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

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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

51 The rate of torque development (RTD) measures the ability of muscles to rapidly increase
52 force around a joint (Tillin and Folland, 2014), and is functionally important where time to develop
53 force is limited, such as sprinting (Tillin, Pain and Folland, 2013) or balance recovery (Sundstrup *et*
54 *al.*, 2010). RTD is often measured in early (0-50 ms), middle (50-100 ms) and late (>100 ms) phases
55 of explosive contraction performed from rest (Maffiuletti *et al.*, 2016), and different physiological
56 factors limit the RTD in these separate phases. One limiting factor is the neural drive to muscle,
57 which positively correlates with early- and middle-phase RTD (Folland, Buckthorpe and Hannah,
58 2014; Del Vecchio *et al.*, 2019). Despite the relevance of neural drive to RTD, the corticospinal
59 mechanisms affecting RTD are not well established.

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

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

76 (0.61-0.70; (Di Virgilio *et al.*, 2022)) to excellent in the soleus and tibialis anterior (0.93-0.95; Mileva,
77 Sumners and Bowtell, 2012; Souron *et al.*, 2016) and one study has reported a CV of 8.6% (Souron *et*
78 *al.*, 2016) in the tibialis anterior. It is unclear whether the reliability of MEP amplitudes and silent
79 periods during explosive contractions will be comparable to that observed at the MVC plateau.
80 Typically, the reliability of torque and EMG amplitude measurements is lower in early compared to
81 later phases of explosive contraction, and generally lower for explosive contractions compared to
82 the MVC plateau (De Ruiter *et al.*, 2004; Buckthorpe *et al.*, 2012), so a similar pattern may be
83 observed for the reliability of MEP amplitudes and silent periods.

84 TMS responses are typically averaged across multiple separate stimuli (in separate
85 contractions) to minimise the influence of random variation and improve reliability. Thus, when
86 assessing the test-retest reliability of MEP amplitudes and silent periods, it is important to consider
87 how many stimulations might be required to achieve adequately reliable averages. A relatively high
88 number of stimuli (≥20) are needed to obtain moderate to good test-retest reliability (ICC = 0.50 -
89 0.75; Goldsworthy, Hordacre and Ridding, 2016; Biabani *et al.*, 2018) of MEP amplitudes recorded
90 when the participant is passive. In contrast, as few as four stimuli have been shown to produce good
91 (≥0.75) test-retest ICC for MEP amplitude during submaximal contractions held at a constant force
92 (Wheaton *et al.*, 2009; Lewis, Signal and Taylor, 2013; Temesi, Ly and Millet, 2017). The effect of the
93 number of contractions on test-retest reliability of average TMS responses has not been determined
94 during either MVC or explosive contractions. Previous studies of TMS responses during MVCs have
95 typically only collected and averaged 3-5 responses (Luc *et al.*, 2014; Tallent *et al.*, 2017; Škarabot *et*
96 *al.*, 2019). This could be due to time constraints or concerns over fatigue with multiple contractions;
97 however, it may be at the expense of poor reliability. Thus, there is a need to assess the effect of
98 the number of contractions on the test-retest reliability of average TMS responses during MVCs and
99 explosive contractions.

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

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

115 **Methods**

116 *Participants*

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

125 participants undertook experimental trials exclusively during their self-reported early to mid-
126 follicular phase (first ten days from the first day of menstruation), when endogenous hormone
127 concentration is low and relatively stable (de Jonge, Thompson and Han, 2019). The University of
128 Roehampton ethics committee approved the study, and all participants provided written informed
129 consent before participating.

130 *Overview*

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

140 *Torque measurements and surface electromyography (EMG)*

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

149 The skin was prepared by shaving, cleaning (70% ethanol), and lightly abrading the area
150 where EMG electrodes were placed. A single, bipolar silver-silver-chloride gel-electrode
151 configuration (2-cm diameter and 2-cm inter-electrode distance; Dual Electrode, Noraxon, Arizona,
152 USA) was placed over the belly of each of the rectus femoris (RF), vastus lateralis (VL) and vastus
153 medialis (VM), based on SENIAM guidelines (Hermens *et al.*, 1999). The EMG system has an
154 inherent 312-ms delay, so whilst suitable for measurements involved in the study, EMG signals
155 sampled by it could not be used to detect activation on-set and trigger the TMS in real-time during
156 the explosive contractions (explained below in Experimental procedures). Thus, a second wired
157 bipolar EMG electrode (2 cm diameter and 2 cm inter-electrode distance; Dual Electrode, Biometrics
158 Ltd, Gwent, UK) was placed on the belly of the VM to trigger the TMS during contraction. Electrode
159 locations were marked with a permanent marker pen, and participants were asked to maintain these
160 marks throughout the study by re-applying them if necessary. The three wireless EMG signals were
161 transmitted to a desktop receiver (TeleMYO D.T.S., Noraxon, Arizona, USA) and sampled, along with
162 the single wired EMG signal, at 2000 Hz via the same A/D convertor and software as the force signal.
163 Once offline, wireless EMG signals were filtered (fourth-order Butterworth, band-pass, 6-500 Hz) and
164 time-corrected for the 312-ms delay inherent in the Noraxon system.

165 *Transcranial magnetic stimulation (TMS)*

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

174 (established in the warm-up; see below) with superimposed TMS on each contraction at a
175 submaximal (range 50-60%) stimulator output. The position determined the optimal coil position for
176 the rest of the session, which was subjectively the highest consistent MEP amplitudes (peak-to-peak)
177 over the four superimposed contractions for all three muscles (RF, VL and VM). The optimal coil
178 position was marked by drawing the edge of the coil over the swim cap, ensuring accurate relocation
179 throughout the session. The active motor threshold (AMT) was then determined via a series of 20%
180 MVC torque contractions superimposed with TMS stimulator output starting at 39%. The AMT was
181 defined as the minimum TMS intensity required to elicit five visible MEPs amongst the background
182 EMG activity of the VM and RF, out of ten consecutive superimposed contractions. If the muscles
183 had less or more than five visible MEPs, the machine intensity was reduced or increased by 2% of
184 the machine output. We prioritised the VM and RF (not the VL) as these muscles produced more
185 consistent and visible MEPs in pilot testing. Where it was impossible to match AMT for VM and RF,
186 we settled on being one visible MEP away from 5/10 (e.g., 6/10 VM and 4/10 RF). The same
187 investigator held the coil by hand throughout the measurement sessions, continuously monitoring
188 its position and orientation. For TMS delivered during maximal and explosive contractions (see
189 experimental protocol), the intensity was set at 140% of the stimulator output at AMT (Groppa *et*
190 *al.*, 2012; Rossini *et al.*, 2015; Rossi *et al.*, 2020). These procedures were repeated for all sessions.

191 *Femoral nerve electrical stimulation*

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

199 investigator as a series of twitches at incremental intensities were evoked until there was a plateau
200 in the peak-to-peak M-wave amplitude (M_{max}) of all three muscles (RF, VL, VM). The stimulator
201 intensity was then increased to 150% of the intensity at M_{max}, ensuring supramaximal intensity.
202 Three supramaximal twitch contractions were then evoked, each separated by 15 s, and M_{max} was
203 averaged across the three contractions for each muscle. The procedures were repeated for all
204 sessions.

205 *Experimental protocol*

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

223 [INSERT FIGURE 1 HERE]

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

230 [INSERT FIGURE 2 HERE]

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

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

245 *Data screening and analysis*

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

257 number of usable contractions. This was 9 (early), 10 (middle), and 15 (late) for the explosive
258 contractions, and 15 for the MVCs.

259 For useable contractions, MEP amplitude was defined as the peak-to-trough of the
260 superimposed response (Fig. 2) and is reported in absolute terms and normalised to M_{max}. The silent
261 period was measured from the point of stimulation to the resumption of EMG activity after the MEP
262 off set. To determine the resumption of EMG activity, the second derivative of EMG amplitude over
263 time was established (1-ms time constant) and then the signal was rectified. EMG activity
264 resumption was defined when the amplitude of the second derivative increased above 5 SD of the
265 mean baseline (calculated in a 500 ms period prior to the contraction) for 70% of the next 10 ms
266 (adapted from Damron *et al.*, 2008; Fig.3). This automated process was confirmed via manual
267 inspection of the signal as recommended by Damron et al., (2008). MEP amplitude (absolute and
268 normalised to M_{max} separately) and silent periods were extracted on a muscle level before being
269 averaged across the three muscles to obtain a quadriceps mean. This is standard practice in studies
270 looking to relate general quadriceps EMG data to net knee extensor torque and RTD (Tillin *et al.*,
271 2010; Tillin, Pain and Folland, 2012, 2013, 2018; Folland, Buckthorpe and Hannah, 2014; Behrens *et*
272 *al.*, 2015; Morales-Artacho *et al.*, 2018; Cossich and Maffiuletti, 2020).

273 *Averaging methods*

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

282 with the highest average torque over 1 s prior to the MEP. For the EMG averaging method, the best
283 explosive contractions were those with the highest EMG RMS amplitude (averaged of all three
284 muscles) from on-set to the start of the MEP (i.e., 0-23, 0-98, 0-173 ms, in early, middle, and late,
285 respectively), whilst the best three MVCs were those with the greatest EMG RMS in the 1 s period
286 before MEP.

287 [INSERT FIGURE 3 HERE]

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

293 *Statistical analysis*

294 Paired t-tests were used to compare the differences between the test and re-test sessions
295 within each condition for each dependent variable. The relative reliability of TMS responses was
296 assessed via the relative test-retest interclass correlation coefficient (ICC) determined via a two-way
297 mixed-effects model, with the absolute agreement, as suggested for a test-retest design (Koo and Li,
298 2016). ICCs were interpreted as <0.5 poor, ≥0.5 but <0.75 moderate, ≥0.75 and <0.9 good, and ≥0.9
299 excellent (Koo and Li, 2016). The coefficient of variation (CV) determined the absolute reliability
300 (degree of fluctuation of repeated measurements within individuals) of dependent variables and was
301 calculated for each participant (100 * test-retest standard deviation (SD) / test-retest mean) before
302 being averaged across participants.

303 Separate ICCs and CVs were produced for each averaging approach; consecutive running
304 averages and the best three based on both torque and EMG averaging methods. Logarithmic
305 functions ($y = a\ln(x) + \mathrm{k}$) were fit to data describing the relationship between ICC or CV and the
306 number of consecutive contractions/stimuli over which the dependent variables were averaged to
307 establish trends in the data and enable extrapolation of the ICC or CV beyond the maximum number
308 of useable contractions. Only statistically significant (p<0.05) logarithmic functions are reported in

309 the results. Logarithmic functions were chosen as they provided excellent fits with significant
310 ($p<0.05$) relationships (r^2=0.97-0.75 and SD=0.08) and did not cross zero values of CV (as with, e.g.,
311 exponential fits), which are physiologically impossible. Best-fit logarithmic functions were made in
312 SPSS v.26 (IBM Inc., Armonk, NY, USA). For ICCs, a confidence interval of 95% was used for the lower
313 bound (Lb) and upper bound (Ub), and CVs were calculated using MATLAB (R2021a Update 5,
314 MathWorks Inc., Natick, MA, USA). The significance level was set at $p \leq 0.05$. Data are presented in
315 the results as mean ± SD.

316 **Results**

317 There was no significant difference ($p \geq 0.168$) between the test and retest sessions for any of
318 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*

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

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

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

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

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

349

[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).*

<u>Reliability for best three contractions using EMG or torque methods.</u>

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

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

393 *Reliability during MVCs*

394 To date, only a handful of studies have investigated the test-retest reliability of MEPs evoked
395 at the MVC plateau, and these have all been based on measures averaged across 3-5 MVCs (Kamen,
396 2004; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016). For comparison within this section,
397 we will focus on our findings within this range of 3-5 contractions, with the influence of higher
398 numbers of contractions being discussed later.

399 Within 3-5 MVCs, we observed moderate ICC (0.50-0.75) for absolute and normalised MEP
400 amplitudes, which is within the range (0.47-0.79) of what others have observed (Kamen, 2004;
401 Sidhu, Bentley and Carroll, 2009; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016; Table 5 in
402 supplementary material). We also observed moderate ICC for silent periods at MVC plateau (0.66-
403 0.70) which was comparable to what has previously been reported for the rectus femoris (0.70;
404 Mileva, Sumners and Bowtell, 2012; 0.61-70; Di Virgilio *et al.*, 2022) and lower than reported for the
405 tibialis anterior (0.93-0.95; Souron *et al.*, 2016; Table 5 in supplementary material). For test-retest
406 CV, within 3-5 MVCs, we observed 11-16% values for absolute and normalised MEP amplitude and
407 below 5% for the silent period. One previous study has reported test-retest CV for TMS responses at

408 MVC plateau in the tibialis anterior (Souron *et al.*, 2016) and reported slightly lower CVs for MEP
409 amplitude (10-11%) and a slightly higher CV for silent period (9%), compared to our results for the
410 quadriceps. A more detailed comparison of our results and those of previous studies is difficult
411 because of the disparity in methods for factors such as the type of ICC used, muscles involved, TMS
412 intensity used, and method of calculating silent period. Nevertheless, based on our results and
413 those of previous studies, averaging data across only 3-5 MVCs should provide moderate ICCs and
414 CVs<16% for MEP amplitudes and silent periods.

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

429 *Reliability during explosive contractions*

430 To our knowledge, no prior study has investigated the reliability of TMS responses during
431 explosive contractions. We had suspected the reliability of TMS responses to be lowest in early-
432 phase explosive contractions and highest at the MVC plateau, as this is commonly observed for

433 torque and EMG amplitudes in these different conditions (Folland, Buckthorpe and Hannah, 2014;
434 Tillin and Folland, 2014). However, our findings indicated the test-retest reliability of TMS responses
435 was similar for the different conditions, an observation that appeared unrelated to the number of
436 contractions averaged. Just prior to the onset of rapid contractions, in a period of time that overlaps
437 with the early phase of EMG measurements in the current study, there is an increase in subthreshold
438 cortical excitability and increased probability in evoking a detectable MEP (Mackinnon and Rothwell,
439 2000). Early subthreshold cortical excitability changes consistently during explosive contractions,
440 but the probability of conversion to detectable voluntary motor output in surface EMG is less
441 reliable (Folland, Buckthorpe and Hannah, 2014; Tillin and Folland, 2014). At later phases in the
442 contraction, corticospinal excitability and voluntary motor output variability align more closely,
443 explaining why early phase EMG and torque measurements are less reliable compared to MEP
444 amplitudes, which remain consistent across phases.

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

454 *Number of averaged MEPs*

455 For absolute and normalised MEPs, ICCs generally increased, and CVs decreased, with an
456 increasing number of contractions, in all conditions except the late phase. This is in agreement with
457 studies on MEP amplitudes recorded at rest or at submaximal force, which also observed improved

458 reliability by increasing the number of averaged stimuli (Goldsworthy, Hordacre and Ridding, 2016;
459 Biabani et al., 2018; Brownstein et al., 2018). Our results show that the conditions in our study,
460 except for the late-explosive phase, require 11-15 contractions (absolute MEP amplitude) and 18-23
461 contractions (normalised MEP amplitude) to ensure both good ICCs (≥0.75) and CVs≤10%. Further,
462 although caution is required when extrapolating the logarithmic functions reported in this study well
463 beyond the number of contractions measured, the shape of the logarithmic functions indicates little
464 benefit to reliability will be gained by sampling beyond the abovementioned ranges. Variability in
465 the MEP responses in our study are likely affected by the TMS intensity we used (140% AMT), which
466 evokes MEPs in the quadriceps half-way up the impulse-response relationship for the quadriceps
467 (Temesi, Ly and Millet, 2017). As this is the steepest section of the impulse-response relationship,
468 small changes in corticospinal excitability can greatly affect MEP amplitude (Temesi, Ly and Millet,
469 2017). Combined with the intrinsic fluctuations in ongoing oscillatory activity within the cortical area
470 (Hordacre et al., 2017), this likely explains the inherent variability in MEP amplitudes, which seems
471 to limit test-retest reliability, even with consecutive averaging. Nevertheless, based on our results,
472 we recommend future investigators consider collecting and averaging MEP amplitudes across more
473 than the standard 3-5 MVCs (or explosive contractions) used in previous studies, if this can be
474 managed within their protocol, with an optimal range likely falls between 10-20.

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

483 In the silent period, ICC and CV remained constant despite increases in the number of
484 averaged stimuli. Thus, variability in the silent period appears to be consistent and remain within a
485 narrow range of possibilities from contraction to contraction. Factors that are likely contributing to
486 the variability in silent period include the intensity of stimulation – with lower intensities resulting in
487 higher variability (Damron *et al.*, 2008) – and differences in motor threshold compared to MEP as
488 changes in response curve reduce variability (Wassermann *et al.*, 1993). Our results, obtained at
489 140% AMT, show that collecting data or more than 3 explosive contractions or MVCs will nt provide
490 meaningful improvements in the reliability of silent period measurament.

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

503 *Conclusion*

504 Regardless of the averaging method or reliability metric (ICC and CV), no specific condition
505 produced consistently more reliable MEP amplitudes (absolute or normalised), and silent periods
506 than another. Generally, increasing the number of averaged consecutive contractions increased the
507 reliability for MEPs amplitude (absolute and normalised) in all conditions except for late explosive

phase, but did not improve the reliability of silent period duration. Thus, it is advisable to collect more than the typical 3-5 contractions, as used by previous studies involving TMS during MVC, to maximise reliability for MEP amplitude; however, 3-5 contractions will suffice for silent period measurements. Alternatively, investigators could opt to average the best 3 contractions based on those with the highest torque or EMG prior to MEP, which provides comparable reliability to averaging the first 10 contractions.

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656

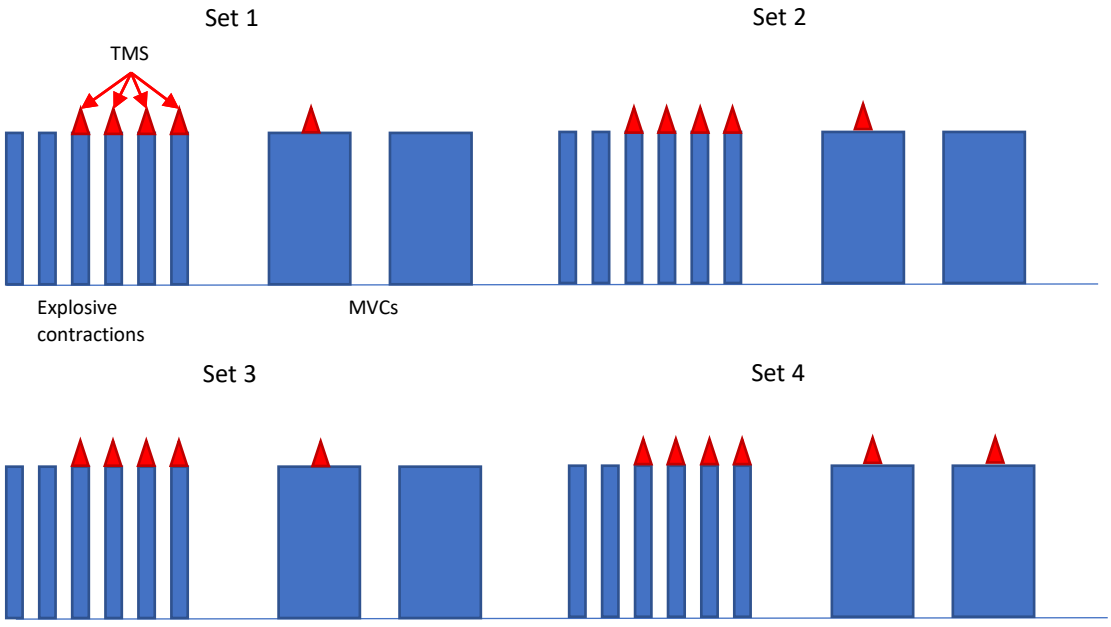

Fig 1

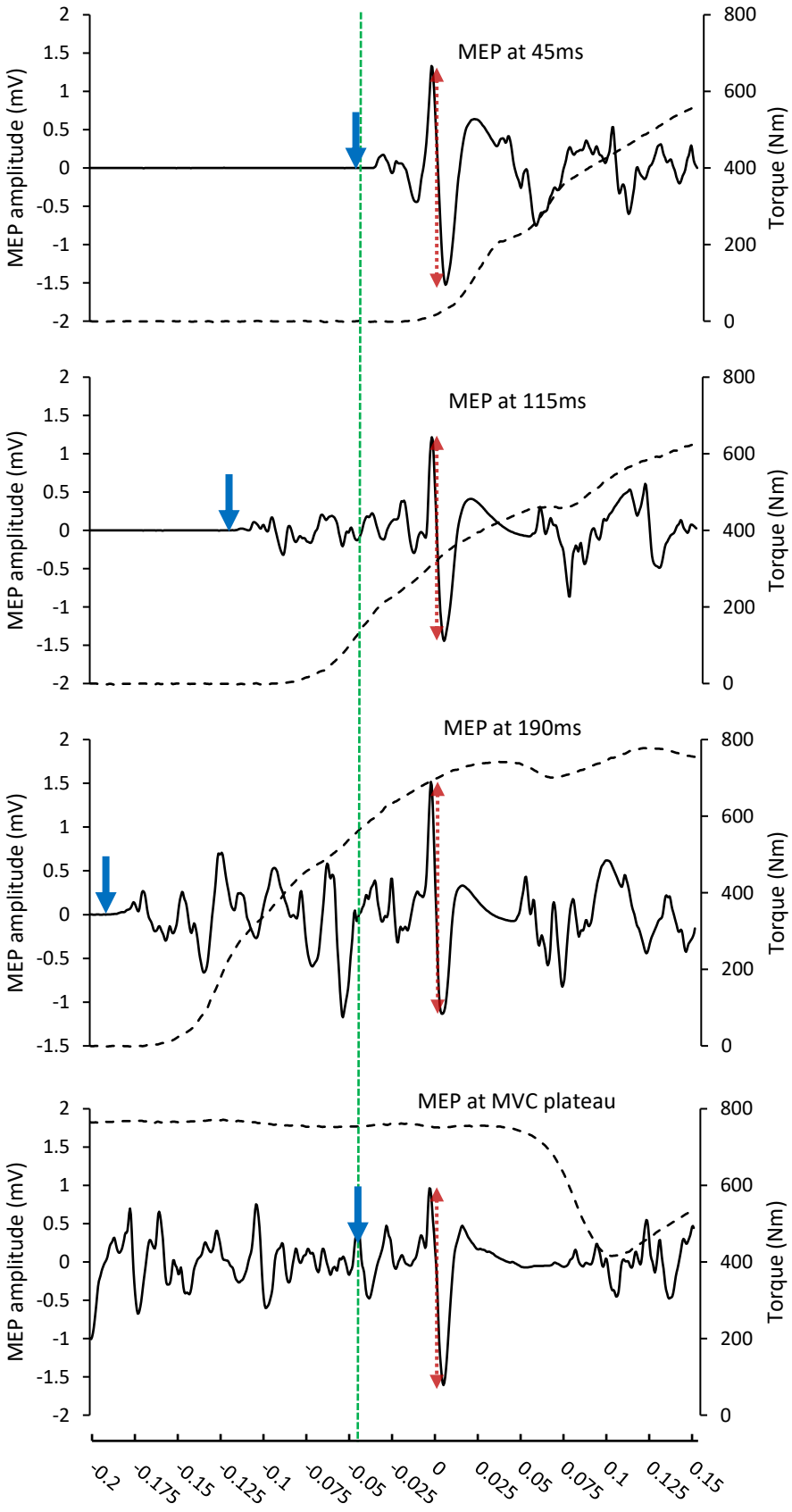
MEP at 45ms
MEP at 115ms
MEP at 190ms
MEP at MVC plateau
MEP amplitude (mV)
Torque (Nm)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
800
600
400
200
0
-0.2
-0.175
-0.15
-0.125
-0.1
-0.075
-0.05
-0.025
0
0.025
0.05
0.075
0.1
0.125
0.15

Fig 2

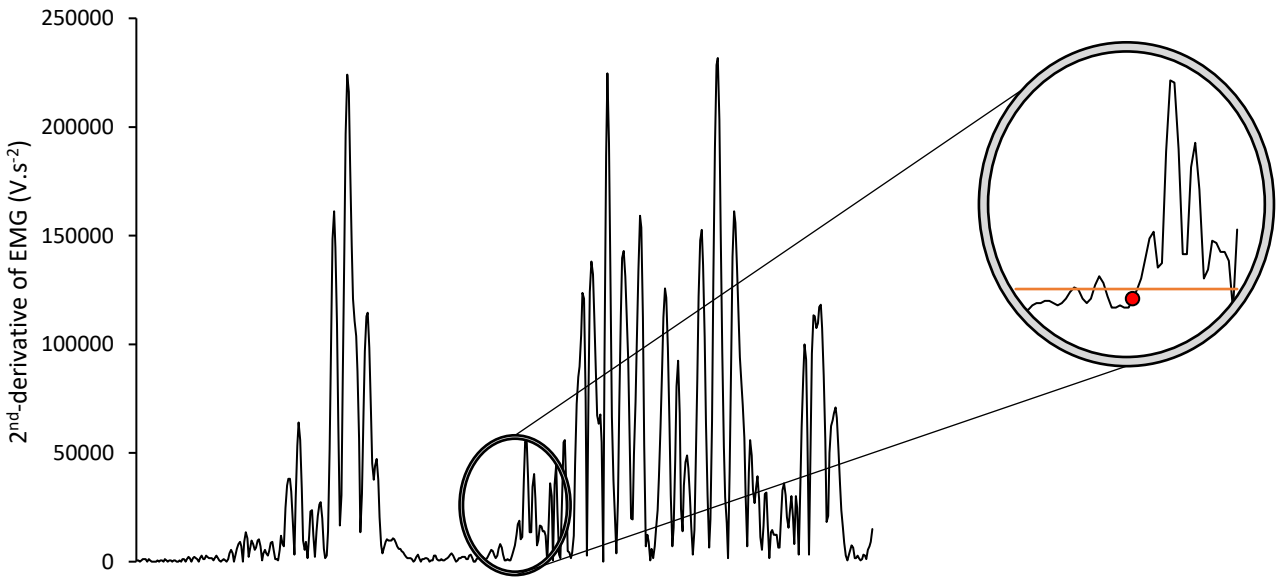
250000
200000
150000
100000
50000
0
2nd-derivative of EMG (V.s-2)

Fig 3

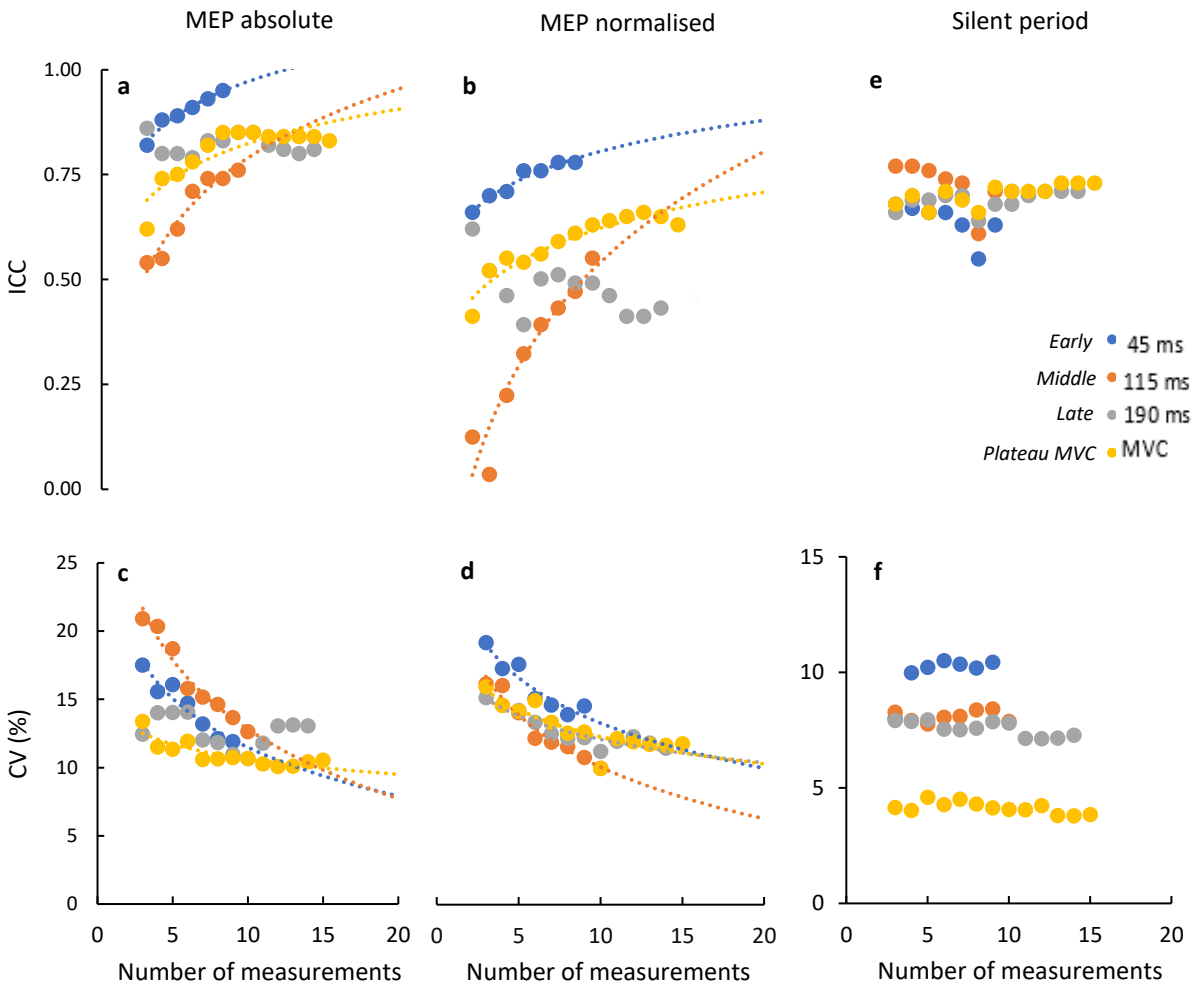
MEP absolute
MEP normalised
Silent period
a
b
e
c
d
f
ICC
CV (%)
1.00
0.75
0.50
0.25
0.00
25
20
15
10
5
0
Number of measurements
Early 45 ms
Middle 115 ms
Late 190 ms
Plateau MVC MVC

Fig 4