Title: Test-retest reliability of TMS motor evoked responses and silent periods during explosive voluntary isometric contractions

Castelli[1] F., Mian[1] O.S., Bruton[1,2] A., Chembila Valappil[1] A.C., Tillin[1] N.A.

[1] University of Roehampton, Life Sciences, Whitelands College, Holybourne Avenue, London, SW15 4JD, United Kingdom

[2] Brunel University London, Centre for Cognitive and Clinical Neuroscience, Sport Health and Exercise Sciences, Uxbridge, UB8 3PH, United Kingdom

Castellf1@roehampton.ac.uk

Abstract

Purpose

This study assessed the intraclass correlation coefficient (ICC) and coefficient of variation (CV) test-retest reliability of TMS responses MEPs and silent periods at early, middle, and late phases of the rising time-torque curve during explosive voluntary contractions. We also investigated how the number of consecutively averaged measurements over 3 to 15 separate contractions influenced reliability.

Methods

On two separate occasions 3-7 days apart, 14 adults completed 48 isometric explosives (1-s) contractions of the knee extensors, superimposed with TMS. The TMS elicited an MEP and silent period in the quadriceps muscles at 45 (early), 115 (middle), or 190 ms (late) during each contraction. TMS was also superimposed at the plateau of 15 separate MVCs. Test-retest ICC and CV were calculated for consecutively averaged MEPs and silent periods.

Results

No one condition was more reliable than another. For MEP amplitude, in all conditions except the explosive late phase, ICCs generally increased, and CV decreased, with an increase in the number of averaged contractions, and were >0.50 ICC and <15% CV within 7 contractions. For silent period, ICCs and CVs were unaffected by the number of consecutively averaged contractions and remained >0.50 ICC and <10% CV.

Conclusion

Test-retest reliability of TMS responses is comparable between phases of explosive contraction and at the plateau of MVC. To enhance MEP reliability, with the increases he number of averaged contractions of except the explosive late phase, we recommend studies average data across > the 3-5 contractions typically reported in the literature for studies using TMS during MVCs.

Key words: Reliability, TMS, explosive contractions, test-retest

Abbreviations

AMT = Active motor threshold

CV = coefficient of variance

EMG = Electromyography

ICC = Inter-class correlation

MEP = Motor evoked potential

MVC = Maximal voluntary contraction

MU = Motor unit

RF = Rectus femoris

RMS = Root mean squared

RTD = Rate of torque development

SD = Standard deviation

SP = Silent period

TMS = Transcranial magnetic stimulation

VM = Vastus medialis

VL = Vastus lateralis

Introduction

The rate of torque development (RTD) measures the ability of muscles to rapidly increase force around a joint (Tillin and Folland, 2014), and is functionally important where time to develop force is limited, such as sprinting (Tillin, Pain and Folland, 2013) or balance recovery (Sundstrup *et al.*, 2010). RTD is often measured in early (0-50 ms), middle (50-100 ms) and late (>100 ms) phases of explosive contraction performed from rest (Maffiuletti *et al.*, 2016), and different physiological factors limit the RTD in these separate phases. One limiting factor is the neural drive to muscle, which positively correlates with early- and middle-phase RTD (Folland, Buckthorpe and Hannah, 2014; Del Vecchio *et al.*, 2019). Despite the relevance of neural drive to RTD, the corticospinal mechanisms affecting RTD are not well established.

Transcranial magnetic stimulation (TMS), superimposed during voluntary contractions, elicits a motor-evoked potential (MEP) in the surface EMG signal of the target contracting muscle. The MEP amplitude is thought to reflect corticospinal excitability (Rossini *et al.*, 2015), whilst a period of electrical inactivity immediately after the MEP, referred to as the silent period (Damron *et al.*, 2008), is thought to reflect corticospinal inhibitory mechanisms (Säisänen *et al.*, 2008). Despite extensive use of TMS to explore corticospinal excitability/inhibition at the plateau of a maximal voluntary contraction (MVC; Todd, Taylor and Gandevia, 2016), TMS has not been commonly used during the rising torque of explosive voluntary contractions, where RTD is typically measured.

Before using TMS to assess corticospinal excitability/inhibition during explosive contractions, it is important to establish the test-retest absolute and relative reliability of TMS responses (MEP amplitude and silent period) during such conditions; however, this has not been done. In contrast, the test-retest reliability for TMS responses at the plateau of MVCs has been investigated in various muscles. For absolute and normalised MEP amplitude at the MVC plateau in the lower limbs, studies have reported moderate inter-class correlation (ICC; 0.52-0.79) (Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016) and coefficient of variation (CV) of 10-11% (Souron *et al.*, 2016). For the silent period at the MVC plateau, the test-retest ICC has ranged from moderate in the vastus lateralis (0.61-

0.70; (Di Virgilio *et al.*, 2022)) to excellent in the soleus and tibialis anterior (0.93-0.95; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016) and one study has reported a CV of 8.6% (Souron *et al.*, 2016) in the tibialis anterior. It is unclear whether the reliability of MEP amplitudes and silent periods during explosive contractions will be comparable to that observed at the MVC plateau. Typically, the reliability of torque and EMG amplitude measurements is lower in early compared to later phases of explosive contraction, and generally lower for explosive contractions compared to the MVC plateau (De Ruiter *et al.*, 2004; Buckthorpe *et al.*, 2012), so a similar pattern may be observed for the reliability of MEP amplitudes and silent periods.

TMS responses are typically averaged across multiple separate stimuli (in separate contractions) to minimise the influence of random variation and improve reliability. Thus, when assessing the test-retest reliability of MEP amplitudes and silent periods, it is important to consider how many stimulations might be required to achieve adequately reliable averages. A relatively high number of stimuli (≥20) are needed to obtain moderate to good test-retest reliability (ICC = 0.50 - 0.75; Goldsworthy, Hordacre and Ridding, 2016; Biabani *et al.*, 2018) of MEP amplitudes recorded when the participant is passive. In contrast, as few as four stimuli have been shown to produce good (≥0.75) test-retest ICC for MEP amplitude during submaximal contractions held at a constant force (Wheaton *et al.*, 2009; Lewis, Signal and Taylor, 2013; Temesi, Ly and Millet, 2017). The effect of the number of contractions on test-retest reliability of average TMS responses has not been determined during either MVC or explosive contractions. Previous studies of TMS responses during MVCs have typically only collected and averaged 3-5 responses (Luc *et al.*, 2014; Tallent *et al.*, 2017; Škarabot *et al.*, 2019). This could be due to time constraints or concerns over fatigue with multiple contractions; however, it may be at the expense of poor reliability. Thus, there is a need to assess the effect of the number of contractions on the test-retest reliability of average TMS responses during MVCs and explosive contractions.

100 In studies of TMS responses, measures are typically averaged across all stimulations obtained
101 within a testing session. However, a subset of contractions are typically used for studies of explosive
102 contractions, with the best three out of ten contractions being recommended for averaging RTD
103 measurements (Maffiuletti *et al.*, 2016). Therefore, for studies of MEPs recorded during explosive
104 contractions, it may be desirable to investigate MEPs obtained from the best three contractions rather
105 than from all contractions, and investigation into the reliability of this approach is necessary.

106 This study aims to assess the absolute (ICC) and relative (CV) test-retest reliability of TMS
107 responses (MEP amplitude and silent period) recorded at different time points (early, middle, and late
108 phases) during explosive voluntary contractions and at the plateau of MVCs. As part of this aim, we
109 intend to (i) document how the number of consecutive contractions (between 3-15) over which TMS
110 responses are averaged, influences test-retest reliability; and (ii) document test-retest reliability of
111 TMS responses averaged across the best 3, out of 10, contractions. We determine the best three as
112 those with the highest torque (Torque averaging method) or EMG RMS (EMG averaging method) prior
113 to MEP on-set.

114 **Methods**

115 *Participants*

116 Fourteen participants, nine males (age 31 ± 5 years, height, 178.7 ± 6.6cm, and mass 79.2 ±
117 4.7kg) and five females (age 29 ± 6 years, height, 165.5 ± 5.5cm, and mass 59.3 ± 6.2kg) were recruited
118 to take part in this study. All participants habitually performed 120-180 minutes of moderate to high-
119 intensity activity per week, and were deemed recreationally active (McKay *et al.*, 2022). Participants
120 were also free from injury and disease (screened via a questionnaire adapted from Balady *et al.*, 1998)
121 and free from contraindications to TMS (screened via a questionnaire adapted from Rossi *et al.*, 2011).
122 Due to changes in endogenous hormones throughout the menstrual cycle potentially affecting
123 neuromuscular responses to TMS (Ansdell *et al.*, 2019), female participants undertook experimental
124 trials exclusively during their self-reported early to mid-follicular phase (first ten days from the first

125 day of menstruation), when endogenous hormone concentration is low and relatively stable (de
126 Jonge, Thompson and Han, 2019). The University of Roehampton ethics committee approved the
127 study, and all participants provided written informed consent before participating.

128 *Overview*

129 Participants visited the laboratory on three separate occasions and were asked to avoid strenuous
130 exercise and alcohol consumption for 24 hours before each visit. Each session lasted approximately
131 120-150 minutes, with consecutive sessions separated by 3-7 days. The first visit was a familiarisation
132 session, and the second and third were measurement sessions. The measurement sessions were
133 performed at a consistent time of day and involved an identical protocol, with measurements used to
134 assess the test-retest reliability of the variables of interest. Each session involved knee extensor
135 torque and EMG measurement during maximal voluntary and explosive contractions, using TMS to
136 obtain superimposed MEP and femoral nerve stimulation to obtain compound muscle action
137 potentials at rest.

138 *Torque measurements and surface electromyography (EMG)*

139 Participants were tightly secured in a custom-built strength testing chair (Fig. 6b in Maffiuletti *et al.*,
140 2016) with a waist belt and shoulder straps. The hip and knee angles were set at 100° and 105°,
141 respectively (full extension being 180°). All contractions were isometric knee extensions performed
142 with the right leg. An ankle strap joined to a calibrated S-shaped load cell (FSB-1.5kN, Force Logic,
143 Reading, UK) was secured 4 cm proximal to the medial malleolus. The force signal was amplified (x375)
144 and then sampled at 2000 Hz (Mirco3 1401 and Spike2 v.8; CED., Cambridge, UK). Offline, the force
145 was filtered (fourth-order low-pass Butterworth, 250 Hz cut-off), corrected for limb weight, and
146 multiplied by the external moment arm to calculate joint torque.

147 The skin was prepared by shaving, cleaning (70% ethanol), and lightly abrading the area where
148 EMG electrodes were placed. A single, bipolar silver-silver-chloride gel-electrode configuration (2-cm

149 diameter and 2-cm inter-electrode distance; Dual Electrode, Noraxon, Arizona, USA) was placed over
150 the belly of each of the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM), based on
151 SENIAM guidelines (Hermens *et al.*, 1999). The EMG system has an inherent 312-ms delay, so whilst
152 suitable for measurements involved in the study, EMG signals sampled by it could not be used to
153 detect activation on-set and trigger the TMS in real-time during the explosive contractions (explained
154 below in Experimental procedures). Thus, a second wired bipolar EMG electrode (2 cm diameter and
155 2 cm inter-electrode distance; Dual Electrode, Biometrics Ltd, Gwent, UK) was placed on the belly of
156 the VM to trigger the TMS during contraction. Electrode locations were marked with a permanent
157 marker pen, and participants were asked to maintain these marks throughout the study by re-applying
158 them if necessary. The three wireless EMG signals were transmitted to a desktop receiver (TeleMYO
159 D.T.S., Noraxon, Arizona, USA) and sampled, along with the single wired EMG signal, at 2000 Hz via
160 the same A/D convertor and software as the force signal. Once offline, wireless EMG signals were
161 filtered (fourth-order Butterworth, band-pass, 6-500 Hz) and time-corrected for the 312-ms delay
162 inherent in the Noraxon system.

163 *Transcranial magnetic stimulation (TMS)*

164 TMS with a 1-ms pulse width was delivered via a double cone coil (110-mm Magstim 200,
165 Whitland, UK) over the scalp in an optimal position to elicit MEPs in the right quadriceps muscles. The
166 following procedures were completed in every session. Participants wore a swim cap, and the vertex
167 of the head – identified as 50% of the distance between nasion and inion and between right and left
168 perpendicular point – was marked on the swim cap. A 5-by-5-cm grid with 1-cm spacing between grid
169 lines was drawn on the swim cap, lateral (left hemisphere) and posterior from the vertex. The coil
170 was moved posteriorly and laterally from the vertex in ~0.5-cm steps, and in each position, the
171 participant completed four submaximal voluntary contractions at 20% MVC torque (established in the
172 warm-up; see below) with superimposed TMS on each contraction at a submaximal (range 50-60%)
173 stimulator output. The position determined the optimal coil position for the rest of the session, which

174 was subjectively the highest consistent MEP amplitudes (peak-to-peak) over the four superimposed
175 contractions for all three muscles (RF, VL and VM). The optimal coil position was marked by drawing
176 the edge of the coil over the swim cap, ensuring accurate relocation throughout the session. The
177 active motor threshold (AMT) was then determined via a series of 20% MVC torque contractions
178 superimposed with TMS stimulator output starting at 39%. The AMT was defined as the minimum
179 TMS intensity required to elicit five visible MEPs amongst the background EMG activity of the VM and
180 RF, out of ten consecutive superimposed contractions. If the muscles had less or more than five visible
181 MEPs, the machine intensity was reduced or increased by 2% of the machine output. We prioritised
182 the VM and RF (not the VL) as these muscles produced more consistent and visible MEPs in pilot
183 testing. Where it was impossible to match AMT for VM and RF, we settled on being one visible MEP
184 away from 5/10 (e.g., 6/10 VM and 4/10 RF). The same investigator held the coil by hand throughout
185 the measurement sessions, continuously monitoring its position and orientation. For TMS delivered
186 during maximal and explosive contractions (see experimental protocol), the intensity was set at 140%
187 of the stimulator output at AMT (Groppa *et al.*, 2012; Rossini *et al.*, 2015; Rossi *et al.*, 2020). These
188 procedures were repeated for all sessions.

189 *Femoral nerve electrical stimulation*

190 Single, square-wave pulses (200 µs duration) were delivered (DS7AH, Digitimer, Hertfordshire,
191 UK) over the femoral nerve in the inguinal triangle to evoke twitch contractions and obtain compound
192 muscle action potentials (M-waves) at rest. The anode (5 x 8 cm carbon rubber; EMS Physio Ltd.,
193 Oxfordshire, UK) was placed over the head of the greater trochanter. The optimal location of the
194 cathode (1 cm diameter tip; S1 Compex Motor PointPen, Digitimer, UK) was determined as that
195 which evoked the greatest peak twitch torque for a given submaximal stimulation intensity (80-120
196 mA). The cathode was taped down and held in position by the same investigator as a series of twitches
197 at incremental intensities were evoked until there was a plateau in the peak-to-peak M-wave
198 amplitude (M_{max}) of all three muscles (RF, VL, VM). The stimulator intensity was then increased to

199 150% of the intensity at M_{max}, ensuring supramaximal intensity. Three supramaximal twitch
200 contractions were then evoked, each separated by 15 s, and M_{max} was averaged across the three
201 contractions for each muscle. The procedures were repeated for all sessions.

202 *Experimental protocol*

203 Participants first completed a warm-up involving a series of explosive, submaximal, and maximal
204 voluntary contractions (the latter being used to establish MVC torque), followed by procedures for
205 obtaining optimal TMS coil position, ATM, and M_{max}. Participants then completed a series of MVCs
206 and explosive voluntary contractions with and without superimposed TMS. The instruction for MVCs
207 was to "push as hard as possible" for 3-5 s and explosive contractions to "push as fast and hard as
208 possible", emphasising fast for 1 s. The protocol was organised into three blocks of contractions (Fig.
209 1). Each block involved 24 explosive contractions (8 without and 16 with superimposed TMS) and 8
210 MVCs (3 without and 5 with superimposed TMS), distributed across four sets. Each set involved six
211 explosive contractions (the final four with TMS stimulation) and two MVCs. In sets 1-3, one of the two
212 MVCs had superimposed TMS (randomly ordered), and in set four, both MVCs had superimposed TMS
213 (Fig. 1). Participants rested 10 s between explosive contractions, 30 s between MVCs, 120 s between
214 sets, and 300 s between blocks. Each block was identical except for the timing of TMS application
215 during explosive contractions, with each block using a different TMS timing condition for these
216 contractions (see next section). The order of the blocks was randomised across participants but held
217 constant across sessions for the same participant. Overall, the 3-block protocol yielded 24 explosive
218 contractions and 9 MVCs without TMS, and 48 explosive contractions (16 per stimulus time condition)
219 and 15 MVCs with TMS.

220 [INSERT FIGURE 1 HERE]

221 ***Fig 1*** *The order of experimental contractions in a block. There were 3 blocks per experimental session and each block involved*
222 *a different condition for explosive contractions performed with superimposed TMS. In one condition/block TMS occurred in*
223 *the early phase, in another the middle phase, and in another the late phase, of explosive contraction. The order that*
224 *blocks/conditions were completed was randomised. Each block consisted of 4 sets of contractions as shown. The thin blue bar*
225 *represents explosive contractions, while the wide blue bar represents MVCs. The red triangle on the top of the blue bars*
226 *represents the superimposed TMS*

227 [INSERT FIGURE 2 HERE]

228 ***Fig 2*** *Representative traces of motor evoked potentials (full black line) in the vastus medialis and knee extensor torque (empty*
229 *dotted line) in each of the four conditions: Early (45 ms), middle (115 ms), and Late (190 ms) from EMG on-set during explosive*
230 *contractions, and at MVC plateau. The vertical black line across all graphs represents the time of stimulation. The blue line*
231 *in each graph represents when the TMS was triggered in each condition. The red dotted arrows represent the peak-to-peak*
232 *amplitude of the MEPs used for analysis*

233 For superimposed MVCs, the TMS was triggered manually by the same experienced
234 investigator during the torque plateau of the MVC. For explosive contractions, the TMS was triggered
235 automatically when the VM EMG signal of the wired system exceeded a set threshold. The threshold
236 was set in the Spike2 software as the lowest amplitude for that session above the highest peaks and
237 troughs of the baseline noise. Commencing from manually detected EMG on-set (0 ms; determined
238 via the methods of Tillin *et al.*, 2010), the threshold was exceeded after ~5 ms, and triggered the TMS
239 at either 8, 78, or 153 ms (accounting for a 3 ms system delay), for the early, middle, and late-phase
240 conditions, respectively (Fig 2). The centre of the evoked MEPs would then occur at ~45 (early), 115
241 (middle), or 190 ms (late) from manually detected EMG on-set (Fig. 2).

242 *Data screening and analysis*

243 Out of 15 superimposed MVCs, only those where the TMS was delivered >90% of MVC torque
244 were used for further analysis. Out of the 16 superimposed explosive contractions per condition, only
245 those that met the following criteria were used for further analysis: (i) average baseline force did not
246 change by >2 Nm during the 200 ms preceding manually detected force on-set (detected as in Tillin *et*
247 *al.*, 2010); and (ii) there was a genuine attempt at an explosive contraction. A genuine attempt at an
248 explosive contraction was defined as the instantaneous slope of torque-time, just prior to MEP on-set,
249 being within 3 standard deviations (SD) of mean instantaneous slope at the same time point for
250 contractions without TMS. The time points for measuring instantaneous slope were 30, 105, and 180
251 ms after manually detected torque on-set, for early-, middle- and late-phase conditions, respectively.
252 Given the criteria above, the maximum number of MEPs analysed further in each condition was limited
253 to the number of usable contractions in the participant with the lowest number of usable contractions.
254 This was 9 (early), 10 (middle), and 15 (late) for the explosive contractions, and 15 for the MVCs.

255 For useable contractions, MEP amplitude was defined as the peak-to-trough of the
256 superimposed response (Fig. 2) and is reported in absolute terms and normalised to M_{max}. The silent
257 period was measured from the point of stimulation to the resumption of EMG activity after the MEP
258 off set. To determine the resumption of EMG activity, the second derivative of EMG amplitude over
259 time was established (1-ms time constant) and then the signal was rectified. EMG activity resumption
260 was defined when the amplitude of the second derivative increased above 5 SD of the mean baseline
261 (calculated in a 500 ms period prior to the contraction) for 70% of the next 10 ms (adapted from
262 Damron *et al.*, 2008; Fig.3). This automated process was confirmed via manual inspection of the signal
263 as recommended by Damron et al., (2008). MEP amplitude (absolute and normalised to M_{max}
264 separately) and silent periods were extracted on a muscle level before being averaged across the three
265 muscles to obtain a quadriceps mean. This is standard practice in studies looking to relate general
266 quadriceps EMG data to net knee extensor torque and RTD (Tillin *et al.*, 2010; Tillin, Pain and Folland,
267 2012, 2013, 2018; Folland, Buckthorpe and Hannah, 2014; Behrens *et al.*, 2015; Morales-Artacho *et*
268 *al.*, 2018; Cossich and Maffiuletti, 2020).

269 *Averaging methods*

270 For each condition (early, middle, and late explosive phases, and at MVC plateau), mean
271 quadriceps consecutive running averages of the dependent variables were calculated from the first
272 three stimuli/contractions, up to the maximum number of usable contractions: 9 (early), 10 (middle),
273 and 15 (late) for the explosive contractions, and 15 for the MVCs. In the torque and EMG averaging
274 methods, dependent variables were averaged over the best 3 out of the first 10 contractions (9 for
275 early-phase explosive) in each condition. For the torque averaging method, the best 3 explosive
276 contractions were those with the greatest torque just prior to MEP (i.e., at 30, 105, and 180 ms from
277 torque on-set, in early, middle, and late conditions, respectively), whilst the best 3 MVCs were those
278 with the highest average torque over 1 s prior to the MEP. For the EMG averaging method, the best
279 explosive contractions were those with the highest EMG RMS amplitude (averaged of all three

muscles) from on-set to the start of the MEP (i.e., 0-23, 0-98, 0-173 ms, in early, middle, and late, respectively), whilst the best three MVCs were those with the greatest EMG RMS in the 1 s period before MEP.

[INSERT FIGURE 3 HERE]

Fig 1 *Example of how the silent period off-set was detected in the VM of one participant. The data shows the second derivative of the EMG-time trace after rectification. Circled data are an example of SP off-set detection. The orange line represents the detection threshold (5 SD of the mean baseline), whilst the red dot, which is the detected off-set, represents the last time point before the EMG signal crosses threshold, and remains above the threshold for ≥70% of the data points recorded in the next 10 ms*

Statistical analysis

Paired t-tests were used to compare the differences between the test and re-test sessions within each condition for each dependent variable. The relative reliability of TMS responses was assessed via the relative test-retest interclass correlation coefficient (ICC) determined via a two-way mixed-effects model, with the absolute agreement, as suggested for a test-retest design (Koo and Li, 2016). ICCs were interpreted as <0.5 poor, ≥0.5 but <0.75 moderate, ≥0.75 and <0.9 good, and ≥0.9 excellent (Koo and Li, 2016). The coefficient of variation (CV) determined the absolute reliability (degree of fluctuation of repeated measurements within individuals) of dependent variables and was calculated for each participant (100 * test-retest standard deviation (SD) / test-retest mean) before being averaged across participants.

Separate ICCs and CVs were produced for each averaging approach; consecutive running averages and the best three based on both torque and EMG averaging methods. Logarithmic functions ($y = a\ln(x) + \mathrm{k}$) were fit to data describing the relationship between ICC or CV and the number of consecutive contractions/stimuli over which the dependent variables were averaged to establish trends in the data and enable extrapolation of the ICC or CV beyond the maximum number of useable contractions. Only statistically significant ($p<0.05$) logarithmic functions are reported in the results. Logarithmic functions were chosen as they provided excellent fits with significant ($p<0.05$) relationships (r^2=0.97-0.75 and SD=0.08) and did not cross zero values of CV (as with, e.g., exponential

307 fits), which are physiologically impossible. Best-fit logarithmic functions were made in SPSS v.26 (IBM
308 Inc., Armonk, NY, USA). For ICCs, a confidence interval of 95% was used for the lower bound (Lb) and
309 upper bound (Ub), and CVs were calculated using MATLAB (R2021a Update 5, MathWorks Inc., Natick,
310 MA, USA). The significance level was set at $p \leq 0.05$. Data are presented in the results as mean ± SD.

311 **Results**

312 There was no significant difference (p≥0.168) between the test and retest sessions for any of
313 the dependent variables in each condition (Table 1).

	MEP amplitude (mV)		*MEP amplitude (% M_{max})*		*Silent Period (s)*	
	Test	*Re-test*	*Test*	*Re-test*	*Test*	*Re-test*
Early	*0.96 ± 0.40*	*0.92 ± 0.38*	*30.2 ±8.3*	*30.2 ± 9.4*	*0.078 ± 0.002*	*0.078 ± 0.002*
Middle	*0.97 ± 0.47*	*0.95 ± 0.40*	*30.0 ± 6.9*	*31.7 ± 7.8*	*0.083 ± 0.002*	*0.083 ± 0.001*
Late	*1.01 ± 0.45*	*0.96 ± 0.41*	*31.8 ± 7.7*	*31.3 ± 6.9*	*0.088 ± 0.002*	*0.089 ± 0.001*
MVC	*1.10 ± 0.46*	*1.02 ± 0.39*	*35.4 ± 7.3*	*35.5 ± 10.3*	*0.091 ± 0.005*	*0.093 ± 0.004*

314 *Table 1 shows the absolute and normalised MEP amplitude, and silent period for each condition in each session (test and re-*
315 *test). Normalised MEP amplitude is a % of maximal M-wave (M_{max}). Conditions are early (45), middle (115), and late (190)*
316 *phases of explosive contractions, and at the MVC plateau. Data are mean ± SD of the first 10 (9 for early) contractions*
317 *performed in each condition.*

318 *Reliability as a function of the number of MEPs*

319 The test-retest ICCs for absolute and normalised MEP amplitude generally increased with the
320 number of averaged MEPs (Fig.3 A, B), whilst the test-retest CV for the same variables generally
321 decreased (Fig.3 C, D). These trends indicate increasing test-retest MEP amplitude reliability with
322 increasing numbers of averaged MEPs. The exception to these general trends was the late phase. In
323 this condition, although the CV of normalised MEP amplitude was related to the number of averaged
324 MEPs (Fig 3D), the CV of absolute MEP amplitude, the ICC of absolute MEP amplitude, and the ICC of
325 normalised MEP amplitude were not related to the number of averaged MEPs (Figs 3 A, B, C). These
326 trends, apparent upon visual inspection, were supported by logarithmic fits to the data, which were
327 significant in all cases aside from the late phase cases highlighted in the previous sentence (Table 2).

328 ICCs were generally higher for absolute MEP amplitude (Fig 3A) than normalised MEP
329 amplitude (Fig 3B). For absolute MEP amplitude, good ICC (ICC>0.75) was found within just three

330 averaged MEPs for all conditions except middle (where it was attained within nine averaged MEPs).
331 For normalised MEP amplitude, good ICC was only achieved in the early condition (attained within
332 seven contractions), whilst other conditions only reached moderate ICCs (ICC>0.50). CV tended to be
333 similar for absolute and normalised MEP amplitudes, reaching 10-15% values for all conditions within
334 seven contractions (Figs 3C, 3D). We were interested in identifying the number of averaged MEPs
335 needed to attain good ICC (ICC>0.75) and CV<10% for all variables and conditions. As is noticeable
336 from Fig 3 A-D, this was not consistently reached using the number of MEPs measured in this study
337 (9-15, depending on the condition). Therefore, the fitted logarithmic functions provided estimates by
338 extrapolating beyond fitted data where necessary (see Table 2 "n of" columns).

339 In contrast to the general trends for MEP amplitude reliability, the silent period reliability did
340 not vary as a function of number of averaged MEPs (Fig.3E, F). In support, attempted logarithmic fits
341 were all non-significant (p>0.05; fits not shown). Moderate ICCs (0.5≤ICC≤0.75) were generally
342 observed for the silent period in all conditions (Fig 3E). CVs for the silent period (Fig 3F) were lower
343 than for MEP amplitude, particularly during MVC.

344

[INSERT FIGURE 4 HERE]

Fig 4 *test-retest ICC (A , B, E) and CV (C, D, F) for dependent variables as a function of the number of consecutive contractions over which data are averaged. Dependent variables are absolute (A and C) and normalised (B and D) MEP amplitude, and silent period (E and F). For normalised data, MEP amplitude is normalised to maximal M-wave. MEP amplitudes and silent periods were recorded at the plateau of MVCs, and at 3 different time points from activation on-set during explosive contractions: early (45 ms), middle (115 ms), and Late (190 ms). Logarithmic functions (y=a ln(x)+k) were fitted to data and are plotted as a dotted line for conditions where the fit was significant (p<0.05)*

ICC

MEP absolute	a	k	R^2	p	n of	MEP normalised	a	k	R^2	p	n of
Early	0.116	0.704	0.96	0.001	2	Early	0.115	0.537	0.95	0.001	7
Middle	0.231	0.265	0.92	0.001	9	Middle	0.392	-0.395	0.84	0.002	19*
Late	0.002	0.819	-0.20	0.950	-	Late	-0.093	0.660	0.13	0.220	-
MVC	0.427	0.200	0.93	0.001	4	MVC	0.154	0.273	0.85	0.002	23*

CV

MEP absolute	a	k	R^2	p	n of	MEP normalised	a	k	R^2	p	n of
Early	-5.17	23.38	0.90	0.001	14*	Early	-4.77	24.28	0.87	0.001	20*
Middle	-7.23	29.55	0.94	0.001	15*	Middle	-5.41	22.61	0.93	0.001	11*
Late	-1.76	15.82	0.16	0.210	-	Late	-3.04	18.71	0.95	0.001	18*
MVC	-2.16	15.19	0.68	0.013	11	MVC	-2.90	19.04	0.79	0.005	23*

*Table 2. Coefficients (a and k), the goodness of fit (R^2) of the logarithmic functions $y = a\,ln(x) + k$. Functions were fit to describe the relationship between test-retest ICC or CV and the number of consecutive contractions over which MEP amplitudes were averaged. The 'n of' is the number of MEP amplitudes averaged to reach ICC≥0.75 or CV≤10%. The * denotes where 'n of' has been extrapolated beyond the measured data. MEP amplitudes were recorded at the plateau of MVCs, and at three different time points from EMG on-set during explosive contractions: Early (45 ms), middle (115 ms), and Late (190 ms).*

Reliability for best three contractions using EMG or torque methods.

Compared to averaging the first 10 consecutive contractions, the ICC tended to be lower (-0.07±0.01) and CV slightly higher (+1.7±0.2%) for either of the best 3 averaging methods, across all conditions and dependent variables (Table 4). There was no consistency for the torque (best 3) averaging method to be better than the EMG averaging method, nor vice versa, with the higher ICC and lower CV between methods being dependent on condition and variable.

Dependent Variables	*Averaging Method*		
Absolute MEP amplitude	*First 10*	*Best 3*	
		EMG	*Torque*
Early	0.95 (0.84-0.98)	0.93 (0.79-0.98)	0.88 (0.65-0.96)
Middle	0.79 (0.47-0.93)	0.74 (0.26-0.89)	0.69 (0.64-0.97)
Late	0.85 (0.61-0.95)	0.83 (0.56-0.94)	0.84 (0.57-0.94)
MVC	0.85 (0.59-0.95)	0.60 (0.14-0.85)	0.77 (0.43-0.92)
Normalised MEP amplitude	*First 10*	*Best 3*	
		EMG	*Torque*
Early	0.78 (0.39-0.92)	0.72 (0.34-0.90)	0.65 (0.21-0.87)
Middle	0.55 (0.06-0.83)	0.37 (-0.1-0.74)	0.28 (-0.3-0.69)
Late	0.49 (-0.05-0.81)	0.49 (-0.07-0.80)	0.40 (-0.17-0.76)
MVC	0.63 (0.14-0.86)	0.53 (0.02-0.82)	0.72 (0.32-0.90)
Silent Period	*First 10*	*Best 3*	
		EMG	*Torque*
Early	0.62 (0.14-0.88)	0.49 (0.15-0.88)	0.64 (-0.2-0.92)
Middle	0.71 (0.28-0.90)	0.63 (0.21-0.89)	0.68 (0.16-0.93)
Late	0.68 (0.21-0.89)	0.63 (0.13-0.87)	0.63 (0.13-0.87)
MVC	0.71 (0.23-0.89)	0.66 (0.20-0.88)	0.77 (0.42-0.92)

Table 3. test-retest ICC, with 95% confidence interval in parenthesis, of dependent variables determined via 3 different averaging methods: average of the first ten consecutive contractions, and average of the best 3 based on those contractions with the highest EMG or torque prior to the MEP. Dependent variables are absolute MEPs, normalised MEPs (normalised to maximal M-wave), and silent period. MEP data were recorded at the plateau of MVCs, and at 3 different time points from EMG on-set during explosive contractions: early (45 ms), middle (115 ms), and late (190 ms)

Dependent Variables	*Averaging Method*		
Absolute MEP amplitude	*First 10*	*Best 3*	
		EMG	*Torque*
Early	11.7±10	11.9±9.6	16.5±14.6
Middle	12.6±8.9	15.5±11	14.3±10.1
Late	10.7±7.8	12.6±8.5	12.6±8.2
MVC	10.7±6	14.7±11.9	10.0±10.1
Normalised MEP amplitude	*First 10*	*Best 3*	
		EMG	*Torque*
Early	14.0±9.8	11.9±10.6	16.9±14.3
Middle	10.0±7.8	12.3±9.9	13.4±11
Late	11.0±5.1	12.1±7.6	13.1±7.8
MVC	10.0±7.2	17.1±10.3	13.1±9.3
Silent Period	*First 10*	*Best 3*	
		EMG	*Torque*
Early	9.7±9.2	10.3±9.3	8.2±7.3
Middle	7.9±7.1	9.4±7.3	9.0±7.2
Late	7.8±5.7	8.5±5.2	8.9±5.3
MVC	4.1±3.4	4.7±4.1	4.3±2.4

Table 4. test-retest CV mean ± SD of dependent variables determined via 3 different averaging methods: average of the first ten consecutive contractions, and average of the best 3 based on those contractions with the highest EMG or torque prior to the MEP. Dependent variables are absolute MEPs, normalised MEPs (normalised to maximal M-wave), and silent period. MEP data were recorded at the plateau of MVCs, and at 3 different time points from EMG on-set during explosive contractions: early (45 ms), middle (115 ms), and late (190 ms)

Discussion

This is the first study to investigate the absolute (ICC) and relative (CV) test-retest reliability of TMS responses (MEP amplitude and silent period duration) recorded at different time points (early,

378 middle, and late) during explosive voluntary contractions, and at the plateau of MVCs. In general, no
379 one condition (explosive early, middle, late, or MVC plateau) produced consistently more reliable
380 (absolute or relative) TMS responses across the different averaging methods than another. In all
381 conditions except the explosive late phase, both absolute and relative reliability of MEP amplitude
382 (absolute and normalised) generally increased with an increase in the number of consecutive stimuli
383 (separate contractions) averaged. In contrast, increasing the number of averaged stimuli did not
384 improve silent period reliability in any condition. This study also found averaging the best 3 out of the
385 first 10 contractions based on EMG amplitude or torque output, produced comparable, albeit slightly
386 poorer, reliability than averaging the same 10 contractions for all TMS responses and conditions.

387 *Reliability during MVCs*

388 To date, only a handful of studies have investigated the test-retest reliability of MEPs evoked
389 at the MVC plateau, and these have all been based on measures averaged across 3-5 MVCs (Kamen,
390 2004; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016). For comparison within this section,
391 we will focus on our findings within this range of 3-5 contractions, with the influence of higher
392 numbers of contractions being discussed later.

393 Within 3-5 MVCs, we observed moderate ICC (0.50-0.75) for absolute and normalised MEP
394 amplitudes, which is within the range (0.47-0.79) of what others have observed (Kamen, 2004;
395 Sidhu, Bentley and Carroll, 2009; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016; Table 5 in
396 supplementary material). We also observed moderate ICC for silent periods at MVC plateau (0.66-
397 0.70) which was comparable to what has previously been reported for the rectus femoris (0.70;
398 Mileva, Sumners and Bowtell, 2012; 0.61-70; Di Virgilio *et al.*, 2022) and lower than reported for the
399 tibialis anterior (0.93-0.95; Souron *et al.*, 2016; Table 5 in supplementary material). For test-retest
400 CV, within 3-5 MVCs, we observed 11-16% values for absolute and normalised MEP amplitude and
401 below 5% for the silent period. One previous study has reported test-retest CV for TMS responses at
402 MVC plateau in the tibialis anterior (Souron *et al.*, 2016) and reported slightly lower CVs for MEP

403 amplitude (10-11%) and a slightly higher CV for silent period (9%), compared to our results for the
404 quadriceps. A more detailed comparison of our results and those of previous studies is difficult
405 because of the disparity in methods for factors such as the type of ICC used, muscles involved, TMS
406 intensity used, and method of calculating silent period. Nevertheless, based on our results and
407 those of previous studies, averaging data across only 3-5 MVCs should provide moderate ICCs and
408 CVs<16% for MEP amplitudes and silent periods.

409 Independently of the number of averaged MEPs, absolute MEP amplitude (V) at MVC plateau
410 showed consistently better ICC values than normalised MEP amplitude (% M_{max}), and this pattern was
411 consistent for other conditions also. In contrast, a previous study of the tibialis anterior (Souron *et*
412 *al.*, 2016) found ICC for MEP amplitude at the MVC plateau to be similar between absolute and
413 normalised data. This difference between our study and Souron et al. (2016) may be due to the
414 different muscle groups used, although there were other methodological differences (e.g., process for
415 determining coil position and intensity), again making a direct comparison difficult. The greater ICC in
416 absolute compared to normalised MEP amplitude that we observed might originate from the ICC
417 calculation itself. ICC scores will be influenced by inter-participant variability, with greater variability
418 likely contributing to greater ICCs (Koo and Li, 2016). Normalising the MEP amplitudes to M_{max} will
419 control for some of the factors that cause inter-participant variability, such as muscle size and
420 adiposity (Besomi *et al.*, 2020), which in turn might reduce ICCs. In contrast to ICC, CV measurements
421 are not influenced by inter-participant variability which may explain why CVs were generally similar
422 for absolute and normalised MEP amplitudes.

423 *Reliability during explosive contractions*

424 To our knowledge, no prior study has investigated the reliability of TMS responses during
425 explosive contractions. We had suspected the reliability of TMS responses to be lowest in early-phase
426 explosive contractions and highest at the MVC plateau, as this is commonly observed for torque and
427 EMG amplitudes in these different conditions (Folland, Buckthorpe and Hannah, 2014; Tillin and

428 Folland, 2014). However, our findings indicated the test-retest reliability of TMS responses was similar
429 for the different conditions, an observation that appeared unrelated to the number of contractions
430 averaged. Just prior to the onset of rapid contractions, in a period of time that overlaps with the early
431 phase of EMG measurements in the current study, there is an increase in subthreshold cortical
432 excitability and increased probability in evoking a detectable MEP (Mackinnon and Rothwell, 2000).
433 Early subthreshold cortical excitability changes consistently during explosive contractions, but the
434 probability of conversion to detectable voluntary motor output in surface EMG is less reliable (Folland,
435 Buckthorpe and Hannah, 2014; Tillin and Folland, 2014). At later phases in the contraction,
436 corticospinal excitability and voluntary motor output variability align more closely, explaining why
437 early phase EMG and torque measurements are less reliable compared to MEP amplitudes, which
438 remain consistent across phases.

439 The only exception to there being no clear differences in reliability between conditions was
440 the observation that CV for the silent period was generally highest for the early phase and lowest for
441 the MVC plateau (Fig. 2F). This may be associated with the lower overall length of the silent period in
442 the early phase compared to the MVC plateau (Table 1). Given the CV is a measure of variability
443 relative to the mean, a condition with a lower mean silent period (as is the case for the explosive early
444 phase), will be susceptible to higher CV values where variability is constant. The shorter silent period
445 in the early phase may be due to neural drive in this phase being predominantly feedforward (Škarabot
446 *et al.*, 2021) and less affected by afferent feedback or intracortical circuitry causing inhibition.

447 *Number of averaged MEPs*

448 For absolute and normalised MEPs, ICCs generally increased, and CVs decreased, with an
449 increasing number of contractions, in all conditions except the late phase. This is in agreement with
450 studies on MEP amplitudes recorded at rest or at submaximal force, which also observed improved
451 reliability by increasing the number of averaged stimuli (Goldsworthy, Hordacre and Ridding, 2016;
452 Biabani et al., 2018; Brownstein et al., 2018). Our results show that the conditions in our study, except

453 for the late-explosive phase, require 11-15 contractions (absolute MEP amplitude) and 18-23
454 contractions (normalised MEP amplitude) to ensure both good ICCs (≥0.75) and CVs≤10%. Further,
455 although caution is required when extrapolating the logarithmic functions reported in this study well
456 beyond the number of contractions measured, the shape of the logarithmic functions indicates little
457 benefit to reliability will be gained by sampling beyond the abovementioned ranges. Variability in the
458 MEP responses in our study are likely affected by the TMS intensity we used (140% AMT), which
459 evokes MEPs in the quadriceps half-way up the impulse-response relationship for the quadriceps
460 (Temesi, Ly and Millet, 2017). As this is the steepest section of the impulse-response relationship,
461 small changes in corticospinal excitability can greatly affect MEP amplitude (Temesi, Ly and Millet,
462 2017). Combined with the intrinsic fluctuations in ongoing oscillatory activity within the cortical area
463 (Hordacre et al., 2017), this likely explains the inherent variability in MEP amplitudes, which seems to
464 limit test-retest reliability, even with consecutive averaging. Nevertheless, based on our results, we
465 recommend future investigators consider collecting and averaging MEP amplitudes across more than
466 the standard 3-5 MVCs (or explosive contractions) used in previous studies, if this can be managed
467 within their protocol, with an optimal range likely falls between 10-20.

468 Unlike the other conditions, MEP amplitude reliability in the late-explosive phase (190 ms)
469 was not improved by increasing the number of averaged contractions. This implies that variability in
470 MEP amplitude during the late phase of explosive contraction remains constant and within a narrow
471 range from contraction to contraction, unlike during the earlier phases. We can only speculate about
472 the physiological factors explaining this observation. However, potentially afferent feedback, which
473 will be greater in the later than earlier phases of explosive contraction (Škarabot *et al.*, 2021), inhibits
474 the neural drive to muscle and so maybe limits the range of possible MEP amplitudes in the late phase
475 of explosive contraction.

476 In the silent period, ICC and CV remained constant despite increases in the number of
477 averaged stimuli. Thus, variability in the silent period appears to be consistent and remain within a

478 narrow range of possibilities from contraction to contraction. Factors that are likely contributing to
479 the variability in silent period include the intensity of stimulation – with lower intensities resulting in
480 higher variability (Damron *et al.*, 2008) – and differences in motor threshold compared to MEP as
481 changes in response curve reduce variability (Wassermann *et al.*, 1993). Our results, obtained at 140%
482 AMT, show that collecting data or more than 3 explosive contractions or MVCs will nt provide
483 meaningful improvements in the reliability of silent period measurament.

484 In studies on explosive contractions, RFD is often averaged from the best 3 out of as many as
485 10 or more contractions (Maffiuletti *et al.*, 2016). Thus, if intending to relate TMS responses measured
486 during explosive contractions to RFD, it may be pertinent to also average the TMS responses in the
487 best 3 contractions. Of the two methods we used for determining the best 3 (out of the first 10)
488 contractions (torque and EMG methods), neither showed consistently better reliability than the other
489 across all conditions and variables. However, both methods for all conditions provided comparable
490 ICCs and CVs to the average of the first 10 contractions (Tables 3 and 4). It therefore appears that
491 selecting the best 3 contractions to average MEP amplitudes minimises the influence of MEP
492 variability to a similar extent as increasing the number of averaged stimuli over consecutive
493 contractions from 3 to 10. Thus, averaging the best three contractions may be a viable option for
494 investigators in the future, particularly if looking to relate MEP amplitude measurements to RTD.

495 *Conclusion*

496 Regardless of the averaging method or reliability metric (ICC and CV), no specific condition
497 produced consistently more reliable MEP amplitudes (absolute or normalised), and silent periods
498 than another. Generally, increasing the number of averaged consecutive contractions increased the
499 reliability for MEPs amplitude (absolute and normalised) in all conditions except for late explosive
500 phase, but did not improve the reliability of silent period duration. Thus, it is advisable to collect
501 more than the typical 3-5 contractions, as used by previous studies involving TMS during MVC, to
502 maximise reliability for MEP amplitude; however, 3-5 contractions will suffice for silent period

503 measurements. Alternatively, investigators could opt to average the best 3 contractions based on
504 those with the highest torque or EMG prior to MEP, which provides comparable reliability to
505 averaging the first 10 contractions.

506

507 The authors have no relevant financial or non-financial interests to disclose.

508

509 Bibliography

510 Ansdell, P. *et al.* (2019) 'Menstrual cycle-associated modulations in neuromuscular function and
511 fatigability of the knee extensors in eumenorrheic women', *Journal of Applied Physiology*, 126(6), pp.
512 1701–1712. doi: 10.1152/japplphysiol.01041.2018.

513 Balady, G. J. *et al.* (1998) 'Recommendations for cardiovascular screening, staffing, writing group',
514 *Medicine and Science in Sports and Exercise1*, 30(6), pp. 1009–1018. Available at:
515 http://journals.lww.com/acsm-
516 msse/Fulltext/1998/06000/AHA_ACSM_Joint_Position_Statement__Recommendations.34.aspx.

517 Behrens, M. *et al.* (2015) 'Caffeine-induced increase in voluntary activation and strength of the
518 quadriceps muscle during isometric, concentric and eccentric contractions', *Scientific Reports*,
519 5(October 2014), pp. 1–10. doi: 10.1038/srep10209.

520 Besomi, M. *et al.* (2020) 'Consensus for experimental design in electromyography (CEDE) project:
521 Amplitude normalisation matrix', *Journal of Electromyography and Kinesiology*, 53(June), p. 102438.
522 doi: 10.1016/j.jelekin.2020.102438.

523 Biabani, M. *et al.* (2018) 'The minimal number of TMS trials required for the reliable assessment of
524 corticospinal excitability, short interval intracortical inhibition, and intracortical facilitation',

525 *Neuroscience Letters*, 674(March), pp. 94–100. doi: 10.1016/j.neulet.2018.03.026.

526 Brownstein, C. G. *et al.* (2018) 'An optimal protocol for measurement of corticospinal excitability,
527 short intracortical inhibition and intracortical facilitation in the rectus femoris', *Journal of the*
528 *Neurological Sciences*, 394(June), pp. 45–56. doi: 10.1016/j.jns.2018.09.001.

529 Buckthorpe, M. W. *et al.* (2012) 'Reliability of neuromuscular measurements during explosive
530 isometric contractions, with special reference to electromyography normalisation techniques',
531 *Muscle and Nerve*, 46(4), pp. 566–576. doi: 10.1002/mus.23322.

532 Cossich, V. and Maffiuletti, N. A. (2020) 'Early vs. late rate of torque development: Relation with
533 maximal strength and influencing factors', *Journal of Electromyography and Kinesiology*,
534 55(October), p. 102486. doi: 10.1016/j.jelekin.2020.102486.

535 Damron, L. A. *et al.* (2008) 'Quantification of the corticospinal silent period evoked via transcranial
536 magnetic stimulation', *Journal of Neuroscience Methods*, 173(1), pp. 121–128. doi:
537 10.1016/j.jneumeth.2008.06.001.

538 Folland, J. P., Buckthorpe, M. W. and Hannah, R. (2014) 'Human capacity for explosive force
539 production: Neural and contractile determinants', *Scandinavian Journal of Medicine & Science in*
540 *Sports*, 24(6), pp. 894–906. doi: 10.1111/sms.12131.

541 Goldsworthy, M. R., Hordacre, B. and Ridding, M. C. (2016) 'Minimum number of trials required for
542 within- and between-session reliability of TMS measures of corticospinal excitability', *Neuroscience*,
543 320, pp. 205–209. doi: 10.1016/j.neuroscience.2016.02.012.

544 Groppa, S. *et al.* (2012) 'A practical guide to diagnostic transcranial magnetic stimulation: Report of
545 an IFCN committee', *Clinical Neurophysiology*, 123(5), pp. 858–882. doi:
546 10.1016/j.clinph.2012.01.010.

Hermens, H. J. *et al.* (1999) 'European Recommendations for Surface ElectroMyoGraphy', *Roessingh Research and Development*, pp. 8–11.

Hordacre, B. *et al.* (2017) 'Variability in neural excitability and plasticity induction in the human cortex: A brain stimulation study', *Brain Stimulation*, 10(3), pp. 588–595. doi: 10.1016/j.brs.2016.12.001.

de Jonge, X. J., Thompson, B. and Han, A. (2019) *Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise*, *Medicine & Science in Sports & Exercise*. doi: 10.1249/MSS.0000000000002073.

Kamen, G. (2004) 'Reliability of motor-evoked during resting and active contraction conditions', *Medicine and Science in Sports and Exercise*, 36(9), pp. 1574–1579. doi: 10.1249/01.MSS.0000139804.02576.6A.

Koo, T. K. and Li, M. Y. (2016) 'A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research', *Journal of Chiropractic Medicine*, 15(2), pp. 155–163. doi: 10.1016/j.jcm.2016.02.012.

Lewis, G. N., Signal, N. and Taylor, D. (2013) 'Reliability of lower limb motor evoked potentials in stroke and healthy populations: How many responses are needed?', *Clinical Neurophysiology*, 125(4), pp. 748–754. doi: 10.1016/j.clinph.2013.07.029.

Luc, B. A. *et al.* (2014) 'Reliability of Corticomotor Excitability in Leg and Thigh Musculature at 14 and 28 Days', *Journal of Sport Rehabilitation*, 23(4), pp. 330–338. doi: 10.1123/JSR.2013-0069.

Mackinnon, C. D. and Rothwell, J. C. (2000) 'Padrão Trifásico - Time_varying changes in corticospinal excitability', pp. 633–645.

Maffiuletti, N. A. *et al.* (2016) 'Rate of force development: physiological and methodological

569 considerations', *European Journal of Applied Physiology*, 116(6), pp. 1091–1116. doi:
570 10.1007/s00421-016-3346-6.

571 McKay, A. K. A. *et al.* (2022) 'Defining Training and Performance Caliber: A Participant Classification
572 Framework', *International Journal of Sports Physiology and Performance*, 17(2), pp. 317–331. doi:
573 10.1123/ijspp.2021-0451.

574 Mileva, K. N., Sumners, D. P. and Bowtell, J. L. (2012) 'Decline in voluntary activation contributes to
575 reduced maximal performance of fatigued human lower limb muscles', *European Journal of Applied*
576 *Physiology*, 112(12), pp. 3959–3970. doi: 10.1007/s00421-012-2381-1.

577 Morales-Artacho, A. J. *et al.* (2018) 'Associations of the Force-velocity Profile with Isometric Strength
578 and Neuromuscular Factors', *International Journal of Sports Medicine*, 39(13), pp. 984–994. doi:
579 10.1055/a-0644-3742.

580 Rossi, S. *et al.* (2011) 'Screening questionnaire before TMS: An update', *Clinical Neurophysiology*,
581 122(8), p. 1686. doi: 10.1016/j.clinph.2010.12.037.

582 Rossi, S. *et al.* (2020) 'Safety and recommendations for TMS use in healthy subjects and patient
583 populations, with updates on training, ethical and regulatory issues: Expert Guidelines', *Clinical*
584 *Neurophysiology*, 132(1), pp. 269–306. doi: 10.1016/j.clinph.2020.10.003.

585 Rossini, P. M. *et al.* (2015) 'Non-invasive electrical and magnetic stimulation of the brain, spinal cord,
586 roots and peripheral nerves: Basic principles and procedures for routine clinical and research
587 application: An updated report from an I.F.C.N. Committee', *Clinical Neurophysiology*, 126(6), pp.
588 1071–1107. doi: 10.1016/j.clinph.2015.02.001.

589 De Ruiter, C. J. *et al.* (2004) 'Initial phase of maximal voluntary and electrically stimulated knee
590 extension torque development at different knee angles', *Journal of Applied Physiology*, 97(5), pp.
591 1693–1701. doi: 10.1152/japplphysiol.00230.2004.

Säisänen, L. *et al.* (2008) 'Factors influencing cortical silent period: Optimised stimulus location, intensity and muscle contraction', *Journal of Neuroscience Methods*, 169(1), pp. 231–238. doi: 10.1016/j.jneumeth.2007.12.005.

Sidhu, S. K., Bentley, D. J. and Carroll, T. J. (2009) 'Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation', *Muscle and Nerve*, 39(2), pp. 186–196. doi: 10.1002/mus.21064.

Škarabot, J. *et al.* (2019) 'Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments', *European Journal of Neuroscience*, 49(10), pp. 1254–1267. doi: 10.1111/ejn.14321.

Škarabot, J. *et al.* (2021) 'The knowns and unknowns of neural adaptations to resistance training', *European Journal of Applied Physiology*, pp. 675–685. doi: 10.1007/s00421-020-04567-3.

Škarabot, J. *et al.* (2023) 'Decoding firings of a large population of human motor units from high-density surface electromyogram in response to transcranial magnetic stimulation', *The Journal of Physiology*, 601(10), pp. 1719–1744. doi: 10.1113/JP284043.

Souron, R. *et al.* (2016) 'Reliability of the functional measures of the corticospinal pathways to dorsiflexor muscles during maximal voluntary contractions', *Journal of the Neurological Sciences*, 369(October), pp. 368–374. doi: 10.1016/j.jns.2016.09.003.

Sundstrup, E. *et al.* (2010) 'Muscle function and postural balance in lifelong trained male footballers compared with sedentary elderly men and youngsters.', *Scandinavian journal of medicine & science in sports*, 20 Suppl 1, pp. 90–97. doi: 10.1111/j.1600-0838.2010.01092.x.

Tallent, J. *et al.* (2017) 'Enhanced corticospinal excitability and volitional drive in response to shortening and lengthening strength training and changes following detraining', *Frontiers in Physiology*, 8(FEB). doi: 10.3389/fphys.2017.00057.

Temesi, J., Ly, S. N. and Millet, G. Y. (2017) 'Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function', *Journal of the Neurological Sciences*, 375, pp. 442–449. doi: 10.1016/j.jns.2017.02.037.

Tillin, N. A. *et al.* (2010) 'Neuromuscular performance of explosive power athletes versus untrained individuals', *Medicine and Science in Sports and Exercise*, 42(4), pp. 781–790. doi: 10.1249/MSS.0b013e3181be9c7e.

Tillin, N. A. and Folland, J. P. (2014) 'Maximal and explosive strength training elicit distinct neuromuscular adaptations, specific to the training stimulus', *European Journal of Applied Physiology*, 114(2), pp. 365–374. doi: 10.1007/s00421-013-2781-x.

Tillin, N. A., Pain, M. T. G. and Folland, J. (2013) 'Explosive force production during isometric squats correlates with athletic performance in rugby union players', *Journal of Sports Sciences*, 31(1), pp. 66–76. doi: 10.1080/02640414.2012.720704.

Tillin, N. A., Pain, M. T. G. and Folland, J. P. (2012) 'Short-term training for explosive strength causes neural and mechanical adaptations', *Experimental Physiology*, 97(5), pp. 630–641. doi: 10.1113/expphysiol.2011.063040.

Tillin, N. A., Pain, M. T. G. and Folland, J. P. (2018) 'Contraction speed and type influences rapid utilisation of available muscle force: Neural and contractile mechanisms', *Journal of Experimental Biology*, 221(24). doi: 10.1242/jeb.193367.

Todd, G., Taylor, J. L. and Gandevia, S. C. (2016) 'Measurement of voluntary activation based on transcranial magnetic stimulation over the motor cortex', *Journal of Applied Physiology*, 121(3), pp. 678–686. doi: 10.1152/japplphysiol.00293.2016.

Del Vecchio, A. *et al.* (2019) 'You are as fast as your motor neurons: speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans',

638 *Journal of Physiology*, 597(9), pp. 2445–2456. doi: 10.1113/JP277396.

639 Di Virgilio, T. G. *et al.* (2022) 'The Reliability of Transcranial Magnetic Stimulation-Derived
640 Corticomotor Inhibition as a Brain Health Evaluation Tool in Soccer Players', *Sports Medicine - Open*,
641 8(1). doi: 10.1186/s40798-021-00399-3.

642 Wassermann, E. M. *et al.* (1993) 'Topography of the inhibitory and excitatory responses to
643 transcranial magnetic stimulation in a hand muscle', *Electroencephalography and Clinical*
644 *Neurophysiology/ Evoked Potentials*, 89(6), pp. 424–433. doi: 10.1016/0168-5597(93)90116-7.

645 Wheaton, L. A. *et al.* (2009) 'Reliability of TMS motor evoked potentials in quadriceps of subjects
646 with chronic hemiparesis after stroke', *Journal of the Neurological Sciences*, 276(1–2), pp. 115–117.
647 doi: 10.1016/j.jns.2008.09.012.

648

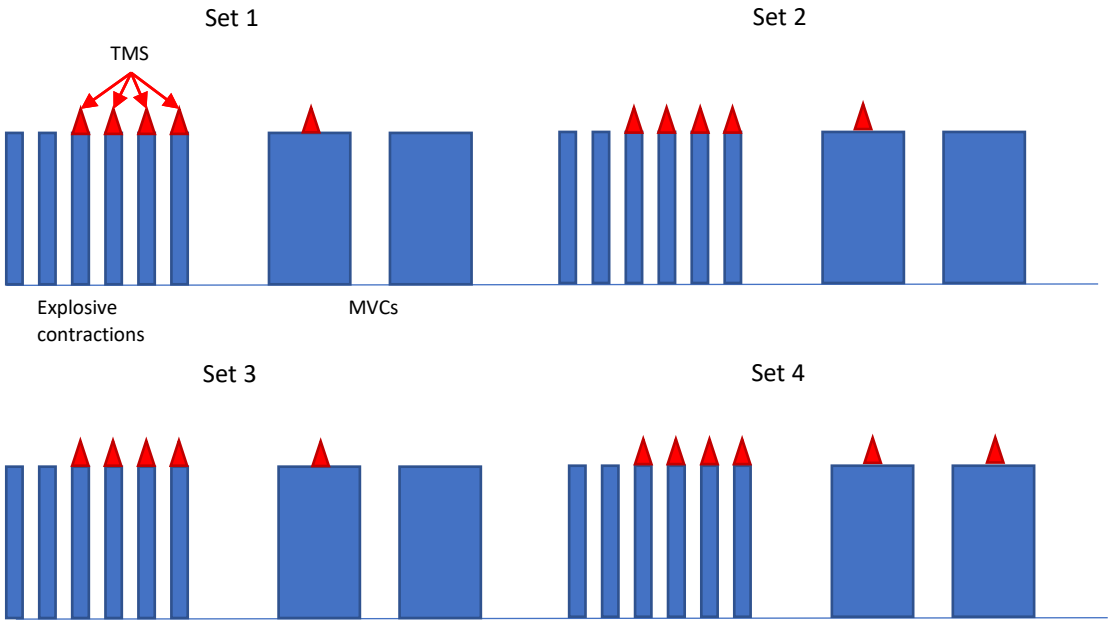

Fig 1

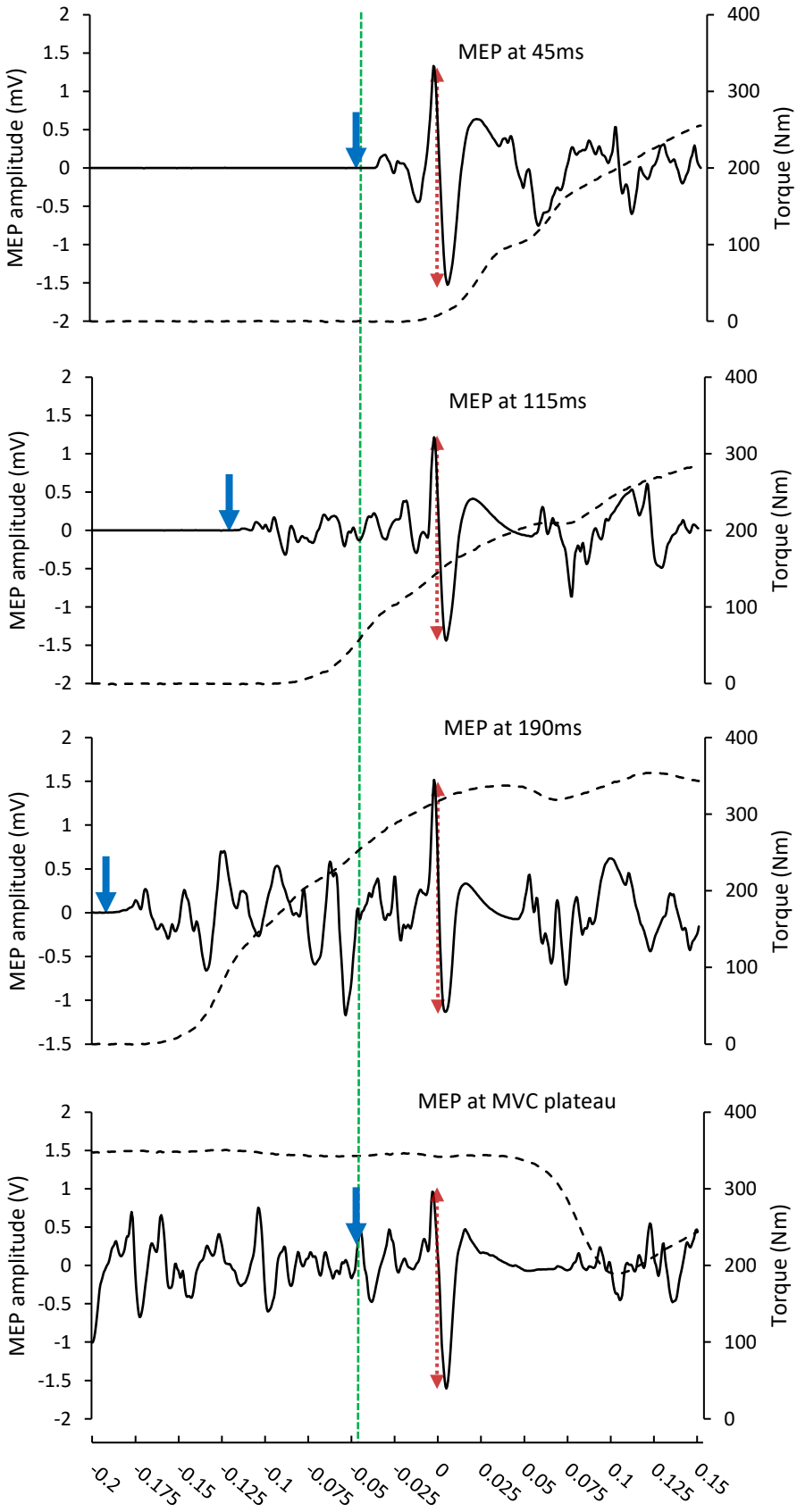
MEP at 45ms
MEP at 115ms
MEP at 190ms
MEP at MVC plateau
MEP amplitude (mV)
MEP amplitude (V)
Torque (Nm)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
400
300
200
100
0
-0.2
-0.175
-0.15
-0.125
-0.1
-0.075
-0.05
-0.025
0
0.025
0.05
0.075
0.1
0.125
0.15

Fig 2

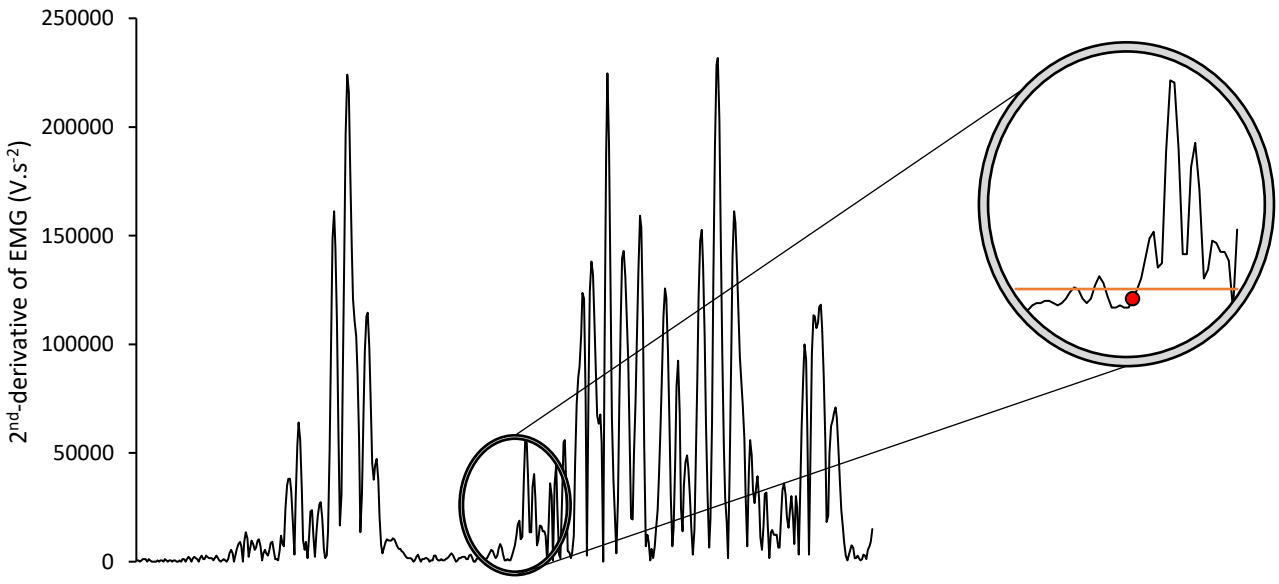
250000
200000
150000
100000
50000
0
2nd-derivative of EMG (V.s-2)

Fig 3

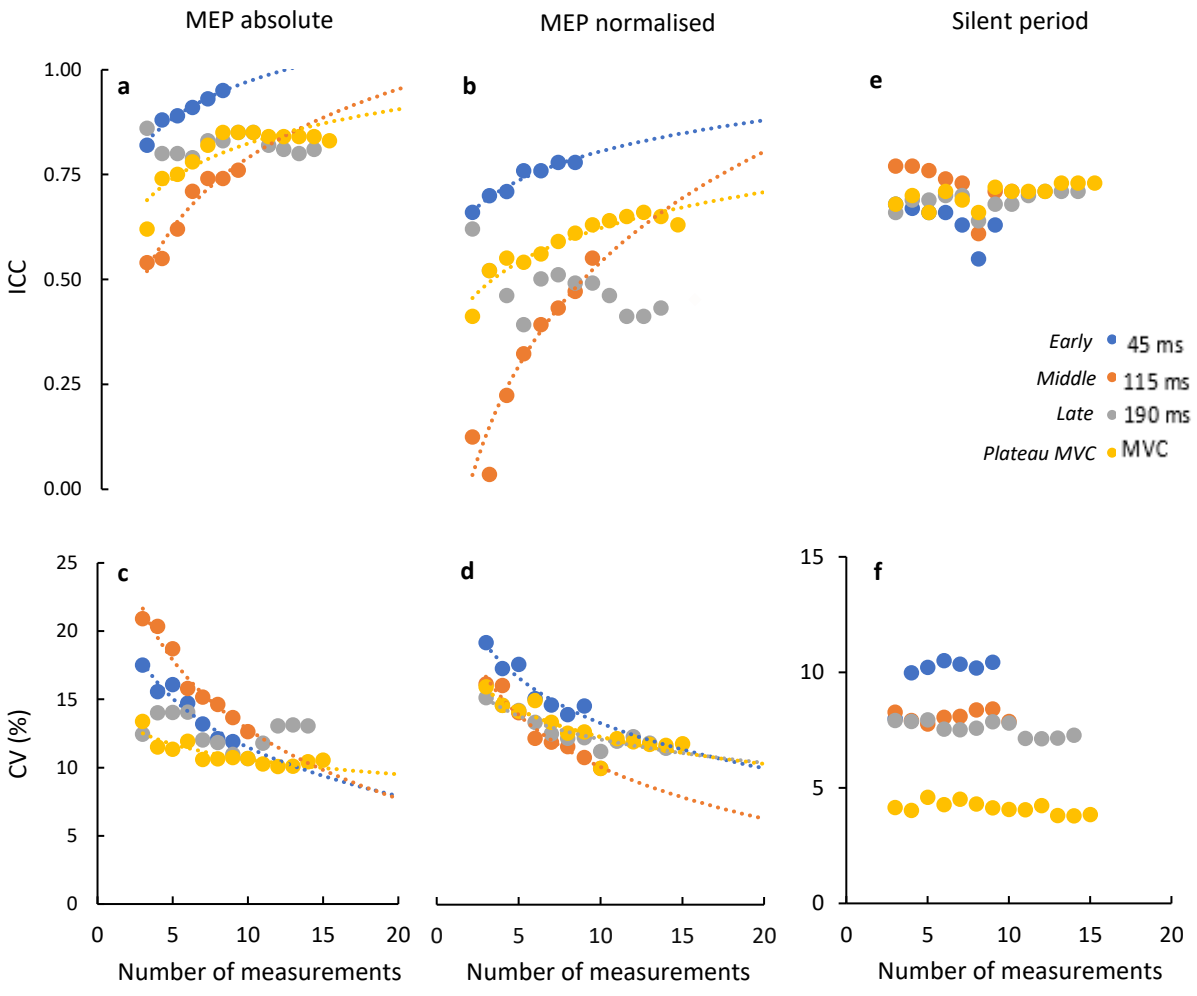
MEP absolute
MEP normalised
Silent period
a
b
e
c
d
f
ICC
CV (%)
1.00
0.75
0.50
0.25
0.00
25
20
15
10
5
0
Number of measurements
Early 45 ms
Middle 115 ms
Late 190 ms
Plateau MVC MVC

Fig 4