Reliability of the functional measures of the corticospinal pathways to dorsiflexor muscles during maximal voluntary contractions

Robin Souron, Adrien Farabet, Guillaume Y. Millet, Thomas Lapole

Reliability of the functional measures of the corticospinal pathways to dorsiflexor muscles during maximal voluntary contractions

Robin Souron[1], Adrien Farabet[1], Guillaume Y Millet[2] & Thomas Lapole[1]

[1]Univ Lyon, UJM-Saint-Etienne, LIBM, F-42023, SAINT-ETIENNE, France

[2]Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Canada

Running title: Reliability of tibialis anterior corticospinal functional properties

Corresponding author:
Thomas LAPOLE
Laboratoire Interuniversitaire de Biologie de la Motricité
Bâtiment IRMIS
10 rue de la Marandière
42270 Saint Priest en Jarez
04 77 42 18 91
thomas.lapole@univ-st-etienne.fr

ABSTRACT

This study aimed to evaluate the intra- and inter-day reliability of transcranial magnetic stimulation (TMS)-related measurements recorded from the tibialis anterior (TA) muscle. Thirteen healthy young men and women (23 ± 4 years) performed 3 testing sessions to assess intra- (i.e., two sessions performed the same day) and inter-day (i.e. two sessions performed one week apart) reliability of (i) dorsiflexion cortical maximal voluntary activation level (VA_{TMS}), (ii) TA corticospinal excitability assessed through the amplitude of the motor evoked potentials (MEP) recorded during 100, 75 and 50% maximal voluntary contractions (MVC), and (iii) intracortical inhibition investigated via the cortical silent period (CSP) recorded at the same %MVC. Absolute (i.e., coefficient of variation (CV) and standard error of the mean (SEM)), and relative (i.e., intraclass correlation coefficients (ICC)) reliability parameters were calculated. VA_{TMS} demonstrated excellent intra- and inter-day reliabilities (ICC: 0.80 and 0.99; CV: 1.7 and 0.8%, respectively). MEPs and CSPs presented moderate to excellent intra- and inter-day reliabilities, while input-output curves extracted parameters presented highly variable outcomes. These results suggest that most TA corticospinal measurements during voluntary contraction can be used to quantify corticospinal adaptations after acute (e.g. fatigue) or long term (e.g. training) interventions.

Keywords: transcranial magnetic stimulation; tibialis anterior; cortical voluntary activation; corticospinal excitability, Boltzmann modelling.

ABBREVIATIONS

CSP	Cortical silent period
CSP_{50}	Cortical silent period during a 50% MVC submaximal contraction
CSP_{75}	Cortical silent period during a 75% MVC submaximal contraction
CSP_{MVC}	Cortical silent period during a MVC
CV	Coefficient of variation
EMG	Electromyographic
ERT	Estimated resting twitch amplitude
I_{50}	Stimulus intensity required to obtain a MEP of half the size of the plateau
ICC	Intraclass correlation coefficient
k	The slope parameter of the Boltzmann modelling
M_{MVC}	M-wave amplitude during a MVC
MEP	Motor evoked potential
MEP_{50}	Motor evoked potential amplitude during a 50% MVC submaximal contraction
MEP_{75}	Motor evoked potential amplitude during a 75% MVC submaximal contraction
MEP_{MVC}	Motor evoked potential amplitude during a MVC
MVC	Maximal voluntary contraction
P	The function plateau of Boltzmann modelling
RMS	Root mean square
SEM	Standard error of the mean
SIT	Superimposed twitch amplitude
SIT_{MVC}	Superimposed twitch amplitude during a maximal voluntary contraction
TA	Tibialis anterior
TMS	Transcranial magnetic stimulation
VA_{TMS}	Voluntary activation assessed with TMS

1. Introduction

It is well established that training, and especially strength training, induces several adaptations to the central nervous system leading, *in fine,* to an augmentation of maximal voluntary contraction (MVC) force. The investigation of maximal voluntary activation is critical to assess neural adaptations. Voluntary activation refers to the neural drive delivered to a muscle during a voluntary contraction. The most common way to estimate maximal voluntary activation is to use the interpolated twitch technique [1], where a supramaximal stimulus is delivered over a motor nerve during an MVC to measure the extra force evoked by the stimulation. Using this method, changes in neural drive can be identified as at or above the site of stimulation of the motor axons, without distinction between spinal and supraspinal mechanisms [2]. Since training may induce both spinal [3] and supraspinal adaptations [4], transcranial magnetic stimulation (TMS) has been proposed as an alternative technique to ascertain whether adaptations occur throughout the corticospinal pathway [5]. Indeed, the evocation of a superimposed twitch elicited by TMS during MVC has been suggested to highlight a suboptimal drive from the motor cortex [5-7], although the cause of VA_{TMS} reduction can be due to modulation at any level of the corticospinal network (i.e. through reduced spinal and/or motoneuronal excitability).

The assessment of cortical voluntary activation using TMS (i.e., VA_{TMS}) cannot be performed for all muscles since some criteria must be met, especially having strong corticomotoneuronal projections [8]. VA_{TMS} has been shown to be reliable for the elbow flexors [9] or the knee extensors [8], but not in dorsiflexor muscles thus far, despite the functional role of these muscles in particular during locomotion [10, 11]. The tibialis anterior (TA), contributing to almost half of the voluntary torque produced in ankle dorsiflexion (the remainder presumably being provided by the long extensors of the toes) [12], is known to have the strongest corticospinal projections among lower limb muscles [13]. Even though dorsiflexor muscles VA_{TMS} has already been assessed in fatigue studies [14, 15], no study has yet investigated VA_{TMS} reliability for this muscle group. Such an approach is necessary since the constant oscillation in the elements of the central nervous system and methodological factors (e.g. subject population, muscle considered, position for magnetic stimulation) are two main sources that may affect the stability of TMS measurements [16]. Indeed, motor-

evoked potential (MEP) elicited by TMS, which is known to represent the excitability of cortical neurons and/or motoneuron pools of the stimulated muscles [17], are inherently variable. For instance, MEP amplitude may be influenced by circadian variation [18]. To reduce variability, MEP amplitude is commonly expressed as ratio to M-wave to rise above the membrane excitability and conductivity influence. MEPs should also be measured during a stable voluntary contraction to further reduce variability [16, 19]. Among the studies that investigated TA MEP reliability [14, 16, 20], only one met these last two criteria and reported high inter-day reliability for MEPs measured during submaximal lengthening and shortening contractions [16]. During maximal isometric contraction, absolute MEP was also reported to be reliable [14], as it was at rest [14, 20]. Hence, intra-day and inter-day reliability of normalized TA MEPs during submaximal and maximal isometric contractions has yet to be determined.. Moreover, although input-output curves (i.e. the relation between TMS intensity and MEP amplitude) [19, 21] are recognized as a more sensitive measure of corticospinal excitability [22, 23], its reliability for the TA muscle has only been proven during a relaxed condition [20].

Although several studies have investigated the reliability of TA TMS-related parameters [14, 16, 20], this topic needs further investigation. Specifically, dorsiflexor VA_{TMS} must be examined to better understand short- and long-term adaptations within the central nervous system during acute and long term interventions. This would provide complementary knowledge with regard to previous TMS studies demonstrating changes in TA corticospinal excitability after strength training [24, 25]. Therefore, the present study is intended to evaluate the intra- and inter-day reliability of VA_{TMS} and associated corticospinal functional properties (e.g., MEP amplitude) for dorsiflexor muscles.

2. Material and methods

2.1. Participants. Thirteen young, healthy young subjects (9 men and 4 women, 23 ± 4 years; height: 177 ± 8 cm; body mass: 69 ± 10 kg) participated in this study. The subjects' level of weekly physical activity was reported to be between 0 and 5 hours per week. Written, informed consent was obtained from all subjects prior to their participation and this study conformed to the standards from latest revision of the Helsinki Declaration and was approved by the local ethics committee. All subjects were free of lower-limb injury during the previous three months, contraindications to TMS, acute and chronic neurological disorders, and trauma. Before testing, each subject was informed about the nature and the aim of this study, as well as risks and discomfort associated with electrical and magnetic stimulation. They were instructed to abstain from caffeine for a minimum of 12 h before each session.

2.2. Design of the study. Subjects came for three visits to the laboratory at the same time of the day with a 1-week interval between visits. The first visit was a familiarization session of the entire experimental protocol. In order to assess the inter-day reliability, the same protocol was performed during the second and third visits, i.e., session 1 (S1) and session 3 (S3), respectively. Except for the TMS recruitment curve and the peripheral nerve stimulation setup (see below), the whole experimental protocol was performed twice with a 30-min resting period between measurement sessions during one of these visits (randomly designed), allowing the assessment of intra-day reliability (S1 and session 2 (S2)) (Fig. 1). The 30 minutes resting period began at the end of S1 and finished at the beginning of S2. Hence, subjects were invited to stay in a sitting position on the custom-built chair during the 30-min period.

2.3. Torque and electromyographic recordings. Dorsiflexion torque was measured during voluntary contractions by a calibrated, instrumented pedal (CS1060 300 Nm, FGP Sensors, Les Clayes Sous Bois, France). Subjects were seated upright in a custom-built chair with a hip, knee, and ankle angle of 90, 120, and 90°, respectively. The foot was securely attached to the pedal with a custom designed hook and loop fastener. Electromyographic (EMG) activity of the right TA and soleus (SOL) was recorded with pairs of self-adhesive surface electrodes (Meditrace 100, Covidien,

Mansfield, USA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the medial malleolus. SOL electrodes were placed 2 cm inferior to the insertion of the gastrocnemii on the Achilles tendon. TA electrodes were placed on the muscle belly parallel to the longitudinal axis of the muscle at one-third of the distance between the head of the fibula and the tip of the medial malleolus, according to SENIAM recommendations [26]. Skin impedance was measured using a classic impedance analyser (Fluke 87V Ex, Fluke, Wallisellen, Switzerland) and low impedance (<5 kΩ) between electrodes was obtained by shaving, gently abrading the skin and then cleaning it with isopropyl alcohol. Signals were amplified with an octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 500), bandpass filtered (5-500 Hz), and analogue-to-digitally converted at a sampling rate of 2000 Hz by PowerLab system (16/30—ML880/P, ADInstruments, Bella Vista, Australia). All data was analyzed offline using Labchart 7 software (ADInstruments).

2.4. Stimulation. Two types of stimulation were used. Specifically, electrical stimulation of the tibialis anterior motor nerve (i.e., peroneal nerve) and transcranial magnetic stimulation (TMS) over the motor hotspot for the TA muscle. For stimulation of the tibialis anterior motor nerve, a bipolar bar stimulating electrode with 30-mm anode-cathode spacing (Bipolar Felt Pad Stimulating Electrode Part number E.SB020/4mm, Digitimer) was positioned next to the fibular head. Single rectangular pulses with 0.5ms duration and 400 V maximal output voltage were delivered to the right peroneal nerve via a constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK). The optimal site of stimulation was determined as the location eliciting the greatest TA M-wave for a given stimulus intensity. This site was marked with semi-permanent ink. To determine the optimal intensity of electrical stimulation, the intensity was increased by 5 mA until the magnitude of resting TA M-wave plateaued. The number of stimulations to find the optimal stimulating intensity varied among subjects (i.e., between 3 and 10). This optimal stimulating intensity was then increased by 50% to ensure supramaximal intensity of stimulation during contraction. Electrical stimulation configuration was determined at the beginning of the S1 and S3 to avoid any potentiation effects. The stimulation intensity used to evoke M-wave during MVC ranged between 30 and 97.5 mA. The mean stimulation intensities were 47.6 ± 20.2 mA in S1 (the same intensity was used for S2) and 48.2

± 17.0 mA in S3. The whole procedure of determining stimulation site and intensity was performed in approximately 3 min.

For the TMS technique, motor evoked potentials (MEPs) and superimposed twitches (SITs) were evoked during voluntary isometric dorsiflexion via a Magstim 200^2 magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with a 110-mm double cone coil. The coil was positioned over the leg area of the left motor cortex along the nasal-inion axis to induce a postero-anterior current. The coil position to obtain an optimal activation of the TA is known to be 0.5-1 cm posterior and along the anteroposterior plane of the vertex [21]. This location was drawn on the swim cap every centimeter from 1 cm anterior to 3 cm posterior to the vertex along the nasal-inion line and also to 2 cm over the left motor cortex area (i.e., 8 marks on the swim cap). Thus, a single stimulation was delivered at each of the specified anatomical positions (i.e. 8 stimulations in total) and the optimal coil position was chosen as the site eliciting the largest SIT and TA MEP amplitude with minimal SOL MEP amplitude in response to stimulation at 50% maximal stimulator output during brief voluntary dorsiflexion at 10% MVC. The whole procedure was performed in approximately 2 min.

Subjects were provided with real-time feedback of their target force, which was displayed on a screen. This position was marked on a swim cap that was secured to the scalp. During voluntary contractions, TMS was delivered once the subject had stabilized the force on the target level. Subjects were instructed to re-contract as strongly and as fast as possible immediately after TMS delivery to permit accurate measurement of the cortical silent period (CSP). Electrodes for peripheral nerve stimulation as well as the TMS coil were placed and hand-held by the same investigator for all subjects and all visits.

2.5. Neuromuscular measurements.

2.5.1. MVC. After a standardized warm-up consisting of 10 submaximal voluntary contractions performed between 30-80% of the MVC obtained during the familiarization session, subjects performed three maximal dorsiflexion contractions lasting approximately 3 s, with a 1-min resting period between trials to avoid fatigue. Electrical stimulation was delivered during MVCs to obtain the maximal M-wave

peak-to-peak amplitude recorded during MVC (M_{MVC}) (Fig. 1.B). MVC was determined as the highest torque value achieved before the electrical stimulation. TA EMG root mean square (RMS) was calculated as the mean over a 500-ms period after maximal force had reached a plateau and was then normalized to M_{MVC}.

2.5.2. Input-output curves. TA TMS input-output curves were only assessed once during each visit to the laboratory to determine the optimal stimulation intensity. Hence, only inter-day reliability was investigated for this curve. TMS was delivered over the hotspot. Since corticospinal excitability is increased with muscle activity [27], input-output curves were determined during brief voluntary dorsiflexion at 20% MVC. Four consecutive 2-3 s contractions, with a 10 second rest between contractions, were performed at 20, 30, 40, 50, 60, 70 and 80% maximal stimulator output (randomly ordered), and a TMS pulse was delivered during each of these contractions (i.e., 28 stimulations were initially delivered). If a TA MEP plateau was not confirmed from these intensities, higher TMS intensities were investigated. The optimal stimulation intensity obtained from the input-output curves was chosen when TA MEP and SIT plateaued. The MEP evoked in the antagonist SOL was also measured to ensure it stayed at a low value (Fig 3). The whole procedure was performed in approximately 10 min.

The aforementioned method has been recently reported to be appropriate for determining TMS intensity in the quadriceps [28]. The size of the TA MEPs was measured as the peak-to-peak amplitude of the non-rectified EMG response and expressed relative to M_{MVC}, and then averaged and plotted against the stimulus intensity. Then Boltzmann modelling was performed on input-output curves in order to apply a nonlinear regression analysis (Fig. 2.A) [21, 24, 29]:

$$\mathrm{MEPampl} = \frac{\mathrm{P}}{1 + \exp\left[\frac{\mathrm{I50} - \mathrm{I}}{\mathrm{k}}\right]}$$

This function allows the determination of the relation between MEP amplitude (MEPampl) and the TMS intensity (I, in percentage of the stimulator output). Three parameters can be extracted from this relationship: the maximum MEP defined by the function (P, in % M_{MVC}), the stimulus intensity at which the MEP size is 50% of the maximal MEP(I_{50}, in percentage of the stimulator output), and the slope parameter (k)

that is inversely proportional to the maximal function steepness [21]. The peak slope of the function was also calculated to provide an indication of the maximal rate of increase in MEP amplitude with stimulus intensity. The peak slope of the function is its tangent at I_{50} [19] and was further calculated by the following equation, taking into account the slope parameter (i.e., k) and the maximum MEP defined by the previous equation (i.e., P) [19, 24]:

$$\text{Peak slope} = \frac{k \times P}{4}$$

2.5.3. Cortical voluntary activation. Cortical voluntary activation (VA_{TMS}) during maximal effort was calculated with the modified twitch interpolation technique as initially developed by Todd et al. [7]. Stimulus intensity used for the investigation of cortical voluntary activation was defined as the lowest stimulus intensity tested eliciting maximal SIT and TA MEP amplitude from the input-output curve test performed for corticospinal excitability measurement. The same intensity was used for intra-session reliability, but was not necessarily the same between S1 and S3 (i.e., inter-day). The stimulus intensities used to assess VA_{TMS} were 65.3 ± 7.7% and 65.3 ± 8.7% of the maximal stimulator output in S1 and S3, respectively. Corticospinal excitability increases substantially during the transition from relaxed to contracted muscle states [30], thus underestimating TMS evoked twitch in the relaxed muscle. Hence, estimated resting twitch (ERT) was determined by linear regression of the relation between SIT amplitude evoked when TMS was delivered at 100, 75 and 50% MVC and voluntary force (Fig. 2.B) [7]. This relation was extrapolated and the y-intercept was interpreted as the ERT amplitude (Fig. 4). VA_{TMS} was assessed with the equation:

$$VA_{TMS} = (1 - SIT_{MVC} / ERT) \times 100$$

where SIT_{MVC} is the superimposed twitch evoked during MVC. Three series were performed and averaged with contractions separated by 30 s and series by 2 min. Thus, the linear regression was run out with the 3 values from each series, and VA_{TMS} was then averaged. ERT was excluded and VA_{TMS} was not calculated when the linear regression was not linear within a series ($r^2 < 0.9$) [31]. One series was excluded from the data analysis for 5 subjects (out of 39 series in total) and the average was done with 2 series rather than 3 in this case. ERT and SIT_{MVC} were determined on each series separately before being averaged. Mean R^2 of the linear

regression was 0.97 ± 0.04, 0.97 ± 0.05 and 0.95 ± 0.03 for S1, S2 and S3, respectively. MEPs and CSPs elicited by TMS during 100, 75 and 50% MVC contractions were also calculated and averaged over the 3 sets. Peak-to-peak amplitude of MEPs were measured and normalized to M_{MVC} (MEP_{MVC}, MEP_{75} and MEP_{50}, respectively). Since it was difficult to precisely determine the beginning and the end of the MEP, due to its polyphasic form, MEP area was not calculated. The duration of the CSPs were determined visually and defined as the duration from the TMS stimulus to the return of continuous voluntary EMG (CSP_{MVC}, CSP_{75} and CSP_{50}, respectively) (Fig. 2.C) Two investigators performed the CSP analysis, and the two values were averaged. The treatment was redone by a 3rd investigator when the difference between the two was higher than 5%. This large error occurred only for one subject (in that case, the average was performed on the values that were these closest to each other), showing that analysing CSP duration visually was not investigator-dependant and was thus an appropriate method.

2.6. Statistical analysis. As recommended by Hopkins [32], intra-session and inter-day reliabilities were assessed using the intra-class correlation coefficient (ICC), standard error of the mean (SEM) and coefficient of variation (CV). The ICC represents a measure of relative reliability showing the degree to which individuals maintain their position in a sample with repeated measurements [33]. According to Fleiss [34], ICC greater than 0.75 represents an excellent reliability; an ICC ranging between 0.74 and 0.40 represents a moderate to good reliability and an ICC smaller than 0.40 represents poor reliability. The SEM scores were also calculated to quantify absolute reliability and give an indication of the precision of a recorded parameter. Thus, a low SEM value means a strong reliability and vice versa [35]. The CV ((standard deviation (SD) / mean) was calculated to represent the degree to which repeated measurements vary for individuals [33]. Data are presented as means ± SD.

3. Results

3.1. MVC and cortical voluntary activation (VA_{TMS})

The results of intra- and inter-day reliabilities of MVC, M_{MVC}, RMS/M_{MVC} and VA_{TMS} are presented in Table 1. Intra-day ICCs were 0.96 and 0.80 while inter-day ICCs were 0.95 and 0.99, for MVC and VA_{TMS}, respectively. For these two parameters, all CVs for intra- and inter-day conditions were lower than 4.1%, and were associated to low SEM values. VA_{TMS} across the three testing sessions was 96.0 ± 4.9%. Mean SIT_{MVC} and ERT values as well as their associated ICC, CV and SEM values are reported in Table 1.

3.2. Corticospinal excitability and intracortical inhibition

Fig 2.C represents typical torque and EMG recordings (i.e., MEP amplitude and silent period duration) in response to TMS during 100, 75 and 50% MVC contractions.

Mean results for CSP and MEP recorded at 100, 75 and 50% MVC are presented in Table 1 as well as their reliability outcomes. For these two parameters, ICCs ranged from 0.51 to 0.99 and CVs from 3.5% to 14.9%. The parameters of the input-output curves are presented in Table 2. R^2 of the Boltzmann sigmoid function were 0.83 ± 0.12 and 0.88 ± 0.10 for S1 and S3, respectively. For the four parameters extracted, ICC values ranged from 0.71 to 0.87 and CVs from 8.0% to 43.9%.

4. Discussion

This study aimed to assess the intra- and inter-day reliability of several TMS-related measures (VA_{TMS}, MEP amplitude, CSP, input-output curve parameters) related to corticospinal output to a voluntarily activated muscle responsible for dorsiflexion in healthy subjects. The main findings of the present study are that i) dorsiflexor muscles VA_{TMS} was reported for the first time to be highly reliable, ii) MEPs and CSPs showed reliable intra- and inter-day outcomes and iii) highly variable results are reported for Boltzman modelling extracted parameters.

4.1. MVC and cortical voluntary activation. As expected, MVC presented good intra-and inter-day reliability. To the best of our knowledge, cortical voluntary activation level assessment using TMS during MVCs has only been demonstrated to be reliable for elbow flexors [9] or knee extensors muscles [6, 8]. Although previous fatigue studies investigated dorsiflexor VA_{TMS} [14, 15], the present study is the first one to investigate its intra- and inter-day reliability. VA_{TMS}, as well as ERT, demonstrated good to excellent intra-and inter-day reliabilities. An important result of the present study is that VA_{TMS} recorded between days (i.e., inter-day reliability) showed excellent reliability (i.e., 0.99 ICC, 0.8% CV and 0.4 SEM). Since neural adaptations have been reported after strength training through CSP [24] and MEP [25] increases, our results suggest that VA_{TMS} may be a relevant parameter to inform on possible changes in the maximal neural drive from the motor cortex in longitudinal studies although this may also reflect on a modification of spinal excitability.

VA_{TMS} was found to be 96.0 ± 4.9% across the three testing sessions, highlighting the inability of the nervous drive originating from the motor cortex to fully activate motoneurons [5]. Voluntary activation measured in our study appears to be higher compared to cortical voluntary activation recorded for elbow flexors (91-95%) [9, 31, 36-38] or knee extensors muscles (89-93%) [6, 8, 39, 40], and seem to be in agreement with others showing that TA activation, quantified by the classical superimposed stimulation method, was reported to be maximal or near maximal in a healthy young population [41]. This may be explained by the fact that the TA has the strongest corticospinal projections among all lower limb muscles [13].

Our values also appear to be greater than those reported by Ross et al., (2007) and Mileva et al., (2012) who found VA_{TMS} to be 75.5 ± 16.0% and 89.5 ± 2.9%, respectively [14, 15]. These discrepancies may be due to differences in the TMS intensity and/or the type of TMS coil used to evaluate VA_{TMS}. Ross et al., (2007) evaluated the VA_{TMS} at 100% of maximal stimulator output using a 70 mm figure-of-eight coil [15], while a 110-mm double-cone coil was used in the present study. This difference in coil size in the present study may have caused an increased activation of the antagonist muscles leading to misinterpretation of the evoked superimposed twitches. In the study of Mileva et al., (2012), TMS intensity was set at 120% of the resting motor threshold [14]. According to a recent study, this may not have been optimal to fully activate the motor cortical neurons since such intensity selection does not precisely identify the minimum stimulus intensity needed to elicit SIT and MEPs of maximal amplitude [28]. The authors of the present study suggest that determining TMS intensity from an input-output curve (i.e., using optimal intensity identified as the minimum stimulus intensity needed to elicit MEPs of maximal amplitude with minimal co-activation) is more appropriate to assess corticospinal excitability and VA_{TMS}.

4.2. Corticospinal excitability. Input-output curves performed in the present study to determine TMS intensity for VA_{TMS} assessment were further used to characterize the excitability of the corticospinal pathway, through the Boltzmann modeling [21, 42]. According to the ICC and SEM, the extracted parameters (i.e., the function plateau, the stimulus intensity required to obtain a MEP amplitude of half the size of the plateau, the slope parameter, and the peak slope) demonstrated good reliability and these values are in agreement with previous studies performed on first dorsal interosseous [19] or TA [20]. However, the present study demonstrated high CVs for the slope parameter and the peak slope (43.9 and 40.5%, respectively). The high reported CVs may question the reliability of these two parameters in the current study. Although Carroll et al., (2001) suggested that the slope parameter (i.e., the k parameter in our study) might be a more reliable index of corticospinal excitability than the peak slope for the first dorsal interosseous, similar reliability was reported for these two parameters in the present study [19]. Nevertheless, regarding the high CV described above, it seems difficult to use these two parameters to investigate the chronic and/or acute adaptations of TA corticospinal excitability.

While input-output curves were used in the present study to investigate TA corticospinal excitability during a low level of contraction, corticospinal excitability was also assessed at higher levels of contraction (100, 75 and 50% MVC) through MEP recordings. The current results present higher MEP values during submaximal (i.e., MEP_{50} and MEP_{75}) than during maximal voluntary contractions (i.e., MEP_{MVC}), which is in agreement with other results obtained on the TA [16]. It has been suggested that the probability for the TMS pulse to be evoked during the refractory period is higher during a maximal (higher discharger rate) rather than a submaximal voluntary contraction, leading to a lower MEP amplitude [8]. MEP_{MVC} presented good intra- and inter-day reliabilities. These results are consistent with a recent study that found that the TA has a good inter-day reliability (ICC: 0.80) for MEPs recorded during MVC [14]. Even if ICC values are lower in our experiment (ICC: 0.51 and 0.52, for intra- and inter-day condition respectively), we can conclude that there was a moderate to good reliability regarding the low associate CV and SEM values, informing about small intra-subject variation. One aim of the present study was to investigate the intra-day reliability of MEP amplitude, which is known to represent corticospinal pathway excitability. Our results have shown that MEPs recorded at maximal or submaximal levels of voluntary contraction may be used as reliable parameters to investigate acute phenomenon such as fatigue. The good reliability observed in the present study is also in agreement with several studies performed on knee extensors [8] or elbow flexors [9]. Yet, unlike the studies where the reliability was independent on force level, in the present study, MEP reliability was higher at low level of voluntary contraction (i.e., MEP_{50} and MEP_{75}) than during MVC (i.e., MEP_{MVC}). The good reliability observed for MEPs recorded during submaximal contraction is consistent with previous studies investigating the knee extensors [8], first dorsal interosseous [43], elbow flexor [9] or TA [16] muscles. The lower reliability observed for MEPs during MVC is consistent with previous studies, suggesting that high contraction intensities may cause a larger desynchronization of the action potential at the membrane level [16, 19]. Hence, the excitability of corticospinal pathway of the TA muscle can be reliably tested through the recording of MEP amplitude in response to TMS during maximal and submaximal contractions for acute interventions (e.g., fatigue) or longitudinal studies (e.g. training).

4.3. Intracortical inhibition. Cortical silent periods (CSP) associated with MEPs were also measured at 100, 75 and 50% MVC (i.e., CSP_{MVC}, CSP_{75} and CSP_{50}, respectively). When applied during a voluntary contraction, TMS may provide information about GABA mediated intracortical inhibition through the assessment of the CSP [44]. Although the exact mechanisms of the CSP remain unclear, it is accepted that the initial part is mainly due to reduced spinal excitability while the final part of the CSP is cortical in origin [45]. CSP results from the activation of inhibitory cortical neurons projecting onto the pyramidal cells in the motor cortex [46], and is thought to be mainly influenced by GABA (gamma aminobutyric acid) neurotransmission [47]. In the present study, CSP_{MVC} was highly reliable, as shown by high ICC and low CV-SEM values for both intra- and inter-day comparisons. To date, only one study recorded CSP in TA during MVC [14] and similar ICC (0.93) were observed. In the present study, CSP_{75} and CSP_{50} were also found to be highly reliable, both between S1 and S2 (intra-day) and between S1 and S3 (inter-day), in agreement with previous studies that assessed CSP during submaximal voluntary contractions in TA [16, 20], first dorsal interrosseous [43] and the extensor digitorum communis [48] muscles. Hence, CSP can be reliably used during maximal and submaximal dorsiflexion to investigate TA intracortical inhibition.

As most reliability studies, the principal limitation of the present work is that TMS-related parameters were investigated for a healthy young population, and that current outcomes may not be applicable to other populations (i.e., clinical, elderly).

In conclusion, the present study reports for the first time the excellent intra- and inter-day reliability for VA_{TMS} of dorsiflexor muscles. The outcomes were found to be highly reliable within and between days. A good reliability in the assessment of corticospinal excitability and intra-cortical inhibition directed towards the TA was also observed. TMS can thus be used to better understand the effects of training interventions on TA neural adaptations (e.g., neural drive from the cortex, corticospinal pathway excitability) in a healthy young population. This has important practical consequences because, besides the fact that TA has the most corticospinal projections among all lower limb muscles, it also plays an important functional role in daily life abilities such as lifting the foot in the swing phase during locomotion.

Acknowledgments

The authors sincerely thank Régis Bonnefoy for technical assistance as well as Aude Carlet-Mallet and Michael James Asmussen for revision. The present study was supported by a doctoral research grant from the University Jean Monnet of Saint-Etienne (France) assigned to Robin Souron.

References

[1] Merton PA. Voluntary strength and fatigue. J Physiol. 1954;123:553-64.

[2] Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81:1725-89.

[3] Giesebrecht S, van Duinen H, Todd G, Gandevia SC, Taylor JL. Training in a ballistic task but not a visuomotor task increases responses to stimulation of human corticospinal axons. J Neurophysiol. 2012;107:2485-92.

[4] Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol. 2002;92:2309-18.

[5] Goodall S, Howatson G, Romer L, Ross E. Transcranial magnetic stimulation in sport science: a commentary. Eur J Sport Sci. 2014;14 Suppl 1:S332-40.

[6] Sidhu SK, Bentley DJ, Carroll TJ. Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle Nerve. 2009;39:186-96.

[7] Todd G, Taylor JL, Gandevia SC. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. J Physiol. 2003;551:661-71.

[8] Goodall S, Romer LM, Ross EZ. Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. Exp Physiol. 2009;94:995-1004.

[9] Todd G, Taylor JL, Gandevia SC. Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. J Appl Physiol. 2004;97:236-42.

[10] Billot M, Simoneau E, Van Hoecke J, Martin A. Coactivation at the ankle joint is not sufficient to estimate agonist and antagonist mechanical contribution. Muscle Nerve. 2010;41:511-8.

[11] Hase K, Stein RB. Analysis of rapid stopping during human walking. J Neurophysiol. 1998;80:255-61.

[12] Marsh E, Sale D, McComas AJ, Quinlan J. Influence of joint position on ankle dorsiflexion in humans. J Appl Physiol Respir Environ Exerc Physiol. 1981;51:160-7.

[13] Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159:197-205.

[14] Mileva KN, Sumners DP, Bowtell JL. Decline in voluntary activation contributes to reduced maximal performance of fatigued human lower limb muscles. Eur J Appl Physiol. 2012;112:3959-70.

[15] Ross EZ, Middleton N, Shave R, George K, Nowicky A. Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. Exp Physiol. 2007;92:417-26.

[16] Tallent J, Goodall S, Hortobagyi T, St Clair Gibson A, French DN, Howatson G. Repeatability of corticospinal and spinal measures during lengthening and shortening contractions in the human tibialis anterior muscle. PLoS One. 2012;7:e35930.

[17] Mills K. Magnetic stimulation of the human nervous system: Oxford University Press; 1999.

[18] Strutton PH, Catley M, Davey NJ. Stability of corticospinal excitability and grip force in intrinsic hand muscles in man over a 24-h period. Physiol Behav. 2003;79:679-82.

[19] Carroll TJ, Riek S, Carson RG. Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. J Neurosci Methods. 2001;112:193-202.

[20] Cacchio A, Cimini N, Alosi P, Santilli V, Marrelli A. Reliability of transcranial magnetic stimulation-related measurements of tibialis anterior muscle in healthy subjects. Clin Neurophysiol. 2009;120:414-9.

[21] Devanne H, Lavoie BA, Capaday C. Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res. 1997;114:329-38.

[22] Boroojerdi B, Battaglia F, Muellbacher W, Cohen LG. Mechanisms influencing stimulus-response properties of the human corticospinal system. Clin Neurophysiol. 2001;112:931-7.

[23] Ridding MC, Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. Electroencephalogr Clin Neurophysiol. 1997;105:340-4.

[24] Christie A, Kamen G. Cortical inhibition is reduced following short-term training in young and older adults. Age. 2014;36:749-58.

[25] Griffin L, Cafarelli E. Transcranial magnetic stimulation during resistance training of the tibialis anterior muscle. J Electromyogr Kinesiol. 2007;17:446-52.

[26] Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol. 2000;10:361-74.

[27] Lazzaro VD, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, et al. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol. 1998;508:625-33.

[28] Temesi J, Gruet M, Rupp T, Verges S, Millet GY. Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. J Neuroeng Rehabil. 2014;11:40.

[29] Kaelin-Lang A, Luft AR, Sawaki L, Burstein AH, Sohn YH, Cohen LG. Modulation of human corticomotor excitability by somatosensory input. J Physiol. 2002;540:623-33.

[30] Ugawa Y, Terao Y, Hanajima R, Sakai K, Kanazawa I. Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. Electroencephalogr Clin Neurophysiol. 1995;97:451-4.

[31] Hunter SK, Butler JE, Todd G, Gandevia SC, Taylor JL. Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. J Appl Physiol. 2006;101:1036-44.

[32] Hopkins WG. Measures of reliability in sports medicine and science. Sports Med. 2000;30:1-15.

[33] Suzuki S, Hayami A, Suzuki M, Watanabe S, Hutton RS. Reductions in recruitment force thresholds in human single motor units by successive voluntary contractions. Exp Brain Res. 1990;82:227-30.

[34] Fleiss JL. The measurement of interrater agreement. Statistical methods for rates and proportions. 1981;2:212-36.

[35] Larsson B, Karlsson S, Eriksson M, Gerdle B. Test-retest reliability of EMG and peak torque during repetitive maximum concentric knee extensions. J Electromyogr Kinesiol. 2003;13:281-7.

[36] Hunter SK, Todd G, Butler JE, Gandevia SC, Taylor JL. Recovery from supraspinal fatigue is slowed in old adults after fatiguing maximal isometric contractions. J Appl Physiol. 2008;105:1199-209.

[37] Keller-Ross ML, Pereira HM, Pruse J, Yoon T, Schlinder-DeLap B, Nielson KA, et al. Stressor-induced increase in muscle fatigability of young men and women is predicted by strength but not voluntary activation. J Appl Physiol. 2014;116:767-78.

[38] Kennedy DS, McNeil CJ, Gandevia SC, Taylor JL. Fatigue-related firing of distal muscle nociceptors reduces voluntary activation of proximal muscles of the same limb. J Appl Physiol. 2014;116:385-94.

[39] Temesi J, Rupp T, Martin V, Arnal PJ, Feasson L, Verges S, et al. Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. Med Sci Sports Exerc. 2014;46:1166-75.

[40] Zghal F, Martin V, Thorkani A, Arnal PJ, Tabka Z, Cottin F. Effects of endurance training on the maximal voluntary activation level of the knee extensor muscles. Eur J Appl Physiol. 2013.

[41] Klass M, Baudry S, Duchateau J. Aging does not affect voluntary activation of the ankle dorsiflexors during isometric, concentric, and eccentric contractions. J Appl Physiol. 2005;99:31-8.

[42] Capaday C. Neurophysiological methods for studies of the motor system in freely moving human subjects. J Neurosci Methods. 1997;74:201-18.

[43] Orth M, Rothwell JC. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. Clin Neurophysiol. 2004;115:1076-82.

[44] Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki H, et al. Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. Brain Res. 2000;859:137-46.

[45] Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res. 1999;128:539-42.

[46] Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol. 1993;466:521-34.

[47] Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. J Physiol. 1999;517 (Pt 2):591-7.

[48] Wolf SL, Butler AJ, Campana GI, Parris TA, Struys DM, Weinstein SR, et al. Intra-subject reliability of parameters contributing to maps generated by transcranial magnetic stimulation in able-bodied adults. Clin Neurophysiol. 2004;115:1740-7.

Figures captions

Fig.1. Overview of the experimental design of the study (A) and the neuromuscular testing protocol (B). The measurements were performed during session 1 (S1), session 2 (S2) and session 3 (S3) except for peripheral nerve stimulation (PNS) and transcranial magnetic stimulation (TMS) setup which were performed only for S1 and S3.

Fig. 2. (A) Typical TA MEP input-output curve. Mean TA MEP amplitudes (white squares) are presented as a function of TMS intensity from 20 to 80% of maximal stimulator output. The *solid line* represents the Boltzman sigmoid function fit to that set of data. *Dashed lines* indicate I_{50} (i.e. stimulus intensity required to obtain a MEP amplitude of half the size of the plateau, ½ P) and P (i.e. the maximum MEP defined by the function). (B) Superimposed twitch force evoked with TMS during maximal (i.e. 100% MVC) and submaximal (i.e. 75% and 50% MVC) voluntary contractions. (C) Example of electromyographic recording in response to transcranial magnetic stimulation (TMS) during maximal (100% MVC) and submaximal voluntary contractions (75% and 50% MVC). The time interval between black and grey arrows represents the way to assess cortical silent period (CSP) duration. Black arrows represent the time of TMS stimulation. Grey arrows represent the resumption of continuous voluntary EMG. Dotted arrows represent the MEP peak-to-peak amplitude. Data are from a representative subject.

Fig. 3. Input-output curve for a representative subject. The optimal stimulation intensity was selected when MEP TA plateaued and when MEP SOL was small. For this subject, 60% of the maximal stimulator output was chosen.

Fig.4. Amplitude of superimposed twitches (SIT) produced by TMS during contraction at 50% (triangles), 75% (circles) and 100% MVC (squares). The linear regression was extrapolated and the y-intercept (dashed line) was interpreted as the ERT amplitude. Data from a representative subject.

Table 1. Intra- and inter-day reliabilities of neuromuscular parameters recorded during maximal (MVC) and submaximal (75% and 50% MVC) voluntary contractions

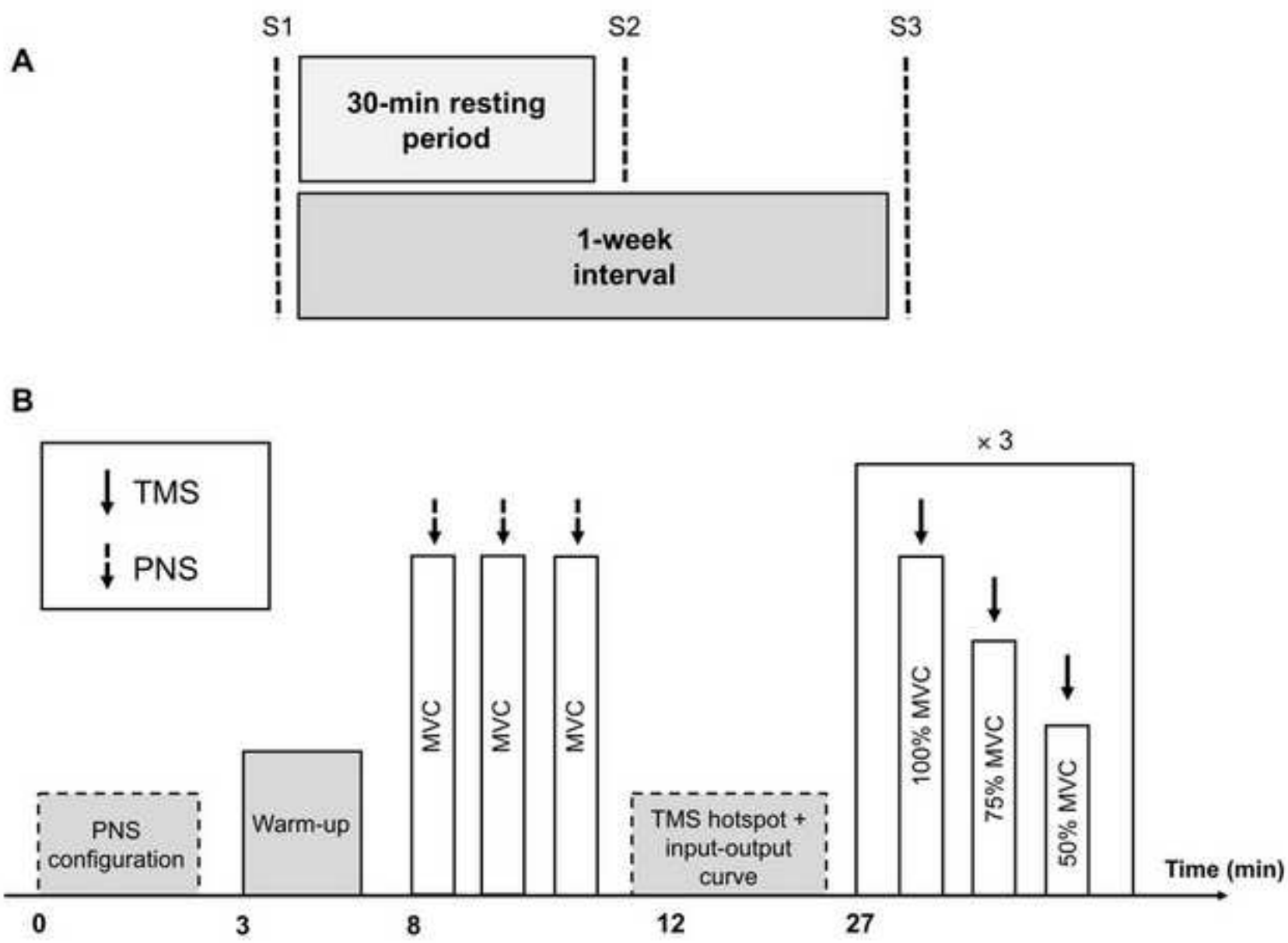
	Mean ± SD			Intra-day			Inter-day		
	S1	S2	S3	ICC	CV (%)	SEM	ICC	CV (%)	SEM
MVC (N.m)	52.4 ± 12.6	52.6 ± 12.9	51.4 ± 12.1	0.96	3.2 ± 2.4	2.7	0.95	4.1 ± 3.0	2.7
RMS / M_{MVC} (%)	9.9 ± 2.3	10.8 ± 2.2	10.5 ± 2.6	0.87	7.7 ± 5.2	0.82	0.68	7.9 ± 6.1	1.4
VA_{TMS} (%)	95.7 ± 5.1	95.9 ± 4.7	96.4 ± 4.9	0.80	1.7 ± 1.5	2.3	0.99	0.8 ± 0.9	0.4
SIT_{MVC} (N.m)	0.76 ± 0.97	0.67 ± 0.88	0.60 ± 0.40	0.73	46.4 ± 40.5	0.5	0.95	38.3 ± 41.7	0.2
ERT (N.m)	16.4 ± 8.1	16.2 ± 4.9	17.4 ± 7.3	0.47	13.2 ± 13.2	4.9	0.94	13.9 ± 14.5	1.8
M_{MVC} (mV)	5.5 ± 1.1	5.7 ± 1.1	6.2 ± 1.7	0.94	6.5 ± 3.7	0.3	0.36	14.1 ± 8.7	1.1
MEP_{50} (mV)	3.1 ± 0.6	3.1 ± 0.8	3.3 ± 1.2	0.83	8.6 ± 6.3	0.28	0.55	13.1 ± 9.3	0.42
MEP_{75} (mV)	3.5 ± 0.8	3.5 ± 0.8	3.6 ± 0.9	0.92	5.6 ± 4.6	0.23	0.92	6.7 ± 6.5	0.26
MEP_{MVC} (mV)	3.1 ± 0.6	3.1 ± 0.8	3.3 ± 1.2	0.80	8.9 ± 6.9	0.31	0.47	11.2 ± 10.7	0.67
MEP_{50} (% M_{MVC})	65.2 ± 14.7	65.3 ± 11.4	61.9 ± 14.9	0.75	10.3 ± 7.5	6.6	0.70	13.4 ± 8.1	8.1
MEP_{75} (% M_{MVC})	75.8 ± 19.1	74.7 ± 13.7	74.4 ± 18.9	0.86	7.1 ± 5.0	6.1	0.88	7.5 ± 7.4	6.4
MEP_{MVC} (% M_{MVC})	57.0 ± 14.0	50.1 ± 12.3	57.0 ± 14.4	0.51	14.9 ± 8.1	8.7	0.52	9.9 ± 10.4	9.2
CSP_{50} (ms)	246 ± 58	266 ± 67	230 ± 58	0.84	6.4 ± 7.4	25.2	0.79	7.8 ± 9.2	26.2
CSP_{75} (ms)	261 ± 64	271 ± 63	246 ± 59	0.99	3.5 ± 3.9	6.4	0.86	7.6 ± 8.3	23.3
CSP_{MVC}(ms)	250 ± 51	267 ± 54	229 ± 62	0.98	7.3 ± 10.9	7.4	0.95	8.6 ± 11.6	13.2

Absolute data and CV are expressed as mean ± standard deviation
S1: session 1; S2: session 2: S3: session 3
MVC: maximal voluntary contraction; RMS: root mean square; VA_{TMS}: cortical voluntary activation; SIT_{MVC}: superimposed twitch amplitude evoked during MVC; ERT: estimated resting twitch amplitude; $M_{MVC\text{-}PP}$: M wave amplitude recorded during MVC; MEP: motor evoked potential recorded at 50% MVC (MEP_{50}), 75% MVC (MEP_{75}) and 100% MVC (MEP_{MVC}). CSP: cortical silent period recorded at 50% MVC (CSP_{50}), 75% MVC (CSP_{75}) and 100% MVC (CSP_{MVC}).
ICC: intraclass correlation coefficients; CV: coefficient of variation; SEM: standard error of the mean.

Table 2. Intra- and inter-day reliabilities for input-output curves parameters.

	Mean ± SD		Inter-day		
	S1	**S3**	**ICC**	**CV (%)**	**SEM**
k	**0.06 ± 0.03**	**0.07 ± 0.03**	**0.73**	**43.9 ± 37.7**	**0.02**
P (%M_{MVC})	**55.8 ± 12.3**	**49.0 ± 12.6**	**0.71**	**14.8 ± 11.3**	**6.7**
I_{50} (% stimulator output)	**37.1 ± 7.4**	**37.2 ± 9.1**	**0.87**	**8.0 ± 6.3**	**3.0**
Peak slope	**0.81 ± 0.55**	**0.88 ± 0.43**	**0.71**	**40.4 ± 37.9**	**0.27**

Absolute data and CV are expressed as mean ± standard deviation
S1: session 1; S3: session 3
K: slope parameter; P: function plateau; I_{50}: stimulus intensity required to obtain a MEP amplitude of half the size of the plateau; ICC: intraclass correlation coefficients; CV: coefficient of variation; SEM: standard error of the mean.


A
S1
S2
S3
30-min resting period
1-week interval
B
TMS
PNS
× 3
MVC
MVC
MVC
100% MVC
75% MVC
50% MVC
PNS configuration
Warm-up
TMS hotspot + input-output curve
Time (min)
0
3
8
12
27

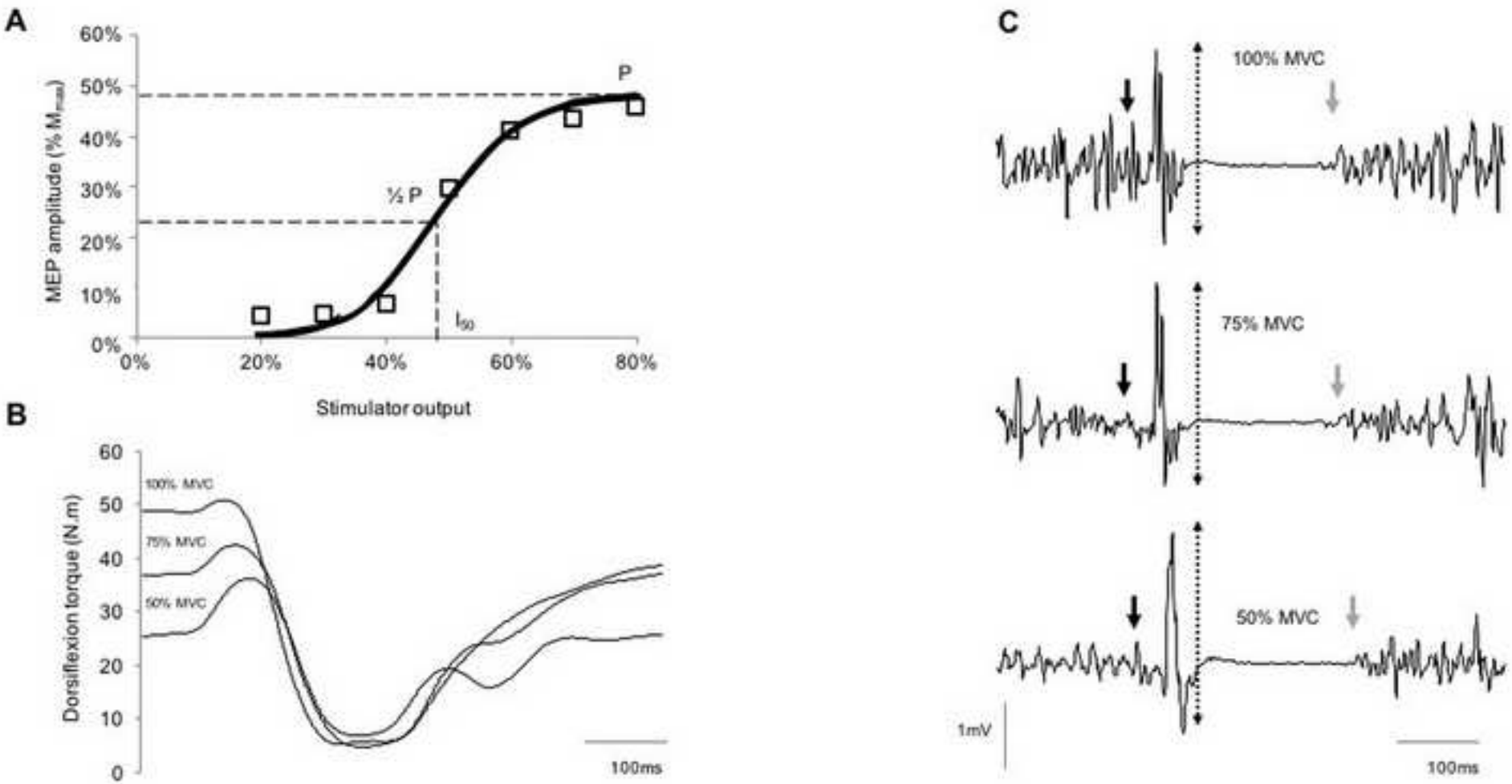

A
60%
50%
40%
30%
20%
10%
0%
MEP amplitude (% M_{max})
P
½ P
I_{50}
0%
20%
40%
60%
80%
Stimulator output
B
60
50
40
30
20
10
0
Dorsiflexion torque (N.m)
100% MVC
75% MVC
50% MVC
100ms
C
100% MVC
75% MVC
50% MVC
1mV
100ms

Figure 3

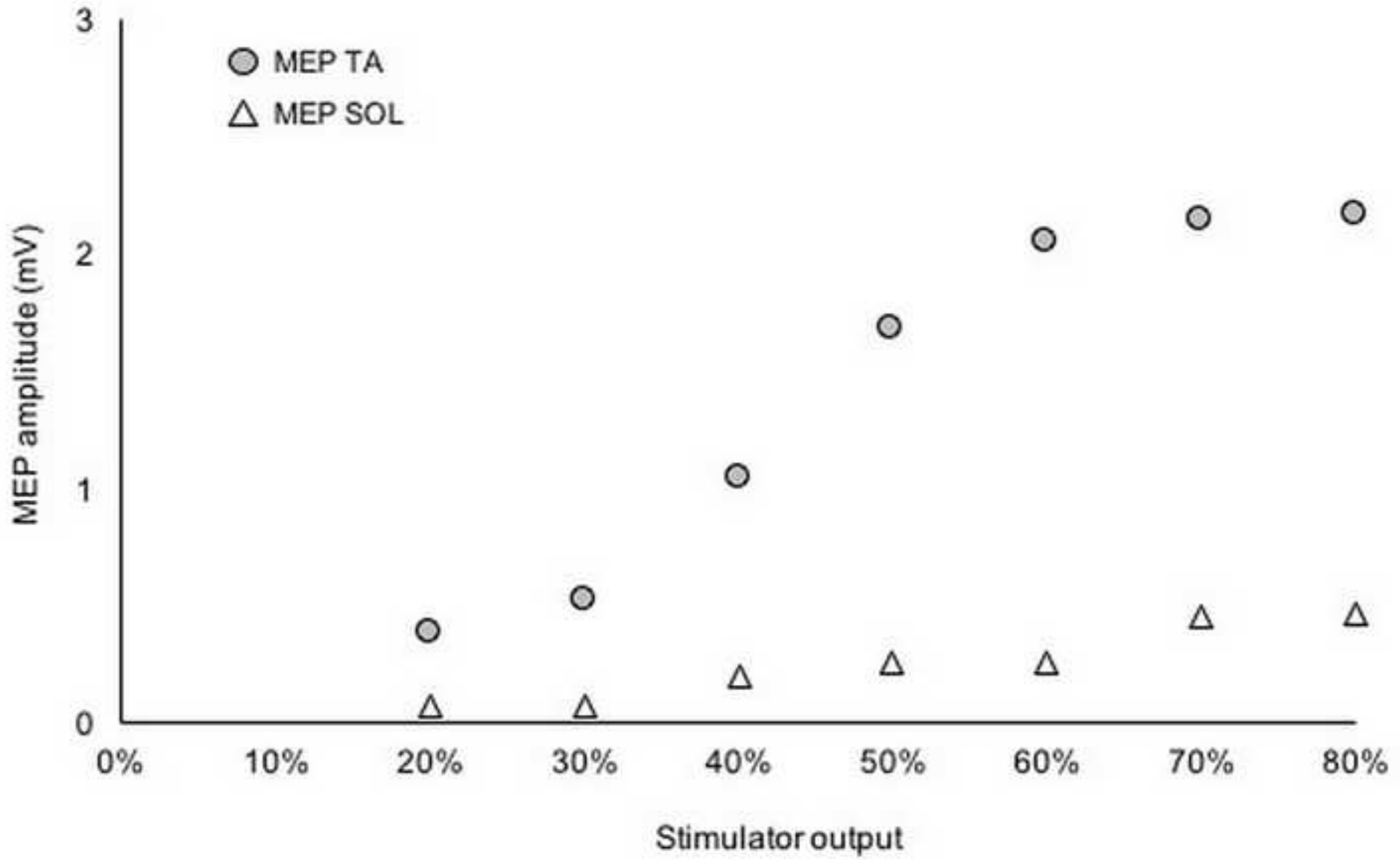

MEP TA
MEP SOL
MEP amplitude (mV)
3
2
1
0
0%
10%
20%
30%
40%
50%
60%
70%
80%
Stimulator output

Figure 4