Title: Test-retest reliability of TMS motor evoked responses and silent periods during

explosive voluntary isometric contractions

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9   **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.

In studies of TMS responses, measures are typically averaged across all stimulations obtained

within a testing session. However, a subset of contractions are typically used for studies of explosive

contractions, with the best three out of ten contractions being recommended for averaging RTD

measurements (Maffiuletti *et al.*, 2016). Therefore, for studies of MEPs recorded during explosive

contractions, it may be desirable to investigate MEPs obtained from the best three contractions rather

than from all contractions, and investigation into the reliability of this approach is necessary.

This study aims to assess the absolute (ICC) and relative (CV) test-retest reliability of TMS

responses (MEP amplitude and silent period) recorded at different time points (early, middle, and late

phases) during explosive voluntary contractions and at the plateau of MVCs. As part of this aim, we

intend to (i) document how the number of consecutive contractions (between 3-15) over which TMS

responses are averaged, influences test-retest reliability; and (ii) document test-retest reliability of

TMS responses averaged across the best 3, out of 10, contractions. We determine the best three as

those with the highest torque (Torque averaging method) or EMG RMS (EMG averaging method) prior

to MEP on-set.

Methods

Participants

Fourteen participants, nine males (age 31 ± 5 years, height, 178.7 ± 6.6cm, and mass 79.2 ±

4.7kg) and five females (age 29 ± 6 years, height, 165.5 ± 5.5cm, and mass 59.3 ± 6.2kg) were recruited

to take part in this study. All participants habitually performed 120-180 minutes of moderate to high-

intensity activity per week, and were deemed recreationally active (McKay *et al.*, 2022). Participants

were also free from injury and disease (screened via a questionnaire adapted from Balady *et al.*, 1998)

and free from contraindications to TMS (screened via a questionnaire adapted from Rossi *et al.*, 2011).

Due to changes in endogenous hormones throughout the menstrual cycle potentially affecting

neuromuscular responses to TMS (Ansdell *et al.*, 2019), female participants undertook experimental

trials exclusively during their self-reported early to mid-follicular phase (first ten days from the first

day of menstruation), when endogenous hormone concentration is low and relatively stable (de

Jonge, Thompson and Han, 2019). The University of Roehampton ethics committee approved the

study, and all participants provided written informed consent before participating.

Overview

Participants visited the laboratory on three separate occasions and were asked to avoid strenuous

exercise and alcohol consumption for 24 hours before each visit. Each session lasted approximately

120-150 minutes, with consecutive sessions separated by 3-7 days. The first visit was a familiarisation

session, and the second and third were measurement sessions. The measurement sessions were

performed at a consistent time of day and involved an identical protocol, with measurements used to

assess the test-retest reliability of the variables of interest. Each session involved knee extensor

torque and EMG measurement during maximal voluntary and explosive contractions, using TMS to

obtain superimposed MEP and femoral nerve stimulation to obtain compound muscle action

potentials at rest.

Torque measurements and surface electromyography (EMG)

Participants were tightly secured in a custom-built strength testing chair (Fig. 6b in Maffiuletti *et al.*,

2016) with a waist belt and shoulder straps. The hip and knee angles were set at 100° and 105°,

respectively (full extension being 180°). All contractions were isometric knee extensions performed

with the right leg. An ankle strap joined to a calibrated S-shaped load cell (FSB-1.5kN, Force Logic,

Reading, UK) was secured 4 cm proximal to the medial malleolus. The force signal was amplified (x375)

and then sampled at 2000 Hz (Mirco3 1401 and Spike2 v.8; CED., Cambridge, UK). Offline, the force

was filtered (fourth-order low-pass Butterworth, 250 Hz cut-off), corrected for limb weight, and

multiplied by the external moment arm to calculate joint torque.

The skin was prepared by shaving, cleaning (70% ethanol), and lightly abrading the area where

EMG electrodes were placed. A single, bipolar silver-silver-chloride gel-electrode configuration (2-cm

diameter and 2-cm inter-electrode distance; Dual Electrode, Noraxon, Arizona, USA) was placed over

the belly of each of the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM), based on

SENIAM guidelines (Hermens *et al.*, 1999). The EMG system has an inherent 312-ms delay, so whilst

suitable for measurements involved in the study, EMG signals sampled by it could not be used to

detect activation on-set and trigger the TMS in real-time during the explosive contractions (explained

below in Experimental procedures). Thus, a second wired bipolar EMG electrode (2 cm diameter and

2 cm inter-electrode distance; Dual Electrode, Biometrics Ltd, Gwent, UK) was placed on the belly of

the VM to trigger the TMS during contraction. Electrode locations were marked with a permanent

marker pen, and participants were asked to maintain these marks throughout the study by re-applying

them if necessary. The three wireless EMG signals were transmitted to a desktop receiver (TeleMYO

D.T.S., Noraxon, Arizona, USA) and sampled, along with the single wired EMG signal, at 2000 Hz via

the same A/D convertor and software as the force signal. Once offline, wireless EMG signals were

filtered (fourth-order Butterworth, band-pass, 6-500 Hz) and time-corrected for the 312-ms delay

inherent in the Noraxon system.

Transcranial magnetic stimulation (TMS)

TMS with a 1-ms pulse width was delivered via a double cone coil (110-mm Magstim 200,

Whitland, UK) over the scalp in an optimal position to elicit MEPs in the right quadriceps muscles. The

following procedures were completed in every session. Participants wore a swim cap, and the vertex

of the head – identified as 50% of the distance between nasion and inion and between right and left

perpendicular point – was marked on the swim cap. A 5-by-5-cm grid with 1-cm spacing between grid

lines was drawn on the swim cap, lateral (left hemisphere) and posterior from the vertex. The coil

was moved posteriorly and laterally from the vertex in ~0.5-cm steps, and in each position, the

participant completed four submaximal voluntary contractions at 20% MVC torque (established in the

warm-up; see below) with superimposed TMS on each contraction at a submaximal (range 50-60%)

stimulator output. The position determined the optimal coil position for the rest of the session, which

was subjectively the highest consistent MEP amplitudes (peak-to-peak) over the four superimposed

contractions for all three muscles (RF, VL and VM). The optimal coil position was marked by drawing

the edge of the coil over the swim cap, ensuring accurate relocation throughout the session. The

active motor threshold (AMT) was then determined via a series of 20% MVC torque contractions

superimposed with TMS stimulator output starting at 39%. The AMT was defined as the minimum

TMS intensity required to elicit five visible MEPs amongst the background EMG activity of the VM and

RF, out of ten consecutive superimposed contractions. If the muscles had less or more than five visible

MEPs, the machine intensity was reduced or increased by 2% of the machine output. We prioritised

the VM and RF (not the VL) as these muscles produced more consistent and visible MEPs in pilot

testing. Where it was impossible to match AMT for VM and RF, we settled on being one visible MEP

away from 5/10 (e.g., 6/10 VM and 4/10 RF). The same investigator held the coil by hand throughout

the measurement sessions, continuously monitoring its position and orientation. For TMS delivered

during maximal and explosive contractions (see experimental protocol), the intensity was set at 140%

of the stimulator output at AMT (Groppa *et al.*, 2012; Rossini *et al.*, 2015; Rossi *et al.*, 2020). These

procedures were repeated for all sessions.

Femoral nerve electrical stimulation

Single, square-wave pulses (200 µs duration) were delivered (DS7AH, Digitimer, Hertfordshire,

UK) over the femoral nerve in the inguinal triangle to evoke twitch contractions and obtain compound

muscle action potentials (M-waves) at rest. The anode (5 x 8 cm carbon rubber; EMS Physio Ltd.,

Oxfordshire, UK) was placed over the head of the greater trochanter. The optimal location of the

cathode (1 cm diameter tip; S1 Compex Motor PointPen, Digitimer, UK) was determined as that

which evoked the greatest peak twitch torque for a given submaximal stimulation intensity (80-120

mA). The cathode was taped down and held in position by the same investigator as a series of twitches

at incremental intensities were evoked until there was a plateau in the peak-to-peak M-wave

amplitude (M_{max}) of all three muscles (RF, VL, VM). The stimulator intensity was then increased to

150% of the intensity at M_{max}, ensuring supramaximal intensity.   Three supramaximal twitch

contractions were then evoked, each separated by 15 s, and M_{max} was averaged across the three

contractions for each muscle. The procedures were repeated for all sessions.

Experimental protocol

Participants first completed a warm-up involving a series of explosive, submaximal, and maximal

voluntary contractions (the latter being used to establish MVC torque), followed by procedures for

obtaining optimal TMS coil position, ATM, and M_{max}. Participants then completed a series of MVCs

and explosive voluntary contractions with and without superimposed TMS. The instruction for MVCs

was to "push as hard as possible" for 3-5 s and explosive contractions to "push as fast and hard as

possible", emphasising fast for 1 s. The protocol was organised into three blocks of contractions (Fig.

1). Each block involved 24 explosive contractions (8 without and 16 with superimposed TMS) and 8

MVCs (3 without and 5 with superimposed TMS), distributed across four sets. Each set involved six

explosive contractions (the final four with TMS stimulation) and two MVCs. In sets 1-3, one of the two

MVCs had superimposed TMS (randomly ordered), and in set four, both MVCs had superimposed TMS

(Fig. 1). Participants rested 10 s between explosive contractions, 30 s between MVCs, 120 s between

sets, and 300 s between blocks. Each block was identical except for the timing of TMS application

during explosive contractions, with each block using a different TMS timing condition for these

contractions (see next section). The order of the blocks was randomised across participants but held

constant across sessions for the same participant. Overall, the 3-block protocol yielded 24 explosive

contractions and 9 MVCs without TMS, and 48 explosive contractions (16 per stimulus time condition)

and 15 MVCs with TMS.

220   [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

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

investigator during the torque plateau of the MVC. For explosive contractions, the TMS was triggered

automatically when the VM EMG signal of the wired system exceeded a set threshold. The threshold

was set in the Spike2 software as the lowest amplitude for that session above the highest peaks and

troughs of the baseline noise. Commencing from manually detected EMG on-set (0 ms; determined

via the methods of Tillin *et al.*, 2010), the threshold was exceeded after ~5 ms, and triggered the TMS

at either 8, 78, or 153 ms (accounting for a 3 ms system delay), for the early, middle, and late-phase

conditions, respectively (Fig 2). The centre of the evoked MEPs would then occur at ~45 (early), 115

(middle), or 190 ms (late) from manually detected EMG on-set (Fig. 2).

Data screening and analysis

Out of 15 superimposed MVCs, only those where the TMS was delivered >90% of MVC torque

were used for further analysis. Out of the 16 superimposed explosive contractions per condition, only

those that met the following criteria were used for further analysis: (i) average baseline force did not

change by >2 Nm during the 200 ms preceding manually detected force on-set (detected as in Tillin *et*

al., 2010); and (ii) there was a genuine attempt at an explosive contraction. A genuine attempt at an

explosive contraction was defined as the instantaneous slope of torque-time, just prior to MEP on-set,

being within 3 standard deviations (SD) of mean instantaneous slope at the same time point for

contractions without TMS. The time points for measuring instantaneous slope were 30, 105, and 180

ms after manually detected torque on-set, for early-, middle- and late-phase conditions, respectively.

Given the criteria above, the maximum number of MEPs analysed further in each condition was limited

to the number of usable contractions in the participant with the lowest number of usable contractions.

This was 9 (early), 10 (middle), and 15 (late) for the explosive contractions, and 15 for the MVCs.

For useable contractions, MEP amplitude was defined as the peak-to-trough of the

superimposed response (Fig. 2) and is reported in absolute terms and normalised to M_{max}. The silent

period was measured from the point of stimulation to the resumption of EMG activity after the MEP

off set. To determine the resumption of EMG activity, the second derivative of EMG amplitude over

time was established (1-ms time constant) and then the signal was rectified. EMG activity resumption

was defined when the amplitude of the second derivative increased above 5 SD of the mean baseline

(calculated in a 500 ms period prior to the contraction) for 70% of the next 10 ms (adapted from

Damron *et al.*, 2008; Fig.3). This automated process was confirmed via manual inspection of the signal

as recommended by Damron et al., (2008). MEP amplitude (absolute and normalised to M_{max}

separately) and silent periods were extracted on a muscle level before being averaged across the three

muscles to obtain a quadriceps mean. This is standard practice in studies looking to relate general

quadriceps EMG data to net knee extensor torque and RTD (Tillin *et al.*, 2010; Tillin, Pain and Folland,

2012, 2013, 2018; Folland, Buckthorpe and Hannah, 2014; Behrens *et al.*, 2015; Morales-Artacho *et*

al., 2018; Cossich and Maffiuletti, 2020).

Averaging methods

For each condition (early, middle, and late explosive phases, and at MVC plateau), mean

quadriceps consecutive running averages of the dependent variables were calculated from the first

three stimuli/contractions, up to the maximum number of usable contractions: 9 (early), 10 (middle),

and 15 (late) for the explosive contractions, and 15 for the MVCs. In the torque and EMG averaging

methods, dependent variables were averaged over the best 3 out of the first 10 contractions (9 for

early-phase explosive) in each condition. For the torque averaging method, the best 3 explosive

contractions were those with the greatest torque just prior to MEP (i.e., at 30, 105, and 180 ms from

torque on-set, in early, middle, and late conditions, respectively), whilst the best 3 MVCs were those

with the highest average torque over 1 s prior to the MEP. For the EMG averaging method, the best

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.

283   [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

fits), which are physiologically impossible. Best-fit logarithmic functions were made in SPSS v.26 (IBM

Inc., Armonk, NY, USA). For ICCs, a confidence interval of 95% was used for the lower bound (Lb) and

upper bound (Ub), and CVs were calculated using MATLAB (R2021a Update 5, MathWorks Inc., Natick,

MA, USA). The significance level was set at $p ≤ 0.05$. Data are presented in the results as mean ± SD.

Results

There was no significant difference (p≥0.168) between the test and retest sessions for any of

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

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

Reliability as a function of the number of MEPs

The test-retest ICCs for absolute and normalised MEP amplitude generally increased with the

number of averaged MEPs (Fig.3 A, B), whilst the test-retest CV for the same variables generally

decreased (Fig.3 C, D). These trends indicate increasing test-retest MEP amplitude reliability with

increasing numbers of averaged MEPs. The exception to these general trends was the late phase. In

this condition, although the CV of normalised MEP amplitude was related to the number of averaged

MEPs (Fig 3D), the CV of absolute MEP amplitude, the ICC of absolute MEP amplitude, and the ICC of

normalised MEP amplitude were not related to the number of averaged MEPs (Figs 3 A, B, C). These

trends, apparent upon visual inspection, were supported by logarithmic fits to the data, which were

significant in all cases aside from the late phase cases highlighted in the previous sentence (Table 2).

ICCs were generally higher for absolute MEP amplitude (Fig 3A) than normalised MEP

amplitude (Fig 3B). For absolute MEP amplitude, good ICC (ICC>0.75) was found within just three

averaged MEPs for all conditions except middle (where it was attained within nine averaged MEPs).

For normalised MEP amplitude, good ICC was only achieved in the early condition (attained within

seven contractions), whilst other conditions only reached moderate ICCs (ICC>0.50). CV tended to be

similar for absolute and normalised MEP amplitudes, reaching 10-15% values for all conditions within

seven contractions (Figs 3C, 3D). We were interested in identifying the number of averaged MEPs

needed to attain good ICC (ICC>0.75) and CV<10% for all variables and conditions. As is noticeable

from Fig 3 A-D, this was not consistently reached using the number of MEPs measured in this study

(9-15, depending on the condition). Therefore, the fitted logarithmic functions provided estimates by

extrapolating beyond fitted data where necessary (see Table 2 "n of" columns).

In contrast to the general trends for MEP amplitude reliability, the silent period reliability did

not vary as a function of number of averaged MEPs (Fig.3E, F). In support, attempted logarithmic fits

were all non-significant (p>0.05; fits not shown). Moderate ICCs (0.5≤ICC≤0.75) were generally

observed for the silent period in all conditions (Fig 3E). CVs for the silent period (Fig 3F) were lower

than for MEP amplitude, particularly during MVC.

[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 (early,

middle, and late) during explosive voluntary contractions, and at the plateau of MVCs. In general, no

one condition (explosive early, middle, late, or MVC plateau) produced consistently more reliable

(absolute or relative) TMS responses across the different averaging methods than another. In all

conditions except the explosive late phase, both absolute and relative reliability of MEP amplitude

(absolute and normalised) generally increased with an increase in the number of consecutive stimuli

(separate contractions) averaged. In contrast, increasing the number of averaged stimuli did not

improve silent period reliability in any condition. This study also found averaging the best 3 out of the

first 10 contractions based on EMG amplitude or torque output, produced comparable, albeit slightly

poorer, reliability than averaging the same 10 contractions for all TMS responses and conditions.

Reliability during MVCs

To date, only a handful of studies have investigated the test-retest reliability of MEPs evoked

at the MVC plateau, and these have all been based on measures averaged across 3-5 MVCs (Kamen,

2004; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016). For comparison within this section,

we will focus on our findings within this range of 3-5 contractions, with the influence of higher

numbers of contractions being discussed later.

Within 3-5 MVCs, we observed moderate ICC (0.50-0.75) for absolute and normalised MEP

amplitudes, which is within the range (0.47-0.79) of what others have observed (Kamen, 2004;

Sidhu, Bentley and Carroll, 2009; Mileva, Sumners and Bowtell, 2012; Souron *et al.*, 2016; Table 5 in

supplementary material). We also observed moderate ICC for silent periods at MVC plateau (0.66-

0.70) which was comparable to what has previously been reported for the rectus femoris (0.70;

Mileva, Sumners and Bowtell, 2012; 0.61-70; Di Virgilio *et al.*, 2022) and lower than reported for the

tibialis anterior (0.93-0.95; Souron *et al.*, 2016; Table 5 in supplementary material). For test-retest

CV, within 3-5 MVCs, we observed 11-16% values for absolute and normalised MEP amplitude and

below 5% for the silent period. One previous study has reported test-retest CV for TMS responses at

MVC plateau in the tibialis anterior (Souron *et al.*, 2016) and reported slightly lower CVs for MEP

amplitude (10-11%) and a slightly higher CV for silent period (9%), compared to our results for the

quadriceps. A more detailed comparison of our results and those of previous studies is difficult

because of the disparity in methods for factors such as the type of ICC used, muscles involved, TMS

intensity used, and method of calculating silent period. Nevertheless, based on our results and

those of previous studies, averaging data across only 3-5 MVCs should provide moderate ICCs and

CVs<16% for MEP amplitudes and silent periods.

Independently of the number of averaged MEPs, absolute MEP amplitude (V) at MVC plateau

showed consistently better ICC values than normalised MEP amplitude (% M_{max}), and this pattern was

consistent for other conditions also. In contrast, a previous study of the tibialis anterior (Souron *et*

al., 2016) found ICC for MEP amplitude at the MVC plateau to be similar between absolute and

normalised data. This difference between our study and Souron et al. (2016) may be due to the

different muscle groups used, although there were other methodological differences (e.g., process for

determining coil position and intensity), again making a direct comparison difficult. The greater ICC in

absolute compared to normalised MEP amplitude that we observed might originate from the ICC

calculation itself. ICC scores will be influenced by inter-participant variability, with greater variability

likely contributing to greater ICCs (Koo and Li, 2016). Normalising the MEP amplitudes to M_{max} will

control for some of the factors that cause inter-participant variability, such as muscle size and

adiposity (Besomi *et al.*, 2020), which in turn might reduce ICCs. In contrast to ICC, CV measurements

are not influenced by inter-participant variability which may explain why CVs were generally similar

for absolute and normalised MEP amplitudes.

Reliability during explosive contractions

To our knowledge, no prior study has investigated the reliability of TMS responses during

explosive contractions. We had suspected the reliability of TMS responses to be lowest in early-phase

explosive contractions and highest at the MVC plateau, as this is commonly observed for torque and

EMG amplitudes in these different conditions (Folland, Buckthorpe and Hannah, 2014; Tillin and

Folland, 2014). However, our findings indicated the test-retest reliability of TMS responses was similar

for the different conditions, an observation that appeared unrelated to the number of contractions

averaged. Just prior to the onset of rapid contractions, in a period of time that overlaps with the early

phase of EMG measurements in the current study, there is an increase in subthreshold cortical

excitability and increased probability in evoking a detectable MEP (Mackinnon and Rothwell, 2000).

Early subthreshold cortical excitability changes consistently during explosive contractions, but the

probability of conversion to detectable voluntary motor output in surface EMG is less reliable (Folland,

Buckthorpe and Hannah, 2014; Tillin and Folland, 2014). At later phases in the contraction,

corticospinal excitability and voluntary motor output variability align more closely, explaining why

early phase EMG and torque measurements are less reliable compared to MEP amplitudes, which

remain consistent across phases.

The only exception to there being no clear differences in reliability between conditions was

the observation that CV for the silent period was generally highest for the early phase and lowest for

the MVC plateau (Fig. 2F). This may be associated with the lower overall length of the silent period in

the early phase compared to the MVC plateau (Table 1). Given the CV is a measure of variability

relative to the mean, a condition with a lower mean silent period (as is the case for the explosive early

phase), will be susceptible to higher CV values where variability is constant. The shorter silent period

in the early phase may be due to neural drive in this phase being predominantly feedforward (Škarabot

et al., 2021) and less affected by afferent feedback or intracortical circuitry causing inhibition.

Number of averaged MEPs

For absolute and normalised MEPs, ICCs generally increased, and CVs decreased, with an

increasing number of contractions, in all conditions except the late phase. This is in agreement with

studies on MEP amplitudes recorded at rest or at submaximal force, which also observed improved

reliability by increasing the number of averaged stimuli (Goldsworthy, Hordacre and Ridding, 2016;

Biabani et al., 2018; Brownstein et al., 2018). Our results show that the conditions in our study, except

for the late-explosive phase, require 11-15 contractions (absolute MEP amplitude) and 18-23

contractions (normalised MEP amplitude) to ensure both good ICCs (≥0.75) and CVs≤10%. Further,

although caution is required when extrapolating the logarithmic functions reported in this study well

beyond the number of contractions measured, the shape of the logarithmic functions indicates little

benefit to reliability will be gained by sampling beyond the abovementioned ranges. Variability in the

MEP responses in our study are likely affected by the TMS intensity we used (140% AMT), which

evokes MEPs in the quadriceps half-way up the impulse-response relationship for the quadriceps

(Temesi, Ly and Millet, 2017). As this is the steepest section of the impulse-response relationship,

small changes in corticospinal excitability can greatly affect MEP amplitude (Temesi, Ly and Millet,

2017). Combined with the intrinsic fluctuations in ongoing oscillatory activity within the cortical area

(Hordacre et al., 2017), this likely explains the inherent variability in MEP amplitudes, which seems to

limit test-retest reliability, even with consecutive averaging. Nevertheless, based on our results, we

recommend future investigators consider collecting and averaging MEP amplitudes across more than

the standard 3-5 MVCs (or explosive contractions) used in previous studies, if this can be managed

within their protocol, with an optimal range likely falls between 10-20.

Unlike the other conditions, MEP amplitude reliability in the late-explosive phase (190 ms)

was not improved by increasing the number of averaged contractions. This implies that variability in

MEP amplitude during the late phase of explosive contraction remains constant and within a narrow

range from contraction to contraction, unlike during the earlier phases. We can only speculate about

the physiological factors explaining this observation. However, potentially afferent feedback, which

will be greater in the later than earlier phases of explosive contraction (Škarabot *et al.*, 2021), inhibits

the neural drive to muscle and so maybe limits the range of possible MEP amplitudes in the late phase

of explosive contraction.

In the silent period, ICC and CV remained constant despite increases in the number of

averaged stimuli. Thus, variability in the silent period appears to be consistent and remain within a

narrow range of possibilities from contraction to contraction. Factors that are likely contributing to

the variability in silent period include the intensity of stimulation – with lower intensities resulting in

higher variability (Damron *et al.*, 2008) – and differences in motor threshold compared to MEP as

changes in response curve reduce variability (Wassermann *et al.*, 1993). Our results, obtained at 140%

AMT, show that collecting data or more than 3 explosive contractions or MVCs will nt provide

meaningful improvements in the reliability of silent period measurament.

In studies on explosive contractions, RFD is often averaged from the best 3 out of as many as

10 or more contractions (Maffiuletti *et al.*, 2016). Thus, if intending to relate TMS responses measured

during explosive contractions to RFD, it may be pertinent to also average the TMS responses in the

best 3 contractions. Of the two methods we used for determining the best 3 (out of the first 10)

contractions (torque and EMG methods), neither showed consistently better reliability than the other

across all conditions and variables. However, both methods for all conditions provided comparable

ICCs and CVs to the average of the first 10 contractions (Tables 3 and 4). It therefore appears that

selecting the best 3 contractions to average MEP amplitudes minimises the influence of MEP

variability to a similar extent as increasing the number of averaged stimuli over consecutive

contractions from 3 to 10. Thus, averaging the best three contractions may be a viable option for

investigators in the future, particularly if looking to relate MEP amplitude measurements to RTD.

Conclusion

Regardless of the averaging method or reliability metric (ICC and CV), no specific condition

produced consistently more reliable MEP amplitudes (absolute or normalised), and silent periods

than another. Generally, increasing the number of averaged consecutive contractions increased the

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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Fig 1

Fig 2

Fig 3

Fig 4