official journal of the International Society of
756 Electrophysiological Kinesiology 16(3), 215-223.

757   Taylor, J.L., Amann, M., Duchateau, J., Meeusen, R., Rice, C.L., 2016. Neural Contributions to Muscle
758   Fatigue: From the Brain to the Muscle and Back Again. Medicine and science in sports and exercise
759   48(11), 2294-2306.
760   Taylor, J.L., Gandevia, S.C., 2004. Noninvasive stimulation of the human corticospinal tract. Journal
761   of Applied Physiology 96(4), 1496-1503.
762   Temesi, J., Vernillo, G., Martin, M., Krüger, R.L., McNeil, C.J., Millet, G.Y., 2019. Sustained Maximal
763   Voluntary Contractions Elicit Different Neurophysiological Responses in Upper- and Lower-Limb
764   Muscles in Men. Neuroscience 422, 88-98.
765   Thorstensen, J.R., Taylor, J.L., Tucker, M.G., Kavanagh, J.J., 2020. Enhanced serotonin availability
766   amplifies fatigue perception and modulates the TMS-induced silent period during sustained low-
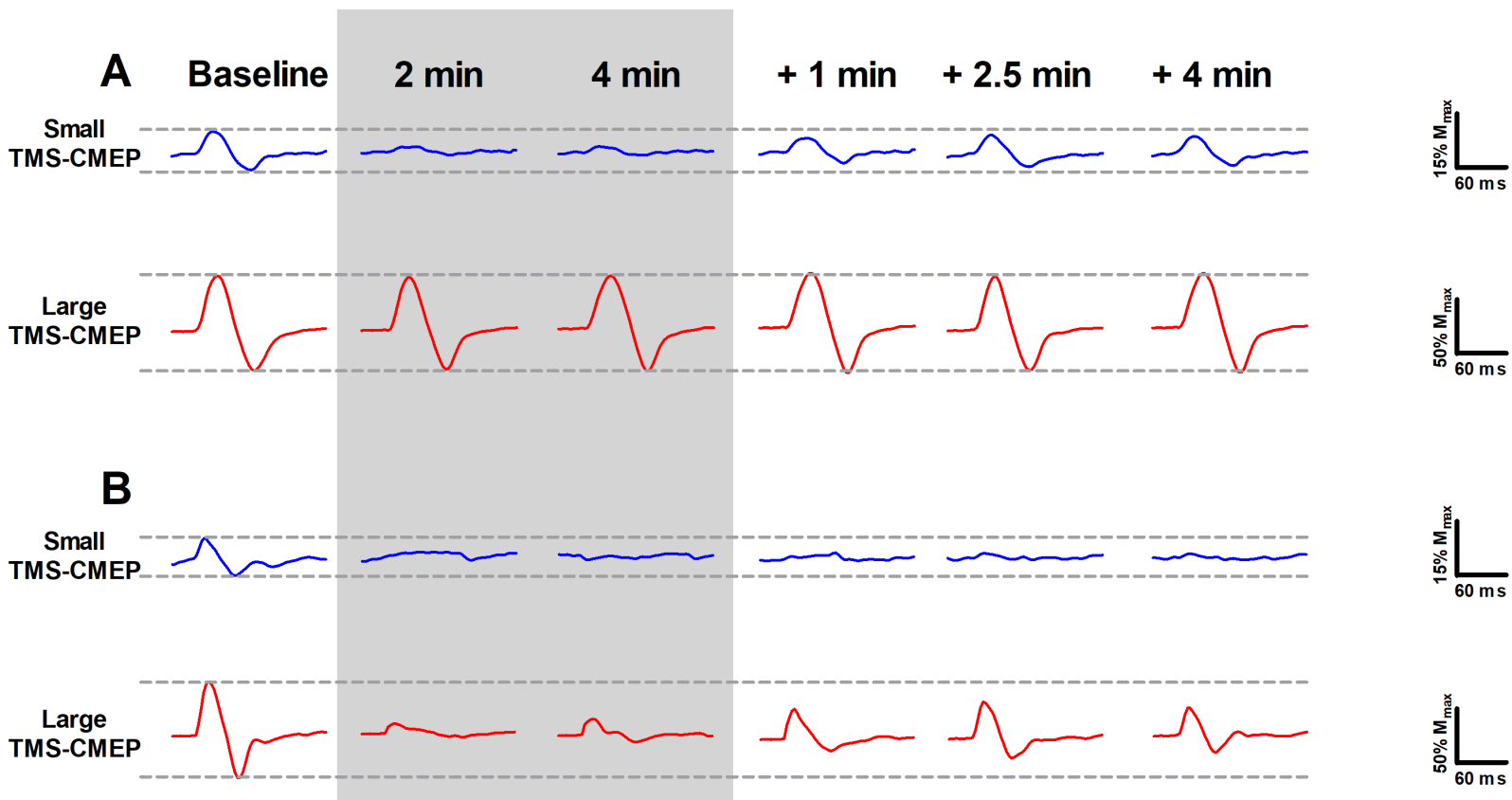767   intensity elbow flexions. The Journal of physiology 598(13), 2685-2701.
768   Weavil, J.C., Amann, M., 2018. Corticospinal excitability during fatiguing whole body exercise.
769   Progress in brain research 240, 219-246.
770   Zytnicki, D., Lafleur, J., Horcholle-Bossavit, G., Lamy, F., Jami, L., 1990. Reduction of Ib autogenetic
771   inhibition in motoneurons during contractions of an ankle extensor muscle in the cat. J Neurophysiol
772   64(5), 1380-1389.
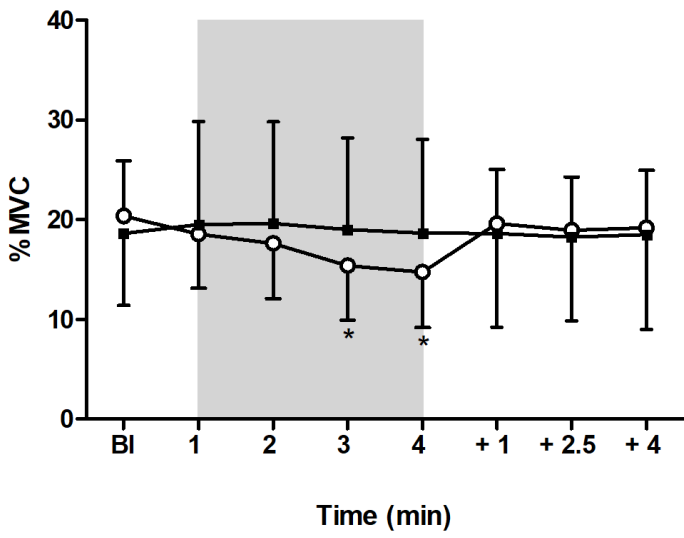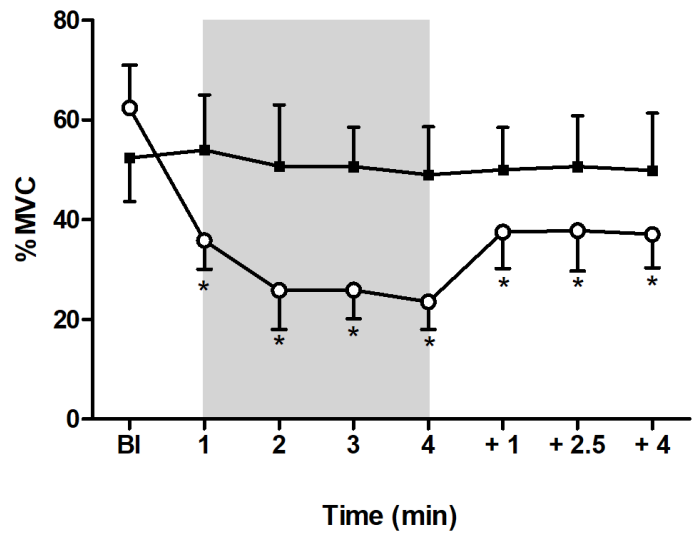
773

774
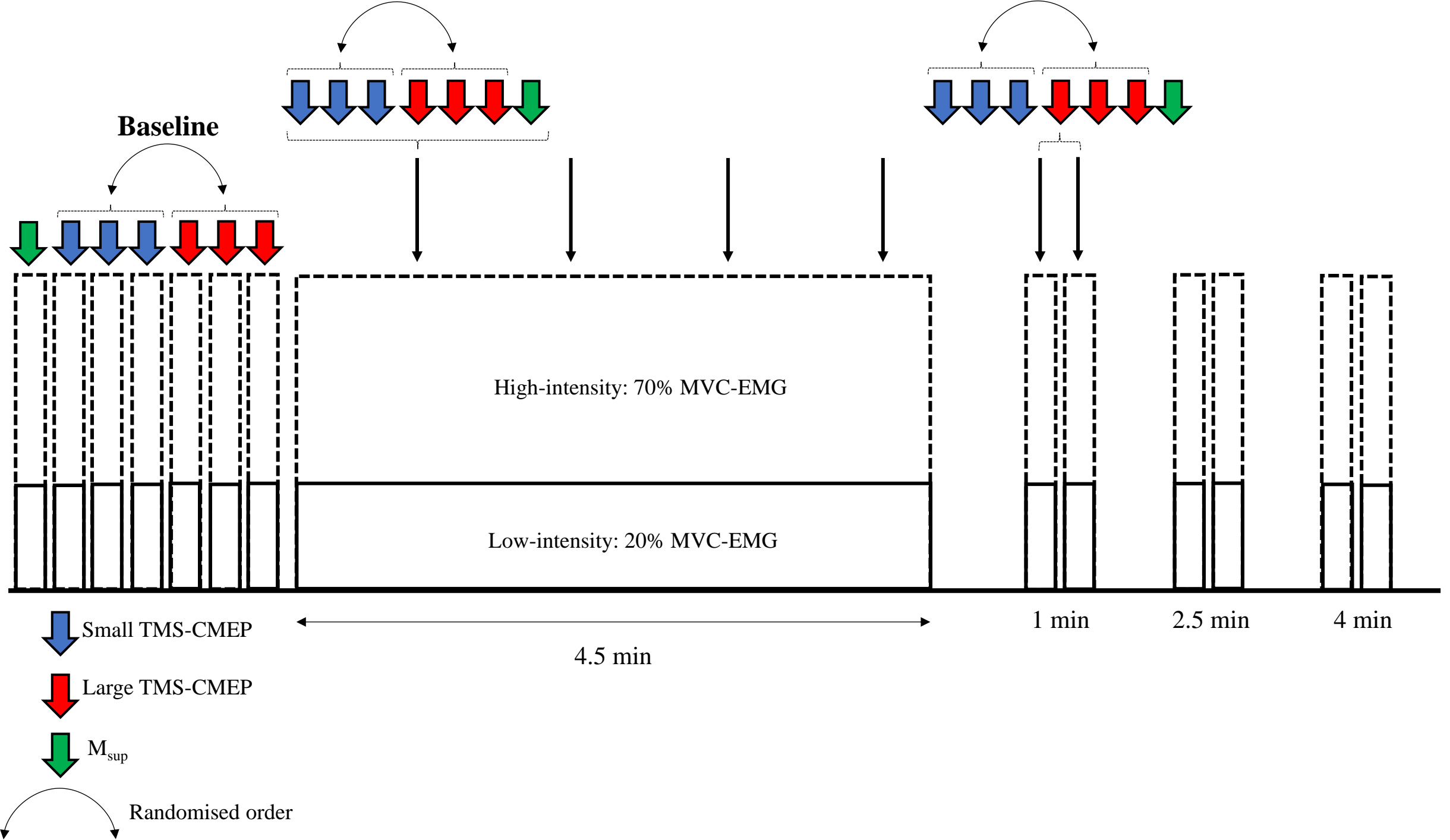
Baseline

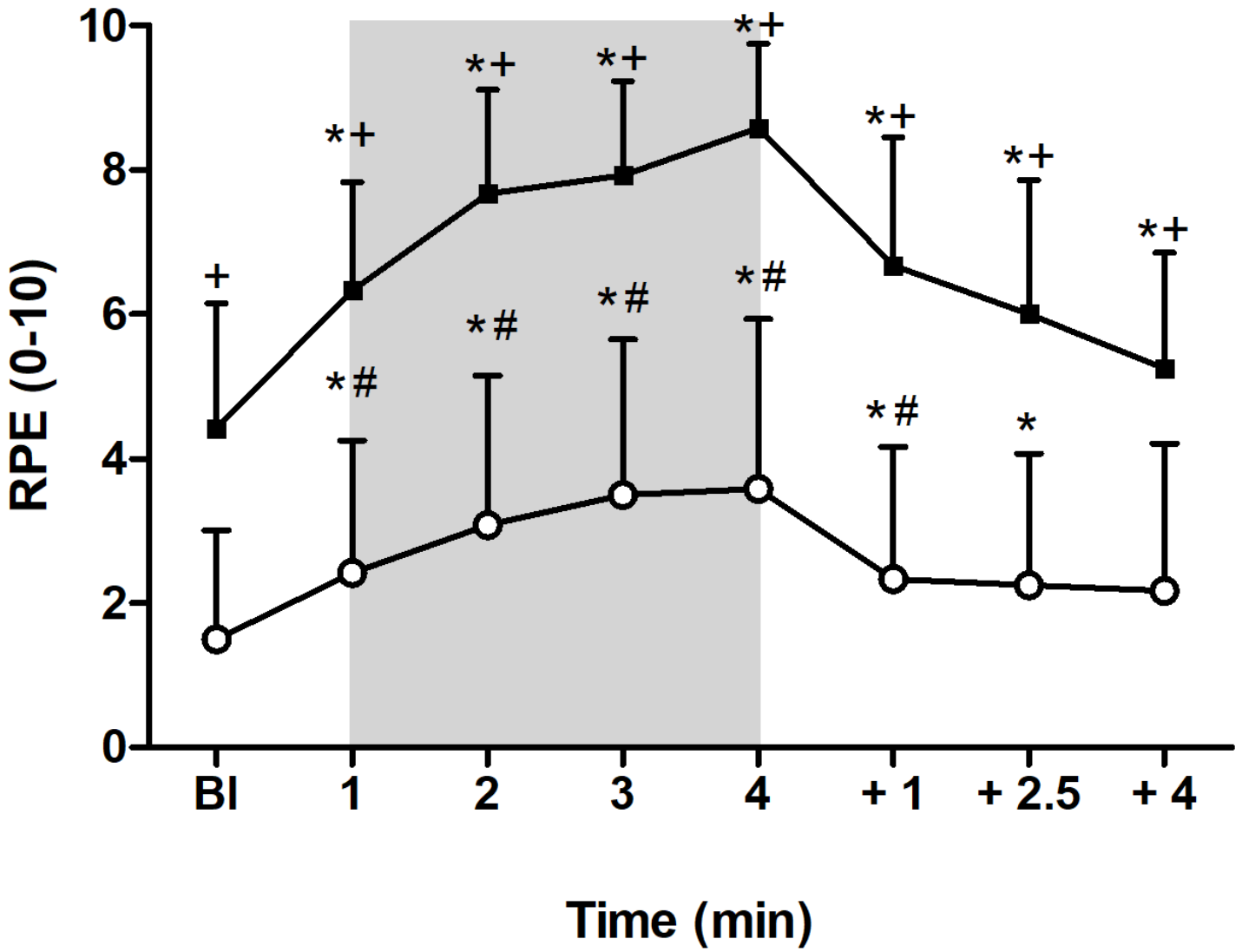High-intensity: 70% MVC-EMG

Low-intensity: 20% MVC-EMG

4.5 min

1 min   2.5 min   4 min

↓ Small TMS-CMEP

↓ Large TMS-CMEP

↓ M$_{sup}$

Randomised order

A

| | Baseline | 2 min | 4 min | + 1 min | + 2.5 min | + 4 min |

Small TMS-CMEP

Large TMS-CMEP

15% M$_{max}$
60 ms

50% M$_{max}$
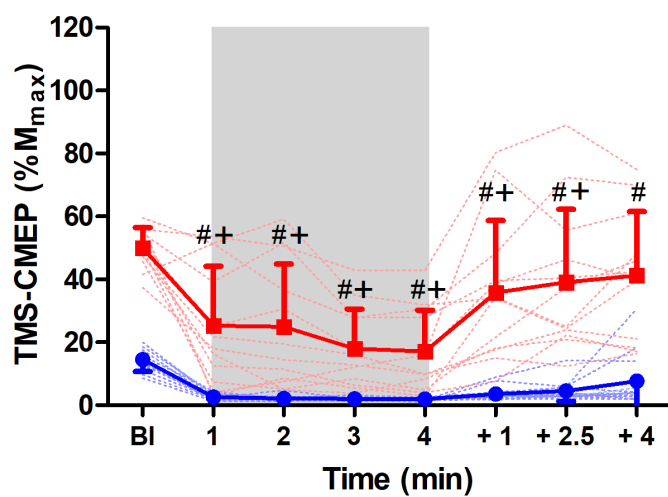60 ms

B

Small TMS-CMEP

Large TMS-CMEP

15% M$_{max}$
60 ms

50% M$_{max}$
60 ms
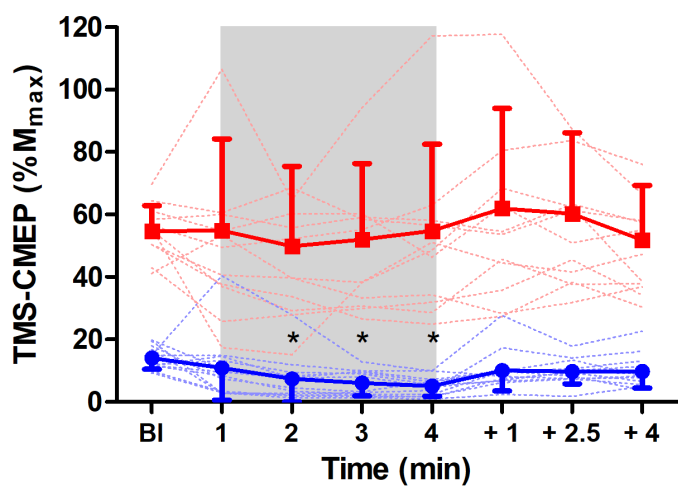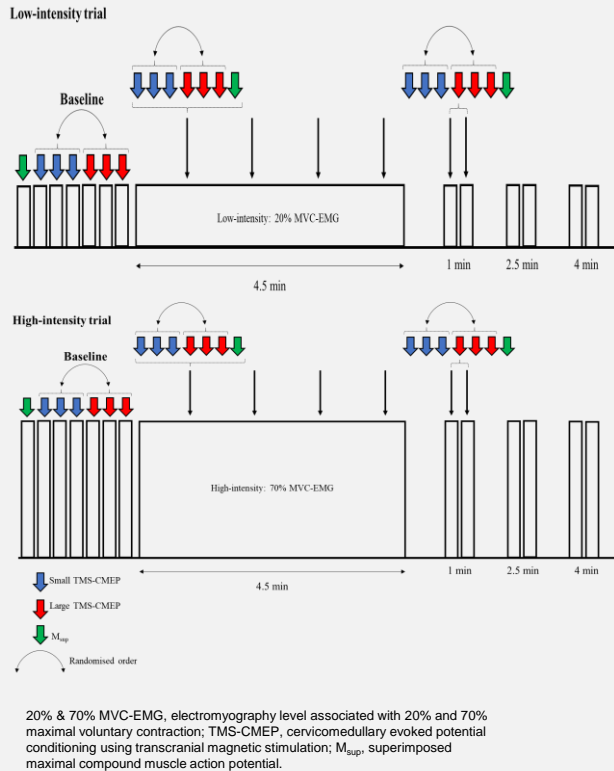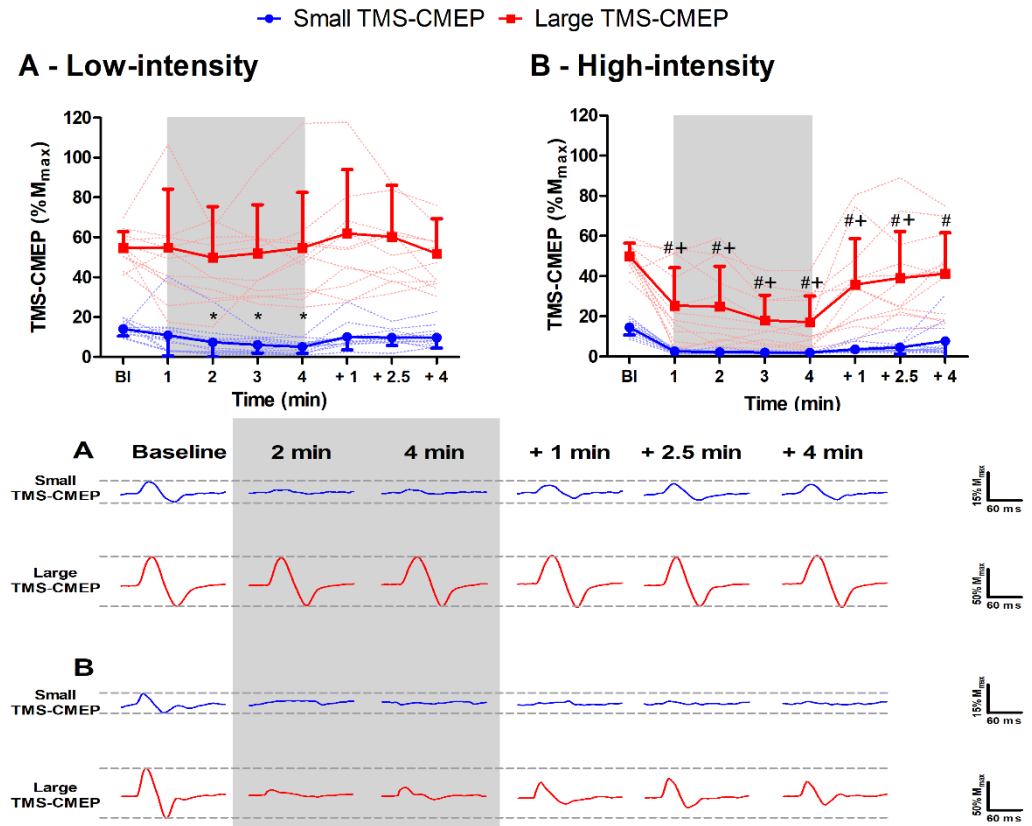
Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity

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.