# *Motoneuron excitability of the quadriceps decreases during a fatiguing submaximal isometric contraction*

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**Running title:** Quadriceps motoneuron excitability during fatigue

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**ABSTRACT:**

During fatiguing voluntary contractions, the excitability of motoneurons innervating arm muscles decreases. However, the behavior of motoneurons innervating quadriceps muscles is unclear. Findings may be inconsistent because descending cortical input influences motoneuron excitability and confounds measures during exercise. To overcome this limitation, we examined effects of fatigue on quadriceps motoneuron excitability tested during brief pauses in descending cortical drive after transcranial magnetic stimulation (TMS). Participants (n=14) performed brief (~5 s) isometric knee extension contractions before and after a 10-min sustained contraction at ~25% maximal EMG of vastus medialis (VM) on one (n=5) or two days (n=9). Electrical stimulation over thoracic spine elicited thoracic motor evoked potentials (TMEP) in quadriceps muscles during ongoing voluntary drive and 100ms into the silent period following TMS (TMS-TMEP). Femoral nerve stimulation elicited maximal M-waves (Mmax). On the two days, either large (~50% Mmax) or small (~15% Mmax) TMS-TMEPs were elicited. During the 10-min contraction, VM EMG was maintained (P=0.39) whereas force decreased by 52% (SD 13%) (P<0.001). TMEP area remained unchanged (P=0.9), whereas large TMS-TMEPs decreased by 49% (SD 28%) (P=0.001) and small TMS-TMEPs by 71% (SD 22%) (P<0.001). This decline was greater for small TMS-TMEPs (P=0.019; n=9). Therefore, without the influence of descending drive, quadriceps TMS-TMEPs decreased during fatigue. The greater reduction for smaller responses, which tested motoneurons that were most active during the contraction suggests a mechanism related to repetitive activity contributes to reduced quadriceps motoneuron excitability during fatigue. By contrast, the unchanged TMEP suggests that ongoing drive compensates for altered motoneuron excitability.

**NEW & NOTEWORTHY:**

We provide evidence that the excitability of quadriceps motoneurons decreases with fatigue. Our results suggest that altered intrinsic properties brought about by repetitive activation of the motoneurons underlie their decreased excitability. Furthermore, we note that testing during

voluntary contraction may not reflect the underlying depression of motoneuron excitability due to changes in ongoing voluntary drive. Thus, this study provides evidence that processes intrinsic to the motoneuron contribute to muscle fatigue of the knee extensors.

**Keywords:** motoneuron, fatigue, quadriceps, EMG, TMS

## INTRODUCTION:

Motoneurons are the final common pathway of descending motor commands (32) and directly innervate muscle fibers. During fatiguing exercise, part of the reduction in maximal force can be attributed to processes within the central nervous system that result in a reduced firing of motoneurons (11). The likelihood that motoneurons will fire in response to a given input is not only dependent on the intrinsic properties of the motoneurons, but also the sum of the multiple inputs received by the motoneurons (7, 17) all of which may be altered during fatiguing exercise (8, 21, 24).

One method to assess the excitability of motoneurons is to stimulate the descending spinal tracts below the motor cortex at either the cervicomedullary junction or over the upper thoracic spine. These stimuli provide descending synaptic input to the motoneurons that can be adjusted by altering stimulation intensity. The number of motoneurons that fire in response to this synaptic input is reflected by the sum of action potentials measured at the muscle level. These responses are commonly referred to as cervicomedullary motor evoked potentials (CMEP) or thoracic motor evoked potentials, (TMEP) (25, 36). A reduction in size of the CMEP or TMEP during fatigue suggests that the motoneuron pool has become less responsive to descending input, but many factors contribute to this reduction (8, 27, 28). One likely factor is change in the intrinsic properties of the motoneurons related to repetitive activation (4, 15, 19, 22, 27, 35). For example, when motoneurons fire repetitively in response to current injection, their firing rates initially decline quickly and then

continue to decline gradually over minutes in a process known as late spike frequency adaptation (22, 35).

For the motoneurons of the quadriceps muscles, the effect of fatigue is not clear as increases (34) and no change (21, 33, 37) in motoneuron excitability have all been reported. In accounting for the heterogeneous results, it is important to note that different exercise modalities (single limb isometric, dynamic, and whole-body exercise) were used in these studies. In addition, these investigations all assessed the motoneurons during contractions when the motoneurons were firing in response to different levels of ongoing excitatory voluntary descending drive (21, 33, 34, 37). While this is often necessary to achieve evoked responses from stimulation, it introduces a confounding effect as changes in voluntary descending drive will influence the measure of motoneuron excitability. This can be seen in an unfatigued state, where the size of the evoked responses first increases and then decreases as the strength of voluntary contraction increases (25, 38). Therefore, measuring motoneuron excitability with changing levels of descending drive, as would occur during fatiguing contractions, means that the evoked response will likely reflect both changes at the motoneurons and changes in voluntary descending drive, and it will be difficult to discriminate the contributions of each.

An experimental technique that reduces the confounding effect of ongoing descending drive on measures of motoneuron excitability is to evoke CMEPs or TMEPs during the brief pause in voluntary descending drive that follows a single transcranial magnetic stimulation (TMS) pulse to the motor cortex during a voluntary contraction. TMS during voluntary contraction causes a short-latency excitatory response which is followed by a brief silent period (~200 ms duration) in the ongoing electromyogram (EMG) activity. During the silent period, inhibition at a cortical level suppresses voluntary cortical output to the motoneurons (9). Hence, with stimulation of the descending tract during this silent period, the resultant response reflects the excitability of motoneurons when they

are not acted upon by descending drive and not actively firing. When this technique was used in the upper arm during both a sustained maximal contraction (28), and a prolonged submaximal contraction (27), the size of the biceps brachii CMEP evoked after TMS was profoundly reduced compared to a CMEP without preceding TMS. Thus, reductions in biceps motoneuron excitability during fatigue were revealed by pausing ongoing descending drive which otherwise may compensate for these reductions. Moreover, smaller CMEPs were reduced more than larger CMEPs (27). Because smaller CMEPs reflected responses from motoneurons that were mostly active in the submaximal contraction whereas the larger CMEP reflected responses from those same active motoneurons plus additional non-active motoneurons, it was concluded that excitability is specifically reduced in the motoneurons of the biceps brachii that are repetitively activated during a fatiguing contraction of submaximal intensity.

Here we aimed to better understand the changes that occur during fatiguing exercise of the quadriceps by assessing quadriceps motoneurons in the absence of voluntary descending drive. Testing was carried out with TMEPs delivered in the silent period following TMS (TMS-TMEP). We hypothesised that during fatigue the quadriceps motoneurons would become profoundly less responsive as indicated by a reduction in the size of the TMS-TMEP. Excitability was also assessed with ongoing drive (TMEP) and we expected that the TMEP would remained unchanged as successful performance of the fatiguing task required excitatory voluntary drive acting on the motoneurons to maintain motoneuron firing. In addition, we used a submaximal task with a constant level of EMG and two different sizes of TMS-TMEPs, small and large, to test the hypothesis that active motoneurons would have a greater reduction in excitability than non-active motoneurons. We expected that during our task, the small TMS-TMEP would be made up of a greater proportion of motoneurons that were active during the task and therefore show greater reductions in size.

## MATERIALS AND METHODS:

### Participants

Seventeen healthy participants were recruited for the study. Three participants were not tested either because responses could not be elicited (n = 2) or due to stimulation discomfort (n = 1). The experiment was completed by fourteen participants (5 female) with an average age of 22.5 (4.8) years (mean and standard deviation). Of those tested, the required baseline response to test smaller and larger portions of the motoneuron pool was achieved in 9 participants (4 females), who were then tested on two separate days, one with large responses and another with small responses chosen in a block randomised order. The other 5 participants were tested on one day only using stimulation intensities to elicit small responses. All studies were approved by Human Research Ethics Committee at the University of New South Wales and conformed to the Declaration of Helsinki (2008). Written consent was obtained from each of the participants.

### Experimental setup

Participants were seated in a custom-built chair with hips at 70 degrees (0 is extended neutral position) and left knee at 70 degrees (knee fully extended is 0 degrees). The left ankle was secured to a force transducer by a Velcro strap and an adjustable strap was placed over the hip and was tightened to secure the participant before contractions. Knee extension force was measured with a linear strain gauge (linear to 1 kN; XTran, Melbourne, Australia). Electromyograms (EMG) of the vastus medialis (VM), vastus lateralis (VL), and the rectus femoris (RF) were recorded via adhesive Ag-AgCl electrodes (20 mm diameter Conmed ClearTrace ECG Sensor Electrodes Utica, NY) arranged in a bipolar fashion. The VM electrodes were positioned two centimetres and seven centimetres proximal to the superior medial border of the patella on the muscle following the orientation of the muscle fibers. The proximal VL and RF electrodes were placed two thirds of the distance from the anterior superior iliac spine to the lateral and superior borders of the patella, respectively, with the second electrodes placed 5 centimetres distal. Placement was confirmed with palpation during a

158 brief knee extension contraction. A 70 mm by 40 mm (3M Universal Electrosurgical Pad, AUS) ground
159 electrode was placed across the upper thigh between the recording electrodes and femoral nerve
160 stimulating electrodes. In all experiments, force and EMG signals were recorded to computer using a
161 16-bit A/D converter (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction
162 with Spike2 software (v. 7.12 Cambridge Electronic Design). EMG signals were amplified (x100) and
163 bandpass filtered (16 - 1000 Hz) using CED 1902 amplifiers (Cambridge Electronic Design) and force
164 and EMG signals were sampled at 1000 and 2000 Hz, respectively. During the experiment, visual
165 feedback of vastus medialis EMG activity was provided to the participant via an external monitor.
166 The EMG signal was root mean square (rms) processed in real time using a 40 ms time constant. The
167 vastus medialis was the main muscle of interest, and stimulation intensity and EMG feedback for the
168 task were set for this muscle.
169
170 *Femoral nerve stimulation.* A constant current stimulator (DS7AH, Digitmer, Welwyn Garden City,
171 UK) was used to deliver single electrical stimuli (500 μs pulse width) to the femoral nerve to record
172 the maximal compound muscle action potential (Mmax) of the three muscles. The anode was a 70
173 mm by 40 mm electrode (3M Universal Electrosurgical Pad, Australia) placed over the gluteus
174 minimus with the top edge along the iliac crest on the left side of the body. The cathode was a
175 custom made circular probe (20 mm diameter) which was placed over the femoral nerve along the
176 inguinal ligament and secured with a Velcro strap. Optimal cathode placement was established by
177 moving the probe along the inguinal ligament and stimulating (30 mA) at each site. The intensity of
178 the stimulation was then progressively increased (10 mA steps) until there was no increase in the
179 peak-to-peak amplitude of the M-wave in all three muscles. Stimulus intensity was then set at 150%
180 of the current required to produce Mmax (60 - 250 mA).
181
182 *Transcranial magnetic stimulation.* Stimulation of the motor cortex was delivered close to the vertex
183 using a double cone coil attached to a BiStim unit with two Magstim 200 stimulators (Magstim,

Dyffed, UK) discharging simultaneously. Optimal TMS location was established by stimulating at positions close to the vertex for the location that produced the largest motor evoked potentials (MEP) in all three muscles at rest. This position, which was typically 1-2 cm to the right of the vertex, was marked on the head and used throughout the experiment. TMS intensity was then adjusted to produce a 200 ms silent period during a brief contraction at the level of VM EMG required to produce 25% maximal force (50 - 80% of stimulator output).

*Thoracic stimulation.* A constant voltage stimulator (D180, Digitimer) was used to stimulate the descending corticospinal tracts to elicit a thoracic motor evoked potential (TMEP) in the three muscles. The anode was placed over the spinous processes between T1 - T2 and the cathode was placed between T5 - T6 using 30 x 25 mm electrodes (3M Universal Electrosurgical Pad). TMS was paired with thoracic stimulation to elicit a TMEP in the silent period (TMS-TMEP). The thoracic stimulation (100 μs duration) was triggered 100 ms after TMS during contraction at the level of EMG required for a force of 25% maximum. During such contractions, thoracic stimulation intensity was set to evoke TMS-TMEPs in VM of either 15% of Mmax area on the small day, or 50% of Mmax area on the large day. This same intensity was used to elicit TMEPs, which were not preceded by TMS.

**Experimental procedures**

The procedures for the two days of the experiment were identical apart from the size of the evoked TMS-TMEP in the VM, either small or large. The experiment began with a maximal voluntary contraction (MVC) to determine maximal force. The participant then used visual feedback displayed on a monitor to perform a 5-s contraction at 25% maximal force. The average VM rmsEMG during this 25% force contraction was then calculated. This level of rmsEMG activity was used as the new target displayed on the monitor. Participants used the real-time visual feedback of the rmsEMG activity for the fatiguing task and all baseline and recovery measures. Once stimulus intensities were established, participants then performed 5 baseline sets of 2 or 3 contractions that included the

assessment of TMS-TMEPs, TMEPs, and then M-waves (only on the first and last set) during separate brief contractions (Figure 1).

The fatigue task required the participants to sustain a 25% EMG contraction for 10 min. From 5 s into the contraction and then every minute after, TMS-TMEP, TMEP, and Mmax were elicited with 5 s between stimuli. At every minute (prior to stimulation) the participants were asked to verbally report their rating of perceived effort (RPE) on a scale from 0 - 10. After the cessation of the sustained task, recovery measures were performed in identical style to baseline measures. These were performed every min starting at 30 s and then every 2 min from 3:30 for 10 min (see Figure 1).

**Data analysis and statistics**

During off-line analysis both Spike2 (v. 7.12) and Signal software (v. 4.06) were used to determine all measures. Mean force and rmsEMG activity for each contraction were calculated over a 1-s period finishing 50 ms before stimulation was delivered. MVC force was calculated as the maximal force of the initial brief contractions. The amplitude and areas of Mmax, TMEP, and TMS-TMEP were measured between cursors placed on the initial deflection from baseline to the second crossing of the horizontal axis (26, 27) but only area was included in the statistical analysis. To account for any changes in the muscle action potential, the TMEPs and TMS-TMEPs were normalised to the nearest recorded Mmax during the protocol. Two sets of statistical analyses were performed.

First, all participants that completed the experiment with small TMS-TMEPs evoked at baseline (n = 14) were analysed together using one-way repeated measures ANOVAs for changes in force, VM rmsEMG, RPE, TMS-TMEP area/Mmax, and TMEP area/Mmax from baseline to the end of the 10-min contraction (GraphPad Prism v. 7.02). Another one-way ANOVA was completed for the same measures but for an effect of time during the recovery period compared to baseline with Greenhouse-Geisser correction. When a main effect was observed, post-hoc testing to determine

time points different from baseline included using paired t test results which were then compared to a Dunnett's table to control for multiple comparisons.

Second, participants that completed two days of the experiment (n = 9) were analysed and days compared. Student's *t* tests were used to compare baseline MVC force, rmsEMG, Mmax, TMS-TMEP, and TMEP between days. Two-way repeated measures ANOVAs with time and day as factors were used to compare rmsEMG, force, RPE, Mmax area, TMS-TMEP area/Mmax, TMEP area /Mmax, TMS-TMEP area/Mmax (% baseline) and TMEP area/Mmax (% baseline) during the 10-min sustained contraction and then again in recovery (GraphPad Prism v. 7.02). When a main effect of day was seen, post-hoc *t* tests with Bonferroni corrections were used to determine differences between days for each time point. In addition, when an effect of day occurred, one-way repeated measures ANOVA was used to assess the effect of time for each day. To determine time points different from baseline, paired *t* test results were compared with a Dunnett's table to control for multiple comparisons. All data in text and in figures are reported as mean (SD). The significance level was set to $P < 0.05$.

**RESULTS:**

In the course of a 10-min sustained submaximal contraction, during which rmsEMG was maintained at a set level corresponding to 25% initial maximal force, perceived effort increased progressively, and force declined. The size of the vastus medialis (VM) TMS-TMEP decreased greatly during the sustained contraction, whereas the size of the TMEP did not change. Similar changes were seen in both the vastus lateralis (VL) and the rectus femoris (RF). In addition, small TMS-TMEPs were more affected than large TMS-TMEPs.

**Small TMS-TMEPs and TMEPs**

During the brief baseline contractions, the average VM rmsEMG was 20.9% (SD 7.1) of the maximal rmsEMG, and the force produced was 27% (SD 3.7) of MVC with the average MVC being 487 N

(SD 164). One-way ANOVA comparing VM rmsEMG in baseline contractions and during the sustained submaximal contraction showed no significant effect of time ($F_{5.2,68.8} = 2.09$, $P = 0.073$) (Figure 2A). VM rmsEMG during recovery contractions was initially higher than baseline, before returning to similar values to baseline ($F_{4.4,58.4} = 2.81$, $P = 0.029$). By contrast, force decreased over the course of the submaximal contraction by 60.1% (SD 19.1) ($F_{2.7,35.2} = 41.71$, $P < 0.001$), and remained lower during recovery contractions compared to baseline ($F_{4.2,55.3} = 11.03$, $P < 0.001$). Rating of perceived effort (RPE) increased during the sustained contraction from 2.2 (SD 1.6) to 7.3 (SD 1.7) on a scale of 0 - 10 ($F_{2.7,35.2} = 67$, $P < 0.001$) (Figure 2A). In recovery, RPE decreased ($F_{2.5,32.7} = 4.94$, $P = 0.009$) and from 1.5 min post contraction, ratings were similar to the reported values at the start of the sustained contraction.

During the sustained contraction, there was a decline in VM TMS-TMEP area expressed as a percentage of Mmax ($F_{2.2,28.1} = 17.31$, $P < 0.001$). Area was reduced from 13.4% Mmax (SD 4.6) at baseline to 4.3% Mmax (SD 5.2) by the end of the fatiguing contraction (Figure 2B). There was a main effect of time during recovery ($F_{2.8,36.5} = 3.65$, $P = 0.023$) with TMS-TMEPs increasing in size towards baseline values. The area of the VM TMEP did not change during the protocol with no effect of time during the sustained contraction ($F_{4.8,62.6} = 1.05$, $P = 0.391$) nor in recovery ($F_{4.3,56.1} = 0.13$, $P = 0.977$).

**Comparison between Large and Small TMS-TMEPs and TMEPs**

Nine of the fourteen participants completed the protocol on two days with the only difference being the size of the baseline VM TMS-TMEP area. Thoracic stimulation intensity was set to elicit a small (~15% of Mmax) or large (~50% of Mmax) TMS-TMEP with the actual means corresponding to 13.8% (SD 4.2) and 39.1% (SD 9.4) of Mmax area respectively ($P < 0.001$) (Table 1). MVC force ($P = 0.562$), normalised VM rmsEMG ($P = 0.079$) and normalised force during baseline contractions ($P = 0.987$) were not different between days. Group means were 442 N (SD 158), 20.9% maximal EMG (SD 6.7)

287 and 26.2% MVC (SD 3.9) respectively. The amplitude and areas of Mmax, TMS-TMEPs, and TMEPs for
288 VM, VL, and RF are reported in Table 1 for participants who completed both days.
289
290 ***TMEP and TMS-TMEP.*** For VM, both the large and small TMS-TMEPs decreased during the sustained
291 contraction (Figures 3A, 4A & C), whereas the large or small TMEPs remained unchanged (Figures 3B,
292 4B & D). Repeated measures ANOVA showed that TMS-TMEPs in VM displayed an effect of time
293 ($F_{11,88} = 15.16$, $P < 0.001$), day ($F_{1,8} = 8.21$, $P = 0.021$) and an interaction ($F_{11,88} = 2.42$, $P = 0.011$) with
294 the large responses decreasing relatively less than the smaller responses (Figure 4C). Large
295 TMS-TMEPs decreased by ~49% from baseline whereas small TMS-TMEPs decreased by ~71%.
296 In recovery, there was an effect of time ($F_{7,56} = 3.27$, $P = 0.005$) but no difference between days
297 ($F_{1,8} = 0.231$, $P = 0.643$). By contrast, the TMEP area (normalised to baseline) (Figure 4D) was
298 unchanged during the sustained contraction ($F_{11,88} = 0.72$, $P = 0.719$) with no difference between
299 days ($F_{1,8} = 0.99$, $P = 0.348$) nor interaction. In recovery, the TMEP areas remained unchanged
300 ($F_{7,56} = 0.42$, $P = 0.882$) with no difference between days ($F_{1,8} = 1.33$, $P = 0.289$).
301
302 In the vastus lateralis, TMS-TMEPs and TMEPs behaved similarly to those in VM. VL TMS-TMEPs
303 showed an effect of time ($F_{11,88} = 16.63$, $P < 0.001$) and day ($F_{1,8} = 9.02$, $P = 0.017$), with the large
304 day having larger relative areas (Figure 5A). In addition, there was a non-significant interaction
305 ($F_{11,88} = 1.74$, $P = 0.078$). Large TMS-TMEPs decreased by ~53% and small TMS-TMEPs decreased by
306 ~71.8%. In recovery, there was an effect of time ($F_{7,56} = 3.18$, $P = 0.029$) with recovery towards
307 baseline, and no difference between days ($F_{1,8} = 0.29$ $P = 0.605$). TMEP area (normalised to baseline)
308 was unchanged during the sustained contraction ($F_{11,88} = 0.71$, $P = 0.725$) with no difference
309 between days ($F_{1,8} = 0.09$, $P = 0.772$). In recovery, the areas remained
310 unchanged ($F_{7,56} = 0.73$, $P = 0.645$) and there was no difference between days
311 ($F_{1,8} = 0.28$, $P = 0.606$).
312

For the rectus femoris, comparison of the normalised TMS-TMEP between small and large responses showed an effect of time ($F_{11,88}$ = 11.08, P < 0.001), but no day effect ($F_{1,8}$ = 0.64, P = 0.448) nor interaction ($F_{11,88}$ = 0.79, P = 0.643) (Figure 5B). Large responses decreased by ~45% and small decreased by ~60%. In recovery, there was no day effect ($F_{1,8}$ = 0.72, P = 0.421) but there was an effect of time ($F_{7,56}$ = 3.44, P = 0.004) such that the TMS-TMEP size increased to values similar to baseline. The TMEP area was unchanged during the sustained contraction ($F_{11,88}$ = 0.76, P = 0.671) with no difference between days ($F_{1,8}$ = 0.07, P = 0.803). In recovery, the areas remained unchanged ($F_{7,56}$ = 1.3, P = 0.267) and displayed no difference between days ($F_{1,8}$ = 1.93, P = 0.202).

***EMG.*** Participants successfully maintained the rmsEMG target during the sustained contraction as VM rmsEMG was unchanged from baseline ($F_{11,88}$ = 0.87, P = 0.574) and was on average ~21% of MVC throughout the sustained contraction. However, there was an unintended significant difference between days ($F_{1,8}$ = 7.78, P = 0.023). VM rmsEMG during the sustained contraction was higher on the day that large responses were evoked by a pooled average of 1.7% (SD 1.9) MVC. For VL, there was no change in rmsEMG during the sustained contraction ($F_{11,88}$ = 1.7, P = 0.086) at ~21% MVC, and no effect of day ($F_{1,8}$ < 0.001, P = 0.971). Additionally, RF rmsEMG was unchanged ($F_{11,88}$ = 1.34, P = 0.217) at ~20% with no difference between days ($F_{1,8}$ = 0.02, P = 0.893). In recovery, VM rmsEMG was higher than baseline particularly at the beginning of recovery ($F_{7,56}$ = 2.51, P = 0.025) and the average size of the increase was 2.5%. In addition, there was an effect of day with the large response day showing higher VM rmsEMG (2.6% SD 1.9) than on the small day ($F_{1,8}$ = 17.24, P = 0.003). During recovery, there was an increase in VL rmsEMG ($F_{7,56}$ = 2.54, P = 0.024), but there was no change in RF rmsEMG ($F_{7,56}$ = 1.45, P = 0.567).

***Force.*** As expected, force declined during the maintained rmsEMG sustained contraction ($F_{3.2,54.2}$ = 29.46, P < 0.001). Force from baseline was approximately halved, falling from 26.2% (SD 4.3) of MVC at baseline, to 12.6% (SD 5.9) by the end of 10-min contraction. This decline was similar

on the two days ($F_{1,8}$ = 0.01, P = 0.956). During the recovery contractions, the force during the brief contraction increased towards baseline values ($F_{4.1,68.7}$ = 10.91, P < 0.001).

***Perceived effort.*** During the sustained contraction, the rating of perceived effort (RPE) increased progressively ($F_{2.9,50.7}$ = 113.3, P < 0.001) during the 10-min contraction from 1.6 (SD 1) to 7.3 (SD 1.5), and there was no difference between days ($F_{1,8}$ = 2.02, P = 0.192). In recovery, there was an effect of time ($F_{2.7,46.9}$ = 6.943, P < 0.001) such that at the start of recovery, RPE was still higher than at the start of the sustained contraction but became similar from 2.5 min onwards.

***Maximal M-wave.*** VM Mmax area decreased slightly by ~6.6% (SD 10.2) by the end of the 10-min contraction ($F_{11,88}$ = 3.21, P = 0.01) with no difference between days ($F_{1,8}$ = 0.09, P = 0.77). During recovery VM Mmax remained below baseline ($F_{7,56}$ = 4.3, P < 0.001). VL Mmax area also decreased by ~2.9% (SD 5.9) ($F_{3.3,56.8}$ = 3.28, P = 0.023) during the contractions, with no difference between days ($F_{1,8}$ = 0.35, P = 0.569). There was no change in the RF Mmax area ($F_{2.4,41.7}$ = 2.41, P = 0.091) and no difference between days ($F_{1,8}$ = 0.48, P = 0.506).

**DISCUSSION:**

In the present study, performance of a fatiguing sustained submaximal contraction of the knee extensors resulted in decreased excitability of quadriceps motoneurons as evident by a reduction in the size of the TMS-TMEP which assessed excitability during brief periods of paused voluntary descending drive. By contrast, when tested with maintained ongoing descending drive, excitability of the motoneurons was unchanged (i.e. the size of the TMEPs without prior TMS remained the same). These findings were consistent for all muscles measured. Furthermore, small TMS-TMEPs, evoked by weak stimulation, declined more than large TMS-TMEPs. This difference suggests that activity-dependent mechanisms contribute to the observed reduction in excitability as active motoneurons were most affected.

***TMS-TMEP***

For the three measured quadriceps muscles, the TMS-TMEPs became smaller during the sustained contraction and thus, indicate reductions in motoneuron excitability. TMS-TMEPs are a measure of motoneuron excitability elicited through stimulation of the corticospinal tracts at a subcortical level during the brief silent period that follows TMS. TMS first elicits an excitatory response from the motor cortex and then a period of inhibition of motor cortical output (39). The inhibition of descending drive from the motor cortex removes one source of excitatory input to the motoneurons at time of assessment making the resulting TMS-TMEP more sensitive to other influences that affect motoneuron excitability including changes of motoneuron properties and changes to other descending or afferent inputs during exercise. Our results for the quadriceps are consistent with those for the biceps brachii when tested in similar circumstances (27) and strongly suggest that during fatiguing contractions of the knee extensor muscles changes occur at the level of the motoneurons and lead to reduced efficacy of descending drive to excite motoneurons. Therefore, to maintain motoneuron output, greater descending drive is required. In the context of past studies looking at the quadriceps, our findings suggest that assessments during ongoing descending drive may underestimate underlying changes in motoneuron excitability during fatigue, but may better represent the efficacy of the multiple inputs onto the motoneurons to maintain motoneuron excitability during contractions.

Small TMS-TMEPs were more affected during fatigue than large TMS-TMEPs. This difference was clear both in vastus medialis, our muscle of interest, and in the vastus lateralis, although it was not significant for the rectus femoris. The rectus femoris is a bi-articular muscle and the RF EMG during that task, as well as the size of the TMS-TMEPs was not controlled which may have introduced variability and thus, explain the non-significant differences. As TMEPs recruit motoneurons synaptically through the activation of descending corticospinal axons, small and large baseline

responses should test different proportions of the quadriceps motoneuron pool. As MEPs, evoked via TMS, recruit motoneurons in the same order as a voluntary contraction (10), and TMEPs and MEPs travel through similar descending corticospinal axons to activate motoneurons (25), we expect TMEPs to also recruit motoneurons in an orderly manner from small, lower threshold motoneurons to large, high threshold motoneurons. During the current study, the sustained contraction was performed to a constant level of EMG in the VM, ~20% of maximum, which was designed to minimise the recruitment of addition motoneurons and therefore keep a similar number of number of active motoneurons throughout the contractions. With the relatively weak submaximal contraction, mostly smaller, low threshold motoneurons would be active (1) and this roughly split the motoneuron pool into two populations, motoneurons that were active during contraction and those that were not recruited. Then by testing with smaller and larger TMS-TMEPs (~13% and ~40% of Mmax respectively), the effects of fatigue could be compared for a mostly active population of motoneurons (recruited into the small response) versus a combination of the active population with a number of inactive motoneurons (recruited into the large response). The relatively greater decline in small TMS-TMEPs suggests that the motoneurons that were most active during the contraction became less excitable. These results for the quadriceps are consistent with similar findings in the upper arm (27) and suggest that similar processes of inhibition related to repetitive firing occurs in motoneurons innervating the arm and leg muscles.

The inhibition of motoneurons related to activity-dependent changes from repetitive firing may be due to changes to the intrinsic properties of the active motoneurons. When motoneurons are exposed to a constant injected current, there is an initial (2s) rapid decline of firing which is then followed by a slow decline in discharge rate over tens of seconds (14, 22, 29). This phenomenon is termed spike frequency adaptation with the slow decline termed late adaptation. Late adaptation is consistent with reduced firing rates of quadriceps motoneurons during a sustained 2 min MVC, and thus is evidence that intrinsic changes contribute to decrease firing rates of motoneurons (5).

Additional evidence consistent with intrinsic motoneuron changes comes from in-vivo single motor unit studies which show that greater descending voluntary drive is required to maintain the firing of a recorded motoneuron over time (15, 19). While the specific mechanisms of late spike frequency adaptation have not been completely identified (e.g. (41)), slow inactivation of $Na^+$ channels may contribute and could alter the threshold for action potential activation (6, 29). A requirement for greater input to generate action potentials is consistent with the decrease in TMS-TMEP seen in our study, where fewer motoneurons are recruited by the same stimulus after the motoneurons have fired repetitively in the sustained contraction.

Another component to the observed depression in motoneuron excitability may be due to inhibitory feedback from group III and IV muscle afferents. As these afferents respond to mechanical and metabolic perturbations their firing is elevated during fatiguing exercise (20, 30). In the upper arm, high rates of firing of these afferents have been associated with reduced excitability of extensor motoneurons, but excitation of flexors (24). As the quadriceps are extensor muscles, they may also be susceptible to inhibition by afferent feedback during exercise (12, 13, 40) c.f (34). Although our current study design does not allow us to comment on the contribution of these afferents to our observed results we would expect afferent feedback to influence the whole motoneuron pool (31) and it could contribute to the depression of both the small and large TMS-TMEPs.

***TMEP***

By contrast to the decline in the TMS-TMEP, the size of the TMEP was unchanged during the sustained contraction. This finding was expected as the task required the maintenance of motoneuron output in the form of maintaining a constant level of EMG. As the unchanged TMEP occurred despite an underlying reduction in motoneuron excitability shown by the TMS-TMEP, we propose that during the fatiguing contraction, increases in voluntary descending drive were required to overcome the motoneuronal depression and maintain the level of EMG. This is further supported

443 by a progressive rise in the perceived effort required to hold the same level of EMG although
444 increased feedback from group III/IV afferents may also be contributing to increases in RPE (2, 3). A
445 similar pattern of progressive rise in RPE during a maintained EMG contraction has been observed
446 during fatiguing submaximal contractions of the elbow flexors (18, 27).
447
448 Our result showing the reduction in TMS-TMEP but an unchanged TMEP highlights the influence of
449 ongoing descending drive on the evoked motoneuron response. Past studies that measure
450 motoneuron excitability during ongoing drive may underestimate the underlying change in
451 motoneuron responsiveness, but better describe the sum of opposing changes in motoneuron
452 properties, afferent feedback, and descending drive on excitability (21). Indeed, Weavil and
453 colleagues (37) provided evidence that the lack of change in CMEPs during fatiguing cycling with
454 increasing EMG was in fact suggestive of reduced excitability, as the same increase in EMG in an
455 unfatigued muscle resulted in a larger CMEP. In other muscles, progressive increases in EMG during
456 a constant force task have been shown to result in increases in the size of CMEP (16, 23). In these
457 circumstances, increasing excitatory descending drive presumably outweighs reductions in
458 underlying motoneuron excitability. The different changes in evoked potentials in different fatiguing
459 tasks emphasises that interpretation of changes in motoneuron excitability is difficult during
460 voluntary contractions when excitability reflects the integration of many varying inputs, as well the
461 intrinsic properties of the motoneurons (6, 33).
462
463 ***Recovery***
464 By 30 s after the end of the sustained contraction, the excitability of the motoneurons had, on
465 average, recovered towards baseline for both the small and large responses and in all muscles
466 (Figure 2A, 4A C, & 5). Previously a single motor unit experiment reported that ~63% of the recovery
467 of triceps brachii motoneurons after sustained firing occurs in the first 28 s of rest with full recovery
468 taking up to four minutes (15). On a practical note, this fast recovery emphasises the need to

measure excitability either during the fatiguing task or immediately after, as assessments even 30s later may underestimate the effects of fatigue.

In addition, we report that there was a markedly reduced rating of perceived effort coupled with unintended higher task EMG during the first few recovery contractions. Together, these suggest an initial overestimation of descending drive needed to reach the target given that motoneuron excitability had recovered from the end of the sustained contraction.

In conclusion, this study shows that motoneurons of the quadriceps become less responsive during a fatiguing contraction. This is seen only when tested in the absence of ongoing descending voluntary drive and is likely due to activity-dependent changes of the intrinsic properties of the motoneurons. Furthermore, the increase in RPE indirectly suggests that to maintain motoneuron firing during fatigue, voluntary descending drive must be increased to overcome the reduced excitability.

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**Disclosures**

The authors report no conflicts of interest.

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579 sodium currents to spike-frequency adaptation in rat hypoglossal motoneurons. *J Neurophysiol* 93:
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581

582 **Figure captions**

583 **Figure 1. Experimental protocol.** At baseline, five sets of brief contractions were performed to a
584 level of rmsEMG required to generate a force of 25% of MVC. During each contraction, either a
585 TMS-TMEP (closed circle), TMEP (open triangle), or maximal M-wave (closed diamond) was elicited.
586 M-waves were only included in two of the baseline sets. During the 10-min sustained contraction,
587 the stimulation sequence of TMS-TMEP, TMEP and M-wave was performed every minute. From 30s
588 post sustained contraction, recovery measures were performed in a similar manner to baseline
589 measurements with M-waves always included in each set. RPE was reported every minute during the
590 fatigue protocol and after each recovery measure.

591 **Figure 2. Task performance and changes in vastus medialis (VM) potentials for all participants**
592 **stimulated to elicit small baseline TMS-TMEPs (n = 14). A**. Force (closed diamonds) and rmsEMG of
593 VM (open triangles) normalised to MVC during the 10-min contraction and recovery contractions.
594 Ratings of perceived effort (RPE; 0 - 10) are displayed on the right y-axis by the grey bars. **B.** Area of
595 VM TMEPs (open circles) and TMS-TMEPs (closed circles) normalised to Mmax area. Grey shading on
596 the x-axis indicates the recovery measures, which were performed in brief contractions. * indicates
597 significant difference from baseline. For RPE, * indicates significant difference from the start of the
598 sustained contraction ($P < 0.05$). Data are mean and SD.

599 **Figure 3. Overlaid raw traces from the vastus medialis in a single participant across the**
600 **experiment. A.** TMS-TMEPs, recorded on the large or small day (arrows indicate thoracic
601 stimulation). TMS-TMEPs were evoked in the silent period following TMS. The MEP evoked by TMS
602 (circles) is coloured in grey for clarity. Note the decline in the TMS-TMEP from baseline during the
603 10-min sustained contraction (large grey shaded box). Dashed horizontal lines indicate the mean
604 amplitude of the baseline TMS-TMEP or TMEP **B.** TMEPs on the large and small day. TMEPs were
605 evoked during ongoing EMG.

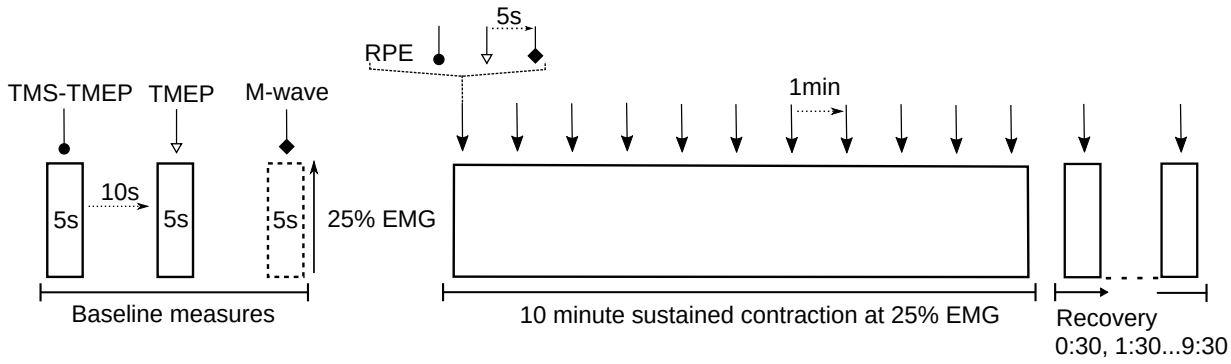
606 **Figure 4. Areas of thoracic motor evoked potentials (TMEPs) and TMS-TMEPS in vastus medialis**
607 **(VM) for the two days.** Each panel presents group data (n = 9; mean and SD) for the large (circles)
608 and small (triangles) days. The top panels show the TMS-TMEP (**A**) and TMEP (**B**) normalised to
609 Mmax. For comparison between the large and small responses the bottom panels show the TMS-
610 TMEP/Mmax (**C**) and the TMEP/Mmax (**D**) when normalised to baseline (bl). * denotes different
611 from baseline. # denotes a significant overall effect of day ($P < 0.05$).

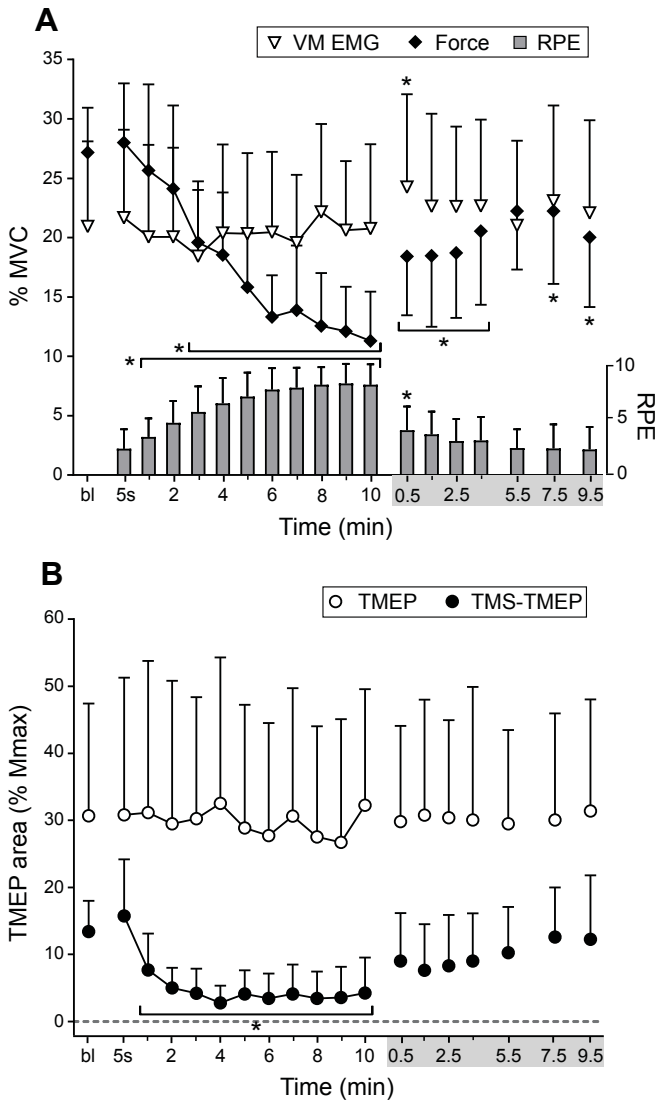
612 **Figure 5. Areas of TMS-TMEPs in vastus lateralis normalised to baseline (bl).** Group data (n = 9;
613 mean and SD) is displayed for the large (circles) and small (triangles) days. * denotes different from
614 baseline. # denotes a significant overall effect of day ($P < 0.05$).

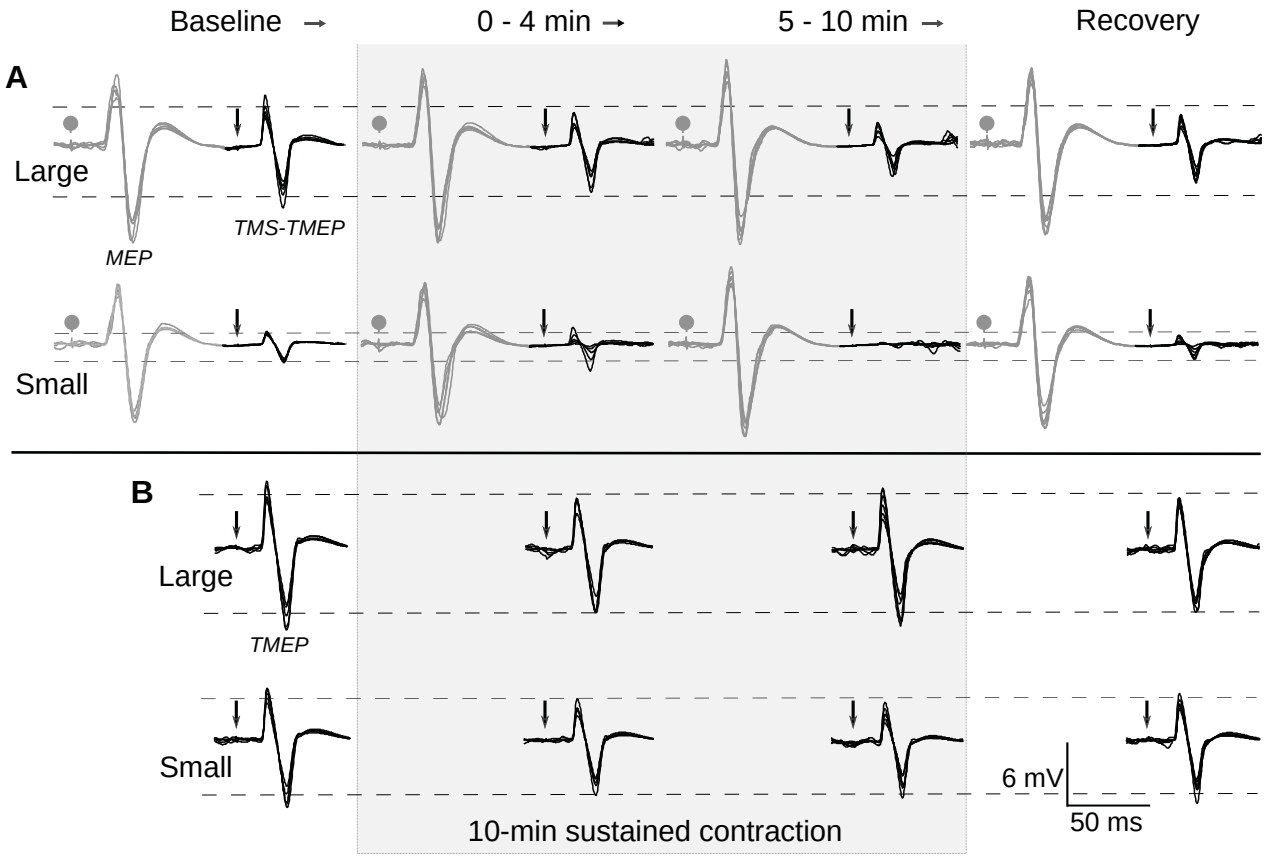
**Table 1- Baseline data for participants who completed both days (n = 9)**

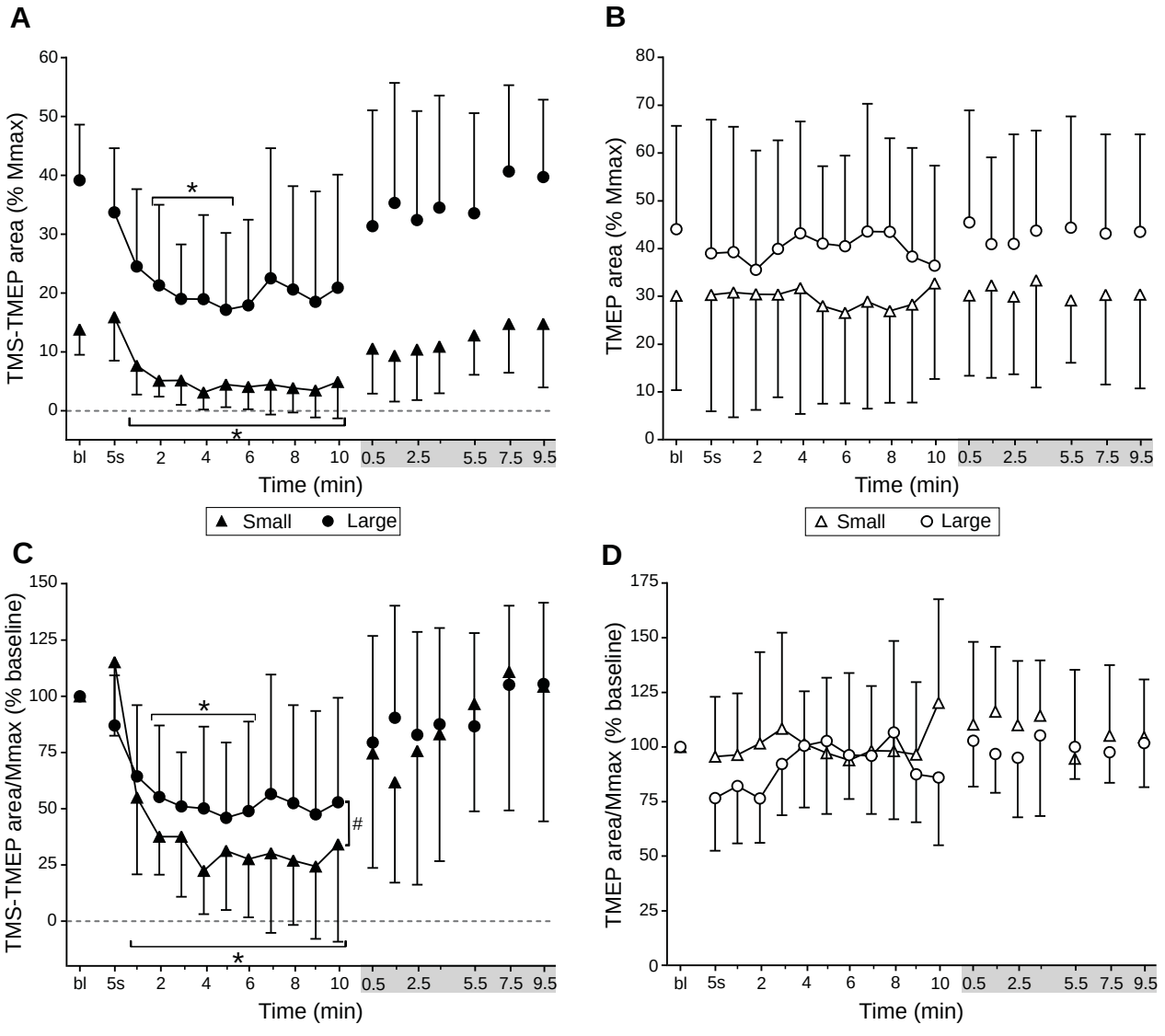
| | Mmax | | TMS-TMEP | | | TMEP | | |
|---|---|---|---|---|---|---|---|---|
| | Amplitude (mV) | Area (mV s) | Amplitude (mV) | Area (mV s) | Area %Mmax | Amplitude (mV) | Area (mV s) | Area %Mmax |
| VM | | | | | | | | |
| Small | 25.1 (6.4) | 0.158 (0.045) | 3.9 (1.7) | 0.021 (0.009) | 13.8 (4.2) | 8 (5.5) | 0.046 (0.032) | 30.1 (19.7) |
| Large | 25.2 (7.2) | 0.155 (0.043) | 10.6 (3.7) | 0.059 (0.019) | 39.1 (9.4) | 11.2 (6.3) | 0.065 (0.035) | 43.9 (21.1) |
| | P = 0.863 | P = 0.62 | **P < 0.001** | **P < 0.001** | **P < 0.001** | **P < 0.001** | **P < 0.001** | **P < 0.001** |
| VL | | | | | | | | |
| Small | 22.3 (5.1) | 0.143 (0.027) | 3.2 (1.3) | 0.018 (0.007) | 12.6 (3.7) | 5.8 (3.5) | 0.036 (0.024) | 25.8 (15.9) |
| Large | 21.9 (5.9) | 0.14 (0.03) | 8.6 (3.3) | 0.051 (0.02) | 35.2 (9.4) | 8.5 (4.5) | 0.053 (0.029) | 37.9 (17.5) |
| | P = 0.618 | P = 0.556 | **P < 0.001** | **P < 0.001** | **P < 0.001** | **P = 0.005** | **P = 0.003** | **P < 0.001** |
| RF | | | | | | | | |
| Small | 10.2 (3.2) | 0.052 (0.02) | 1.6 (0.6) | 0.007 (0.002) | 15.1 (6.2) | 3.2 (1.4) | 0.014 (0.006) | 30.5 (15) |
| Large | 8.8 (4.4) | 0.047 (0.024) | 3.4 (2.1) | 0.015 (0.012) | 35.5 (12.8) | 4.8 (3.1) | 0.022 (0.016) | 48.9 (20.5) |
| | P = 0.369 | P = 0.537 | **P = 0.018** | **P = 0.046** | **P < 0.001** | P = 0.068 | P = 0.118 | **P = 0.016** |

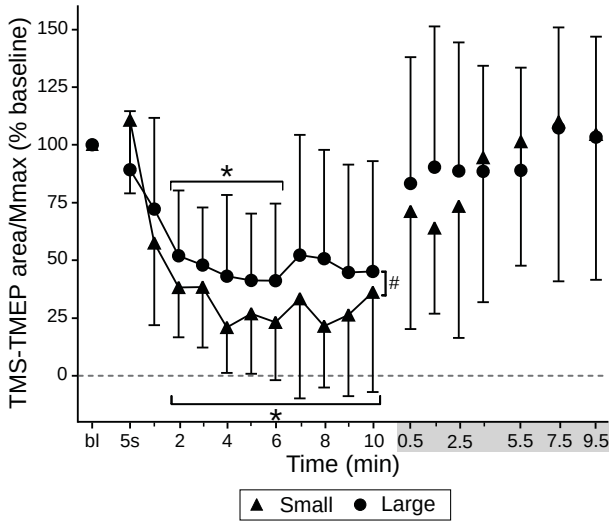
Data are mean (SD). Bold text indicates significant difference between the small and large day P < 0.05.


5s
RPE
TMS-TMEP
TMEP
M-wave
1min
10s
5s
5s
5s
25% EMG
Baseline measures
10 minute sustained contraction at 25% EMG
Recovery
0:30, 1:30...9:30


A
VM EMG
Force
RPE
% MVC
RPE
Time (min)
bl
5s
2
4
6
8
10
0.5
2.5
5.5
7.5
9.5
B
TMEP
TMS-TMEP
TMEP area (% Mmax)
Time (min)


Baseline →
0 - 4 min →
5 - 10 min →
Recovery
A
Large
TMS-TMEP
MEP
Small
B
Large
TMEP
Small
6 mV
50 ms
10-min sustained contraction


A
TMS-TMEP area (% Mmax)
60
50
40
30
20
10
0
*
*
bl
5s
2
4
6
8
10
0.5
2.5
5.5
7.5
9.5
Time (min)
Small
Large
B
TMEP area (% Mmax)
80
70
60
50
40
30
20
10
0
Time (min)
Small
Large
C
TMS-TMEP area/Mmax (% baseline)
150
125
100
75
50
25
0
*
*
#
Time (min)
D
TMEP area/Mmax (% baseline)
175
150
125
100
75
50
25
0
Time (min)


TMS-TMEP area/Mmax (% baseline)
150
125
100
75
50
25
0
*
*
#
bl
5s
2
4
6
8
10
0.5
2.5
5.5
7.5
9.5
Time (min)
Small
Large