Effect of stimulation timing on testing voluntary muscle force generation

Antonio Madrid, Elena Madinabeitia-Mancebo, Verónica Robles-García, Marcelo Chouza-Insua, Javier Cudeiro, Pablo Arias

Universidade da Coruña, NEUROcom (Neuroscience and Motor Control Group) and Biomedical Institute of A Coruña (INIBIC), Department of Physiotherapy, Medicine and Biomedical Sciences and INEF Galicia, A Coruña Spain

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ABSTRACT

Background: The interpolated twitch technique (ITT) is a ubiquitous test for assessing the level of voluntary muscle force generation, in which muscle twitches are evoked via percutaneous electrical stimulation. Traditionally, the stimulation timing during the ITT is not computer-controlled and usually delivered from 5 to 10 s after the maximal voluntary contraction (MVC) of the potentiated muscle.

Methods: In this work, we evaluated the sizes of the evoked twitches in the lower limb with different controlled stimulation time delays with respect to the MVC of the ankle plantar flexors. Fifteen healthy participants were included. We recorded the un-potentiated muscle twitch amplitudes at rest in response to doublet supramaximal stimulation of the tibial nerve, superimposed twitches (SITs) at three different delays from the beginning of the MVC force plateau (0.1, 0.75, and 1.5 s), and resting twitches in the potentiated muscle at four different delays once the MVC was finished (0.1, 2.5, 5.0, and 10.0 s).

Results: The magnitude of the SITs did not vary among the delays tested but varied among the potentiated resting twitch (PRT) amplitudes, with 2.5 s being largest and 0.1 s being the smallest. Remarkably, the resting twitch amplitudes reduced during the session despite the long rest periods between MVCs (5 min).

Conclusion: We conclude that proper control of the stimulation timing is mandatory to increase the sensitivity of the ITT, and a 2.5 s delay from the end of the MVC is recommended for the PRT. Controlling the development of fatigue, which can be intrinsic to testing with repeated MVCs, is also essential. We recommend reducing the number of MVC repetitions and increasing the rest periods between them.

1. Introduction

Muscle fatigue has central and peripheral origins, and it is of paramount importance in different biomedical fields. The interpolation twitch technique (ITT) is a ubiquitous test to evaluate central expressions in muscle force generation (Merton, 1954). The ITT involves percutaneous electrical stimulation of the muscle nerve (or motor point) during a maximal voluntary contraction (MVC). The stimulation produces an increase in the force torque (i.e. a superimposed twitch [SIT]) as it recruits fibres, which despite not being in the refractory period, were not recruited by the maximal voluntary effort. The larger the SIT, the weaker the voluntary activation (VA) in the testing task. To consider the regulatory mechanisms of muscular contractions at the peripheral level (e.g. contractility or neuromuscular transmission), the magnitude of the SIT torque gain is made relative to the gain in force torque produced by the same stimulation with the muscle at rest. Usually, the twitch at the resting state is acquired after the MVC. However, it means recording a muscle twitch during a potentiated state (Baudry and Duchateau, 2004; Folland and Williams, 2007), where the magnitude of the potentiating twitch (PRT) is larger than that of the un-potentiated resting twitch (un-PRT) tested just prior to the MVC. At the physiological level, potentiation permits the maintenance of force outputs even when motor unit firing rates decline (Klein et al., 2001), and its evaluation is influenced by several factors, which were well accounted for in a previous study (Baudry and Duchateau, 2004). These factors include the duration of the potentiating contraction or the time delay to the subsequent stimulation (Baudry and Duchateau, 2004).

Nevertheless, use of the ITT to test VA seems reliable under the same stimulation conditions (Allen et al., 1995; Behm et al., 1996), where

\[
VA(\%) = \left[1 - \left(\frac{\text{SIT_Amplitude}}{\text{PRT_Amplitude}}\right)\right] \times 100
\]
However, the stimulation timing seems to be a critical issue, as 5 s after the contraction, the potentiated muscle state declines logarithmically (Baudry and Duchateau, 2004; Hamada et al., 2000). Therefore, to improve the sensitivity of the ITT, determining the optimal timing for stimulation delivery after the MVC is critical. Theoretically, the sensitivity of the ITT would improve if the PRT is tested under similar muscular conditions that were recorded during the MVC. In most of the cases, stimulation for acquiring the PRT is applied in a range of intervals from 5 to 10 s after the MVC. However, to the best of our knowledge, no studies have systematically investigated the best delay (even <5 s) to acquire the greatest muscle potentiation and thereby increase the sensitivity of the ITT for VA testing. Following the same line of reasoning, it also seems essential to check whether the magnitude of the SIT changes during an MVC of short duration, as usually performed with the ITT.

This short report aimed at clarifying the effect of stimulation timing on the sensitivity of the ITT. In the ankle plantar flexors, we tested the amplitude of the SIT at different time delays from the beginning of the plateau of force during several 4-s MVC repetitions, with 5 min rest between them. Moreover, the effect of the stimulation time delay from the end of the MVC on the PRT size was tested. We hypothesised that the magnitude of the PRT would decrease with the time progression after the MVC. If the magnitude of the PRT is maximal at delays shorter than those frequently used in the field (between 5 and 10 s), setting delays <5 s after the MVC will be recommend during ITT testing. Likewise, we expected an increase in the magnitude of the SIT if the time delay from the MVC peak to stimulation delivery was increased. Finally, changes in muscle temperature are known to modify the contractile properties of the muscle (de Ruijter et al., 1999) and can change the morphology of electrically evoked twitches. Therefore, the muscle temperature was controlled throughout the session.

2. Materials and methods

We performed two experimental sessions. In the first session, the participants familiarised themselves with the task and were evaluated for their tolerance to electrical stimulation at the intensity needed in the study (Button and Behm, 2008). The participants also executed MVCs, as in the main experimental session, as a part of the familiarisation process (Gandevia, 2001). After 10 days, the second (main) session was conducted similarly to the first one but included electrical stimulations at the proper time delays. All the participants provided written consent to participate. The study protocol was in accordance with the Declaration of Helsinki and approved by our institutional ethics committee (CEID17112017).

2.1. Participants

A total of 15 healthy participants completed both sessions (8 women; age range, 20–44 years; mean age, 24 years; weight range, 60–83 kg; height range, 1.64–1.83 m). None of the participants were professional or high-level athletes, but all of them performed occasional leisure activities up to three times/week. To apply the inclusion criteria, they were screened for previous musculoskeletal injuries or drug consumption. Two other participants initially attended the sessions but withdrew from the study, as they could not tolerate the electrical stimulation.

2.2. Protocol

The participants were required to perform plantar flexion MVC with their dominant lower limb while seated. Dominance was determined by the participants’ self-report of their most powerful limb. We used a custom fixation device, including an S-Beam force cell (Biometrics Ltd., Newport, UK), to record ankle force torque and restrain ankle and knee movements. We used goniometers (SG100, Biometrics Ltd., UK) to control the flexo-extension movements around the ankle and knee joints. Sensors were connected to a K800 amplifier (Biometrics Ltd) that sent signals to a CED-1401 mkII (unit-1; Cambridge Electronic Design Limited, Milton, Cambridge, UK). CED unit 1, controlled with the Signal 6 software (Cambridge Electronic Design Limited), sampled signals (at 10 kHz) and stored them for subsequent analyses.

The force signal from the K800 amplifier was also sent in parallel to another CED-1401 mkII (unit-2) controlled with a sequencer (Cambridge Electronic Design Limited), with sampling at 100 kHz. The unit sent triggers to cue for participant execution and induced an electrical stimulation. Triggers were also delivered to CED unit-1 to facilitate posterior offline analyses. The stimulation timing was controlled as follows (Figure 1A):

1.) With the participants at rest, stimulation generated an un-PRT.
2.) After 15 s, a red light-emitting diode (LED) was turned on, prompting the participants to perform an MVC. Electrical stimulation was applied at 0.1, 0.75, or 1.5 s after the MVC force reached its peak and the plateau started. For this purpose, we used a previously described and validated algorithm to compute the rate of force development (Madrid et al., 2018). The participants were required to make the effort as fast as they could and maintained the MVC for as long as the LED remained “On” (4 s).
3.) Next, the LED was turned off as a cue to stop the MVC and relax, and the sequencer initiated stimulation in the potentiated resting muscle state with the same algorithm (Madrid et al., 2018), but this time at the rate of force decrement. Stimulation was applied at four possible time delays after muscle relaxation (0.1, 2.5, 5, or 10 s).

A session included 12 MVC repetitions with verbal encouragement and force execution visual feedback, allowing a 5 min rest in between repetitions. The MVCs were executed without mistakes.

The stimulation timing during the MVC and at the resting potentiated muscle state was pseudo-randomised in order. Randomisation of the 0.1, 0.75, and 1.5 s delays in the first three MVC repetitions (defined as the first set) was repeated in the second, third, and fourth sets, including the fourth to the sixth, seventh to the ninth, and 10th to the 12th MVC repetitions, respectively. For the PRT, randomisation of the 0.1, 2.5, 5, and 10 s stimulation delays was performed likewise; in this case, the three sets included these delays in the first to the fourth, fifth to the eighth, and ninth to the 12th repetitions.

Electrical stimulation of the tibial nerve was applied percutaneously with a Digitimer D57AH stimulator (Digitimer Ltd. Welwyn Garden City, Hertfordshire, UK). Pulses were 100–200 μs in width, delivered with the cathode over the nerve on the popliteal fossa and the anode over the patella. The stimulation was a 100 Hz doublet (Behm et al., 1996; Kennedy et al., 2014) in all the cases. Electromyography (EMG) signals from the soleus (SOL) and tibialis anterior muscles (TA) were acquired with Digitimer D360 amplifiers (gain, ×200–1000; band pass, 3–3,000 Hz) and sent to CED unit-1. At the beginning of the session, the SOL compound muscle action potential (CMAP) was determined on the basis of a stepwise increase in stimulation intensity (with a single pulse) and an intensity 1.2–1.5 times greater than that used in the doublets in the rest of the session (Baudry and Duchateau, 2004). A 3 mm tip-probe (YSI Temperature Probe 400 series, YSI Precision Temperature Group Ltd., Dayton, Ohio, US) connected to a CITER amplifier (CIBERTEC S.A. Madrid, Spain) recorded the temperature over the SOL muscle at all stimulation times, in which the signal was sent to the CED-1401 unit-1 for posterior analyses.

2.3. Analysed variables

We evaluated the following variables in our study: MVC muscle force was defined as the mean force in the 10 ms period just prior to stimulation during the MVC (Madrid et al., 2018). The SIT amplitude was computed as the difference between the peak of the force torque evoked by the stimulation during the MVC and the MVC muscle force. For
the reviewed (Hewlett et al., 2011). The VAS score was determined before visual analogue scale (VAS; 0, not fatigued at all; 10, totally exhausted), PRT. Finally, we also evaluated the rate of perceived fatigue by using a
ture was computed at the time of percutaneous stimulation to acquire the
relaxation after the MVC. We also measured the muscle temperature as
terior to the PRT was calculated to assess the complete muscle
estimated from the skin temperature (de Ruiter et al., 1999). Tempera-
t invading muscle (Jones, 2010). EMG background activity (root mean square)
the relaxation phase of the PRT. It evaluates the contractile properties of
among the participants. The potentiation at 2.5 s was greater than that at 0.1 and 10 s. Furthermore, 0.1 s was smaller than any other delays. H and I) Evaluation of HRT (raw and normalised scores, respectively). J) EMG-background activity at the time of PRT testing after the MVC. K) The levels of perceived fatigue increased at the
the middle of the protocol and increased further at the end of the session. The plotted scores are the mean values across the subjects and the standard error of the
mean.

Figure 1. A) Stimulation timings for recording the un-PRT (blue arrows), SIT (pink arrows), and PRT (black arrows). B and C) Changes in the un-PRT amplitudes during the set progression and raw and normalised scores, respectively. D) Raw scores for the PRT during the set progression. E) PRT to optimise the display of the delay effect. F) PRT recordings in one participant at the different delays and sets. For all the delays, the amplitudes were reduced with the set progression, with the largest and smallest PRTs evoked at 2.5 (blue traces) and 0.1 s delays (red traces). G) Changes in normalised PRT amplitudes at the different delays after the MVC among the participants. The potentiation at 2.5 s was greater than that at 0.1 and 10 s. Furthermore, 0.1 s was smaller than any other delays. H and I) Evaluation of HRT (raw and normalised scores, respectively). J) EMG-background activity at the time of PRT testing after the MVC. K) The levels of perceived fatigue increased at the middle of the protocol and increased further at the end of the session. The plotted scores are the mean values across the subjects and the standard error of the mean.

determining the PRT and un-PRT, we computed the difference between
the peak of the force torque evoked by stimulation at rest and the mean
force in the 10 ms period just prior to stimulation. Half relaxation time
(HRT) was the time lag from the peak torque until 50% of it tested during
the relaxation phase of the PRT. It evaluates the contractile properties of
the muscle (Jones, 2010). EMG background activity (root mean square)
just prior to the PRT was calculated to assess the complete muscle
relaxation after the MVC. We also measured the muscle temperature as
estimated from the skin temperature (de Ruiter et al., 1999). Tempera-
ture was computed at the time of percutaneous stimulation to acquire the
PRT. Finally, we also evaluated the rate of perceived fatigue by using a
visual analogue scale (VAS; 0, not fatigued at all; 10, totally exhausted),
whose validity and reliability for this purpose have been previously
reviewed (Hewlett et al., 2011). TheVAS score was determined before
the first MVC and immediately after the first, sixth, and 12th MVCs. The
introduced score in the analyses was the VAS score incremented at the first,
sixth, and 12th MVCs as compared with the score before the first MVC
(approximately 0).

2.4. Statistical analyses

The variables were analysed after intra-subject normalisation. The
MVC force was normalised to the maximum MVC force score in all the
repetitions. The un-PRT was normalised to the maximum un-PRT ac-
quired during the whole session. The PRT was normalised to the un-PRT
acquired in the same repetition. Furthermore, the HRT was normalised to
the shortest HRT, considering all the repetitions within the session. The
root-mean-square amplitudes of the SOL and TA EMG background ac-
tivity were normalised to the mean score across all the delays for the
given muscle.

After assuming distribution normality with the Kolmogorov-Smirnov
test, the variables were analysed with repeated-measures analysis of
variance (ANOVA) with delay and set as factors. ANOVA for VAS score
only used repetition as the factor of analysis (considered after the first,
sixth, and 12th MVC repetitions). The degrees of freedom were corrected
(if needed) with the Greenhouse-Geisser coefficient. Two variables did
not match normality; thus, they were derived for non-parametric ana-
lyses. By using the Friedman test, we first checked the differences be-
tween the four repetitions of every delay in the different sets. As they did
not differ across the sets, all the repetitions for the same delay were
averaged. Subsequently, the difference between delays (considering the
average) was tested with ANOVA with factor delay after re-checking the
normality assumption (one-sample Kolmogorov-Smirnov test). Pairwise
comparisons between the levels of delay and sets were applied if the
factor was significant. The significance level was set at p < 0.01 (Button
et al., 2013).

3. Results

Table 1 summarises the results. The un-PRT amplitude (either abso-
lute or normalised scores) decreased significantly as the sets progressed
(F2,28 = 24.4, p < 0.001SET and F2,28 = 14.9, p < 0.001SET, respectively;
see Figure 1B and 1C for the pairwise comparisons). This decrease was
also observed in the PRT absolute scores (F2,28 = 7.8, p = 0.002SET;
Figure 1D, 1E, and 1F). Remarkably, the MVC force remained stable
across the delays and sets. However, the MVC scores decreased
(approximately 5%) with the set progression in all the participants, but
the effect was not significant.

Several variables significantly changed across delays. This was the
case for the PRT amplitude in absolute values (F3,42 = 14.1, p < 0.001DEL,
Figure 1E, thick line; Figure 1F presents the recording ex-
amples in one participant). The same was observed when the PRT nor-
malised scores were analysed (F3,42 = 15.9, p < 0.001DEL; Figure 1G). The HRT also changed at the different delays tested, either
analysed in absolute values ($F_{3,42} = 63.5$, $p < 0.001_{\text{DELAY}; \text{Figure 1H}}$) or normalised scores ($F_{3,42} = 63.5$, $p < 0.001_{\text{DELAY}; \text{Figure 1H}}$). The pairwise comparisons between delays (represented in figures) indicate that the 0.1 s delay induced the smallest scores in all these variables (the difference in scores between this delay and any other was always significant). Remarkably, the normalised PRT amplitudes were greater at the 2.5 s delay than at the 10 s delay. Delay effects never changed with the set progression (delay $\times$ set interactions were always not significant). The EMG background activity at PRT testing changed with the different delays. It approached significance in the case of the SO ($F_{3,42} = 3.6$, $p = 0.021_{\text{DELAY}}$) and TA ($F_{3,42} = 3.9, p = 0.049_{\text{DELAY}}$). In the two cases, the 0.1 s delay was larger than the other three delays (Figure 1J), which suggests complete relaxation after the MVC was compromised at the time of 0.1 s PRT testing.

Remarkably, the above-mentioned results were obtained from recordings at a constant muscle temperature of 31.1°C (standard error – SE, 1.4) across delays and sets. At the subjective level, the participants perceived increasing fatigue along the session. Muscle temperature with repeated MVCs despite testing at 5 min intervals to allow rest. This sign of peripheral fatigue is accompanied by increasing levels of perceived fatigue along the session. Muscle temperature is known to modify muscle contractility (de Ruiter et al., 1999) but remained stable in our study; therefore, this can be discarded as a source of changes in the PRT and un-PRT in our study.

Compared with that in the un-PRT, the muscle potentiation produced by the MVC was evident in the PRT along 10 s. We found approximately 14% potentiation if pooled across 2.5, 5, and 10 s delays. This finding is in line with prior reports that examined potentiation after 5 s and later (Duchateau and Baudry, 2014; Hamada et al., 2000). The potentiation magnitude varies depending not only on the time delay from the previous potentiating contraction but also on its duration and the different proportions of slow or fast fibres composing the muscle (Hamada et al., 2000; Moore and Stull, 1984). Although the PRT can be maximal after 10 s MVCs, this time is enough to induce neural expressions of fatigue during the MVC (Madrid et al., 2016); therefore, we restricted MVC duration in our protocol.

Muscle potentiation evolves in time when tested after 5 s from the potentiating contraction (Duchateau and Baudry, 2014; Hamada et al., 2000). Our reasoning was that the closer the stimulation delivery to the potentiating contraction (i.e. MVC), the greater the ITT sensitivity to test the VA. This possibility was confirmed but only partially. The largest PRT amplitudes occurred at 2.5 s with a subsequent decrease. The PRT reduction in amplitude from 2.5 s was significant as compared with that

### Table 1: Statistical significance of the main effects and interaction of the factors with the studied variables.

| Variable | DELAY, P VALUE | SET, P VALUE | SET $\times$ DELAY, P VALUE |
|----------|----------------|--------------|----------------------------|
| MVC (raw magnitude) | 0.050 | 0.373 | 0.896 |
| MVC (normalised)* | 0.054 | 0.332 (0.1 s delay) | 0.156 (0.75 s delay) | 0.131 (1.5 s delay) |
| un-PRT (raw magnitude) | 0.381 | <0.001 | 0.464 |
| un-PRT (normalised) | 0.280 | <0.001 | 0.532 |
| PRT (raw magnitude) | <0.001 | 0.002 | 0.321 |
| PRT (normalised) | <0.001 | 0.574 | 0.272 |
| SOL-EMG background at PRT (normalised) | 0.021 | 0.091 | 0.266 |
| TA-EMG background at PRT (normalised) | 0.049 | 0.112 | 0.441 |
| SIT (raw magnitude) | 0.192 | 0.197 (0.1 s delay) | 0.878 (0.75 s delay) | 0.293 (1.5 s delay) |
| HRT from un-PRT (raw magnitude) | 0.272 | 0.510 | 0.464 |
| HRT from un-PRT (normalised) | 0.248 | 0.451 | 0.431 |
| HRT from PRT (raw magnitude) | <0.001 | 0.209 | 0.361 |
| HRT from PRT (normalised) | <0.001 | 0.179 | 0.381 |
| Muscle temperature | 0.140 | 0.115 | 0.437 |

Significant p-values are presented in bold.

For the variables that did not show normality, the scores for the same delay were tested for differences across sets with non-parametric tests and showed no significant difference. Then, they were averaged, and the difference (across delays) was re-tested.

4. Discussion

The ubiquitous test to evaluate the maximal muscle force produced voluntarily and central fatigue induced by isometric contractions includes SIT and PRT recordings. For this purpose, the ITT is used, which involves stimulation during an MVC and, subsequently, in the potentiating contraction (Allen et al., 1995; Babault et al., 2006; Gandevia et al., 1998; Pasquet et al., 2000). Originally, the technique was developed using percutaneous electrical stimulation; currently, it is sometimes also used with transcranial magnetic stimulation of the M1. The latter estimates the supraspinal failure in muscle activation (Goodall et al., 2009; Sidhu et al., 2009; Todd et al., 2003). However, whether an optimal delay for electrical stimulation exists with regard to the MVC for ITT testing remains to be clarified. If this is the case, its methodological relevance must be determined. Protocols vary among studies; for example, in the case of the PRT, the timing is immediately after the MVC (Babault et al., 2006), in <5 s (Allen et al., 1995), and after 5 s (Gandevia et al., 1998). The timing during the MVC to obtain the SIT varies even further and is sometimes unreported or triggered by hand at the observed plateau of the MVC. Perhaps, this is one of the reasons for the variable responses observed across studies using the ITT. This study aimed to determine the influence of stimulation timing on the amplitude of the SIT and PRT.

The main finding of this study was that stimulation timing to obtain the SIT from the MVC plateau had no impact on the magnitude of the evoked responses in the tested conditions. Stimulation timing is otherwise critical in the case of the PRT, even at the short-delay periods commonly used with the ITT.

A secondary but important finding for experimental designing is the waning of muscle contractility with repeated MVCs despite testing at 5 min intervals to allow rest. This sign of peripheral fatigue is accompanied by increasing levels of perceived fatigue along the session. Muscle temperature is known to modify muscle contractility (de Ruiter et al., 1999) but remained stable in our study; therefore, this can be discarded as a source of changes in the PRT and un-PRT in our study.
at 10 s after the MVC. The shortest of the tested delays from the MVC (0.1 s) produced the smallest muscle twitch amplitudes. We do not have a clear explanation for this finding, but it could be due to the incomplete muscle relaxation in some subjects; note that at a whole-sample level, the background EMG activity during the PRT was greater at 0.1 (approximately 30%) than at any other delay, but not consistently enough across subjects to become significant. This means complete relaxation was difficult to achieve by some subjects. Therefore, we do not recommend the use of extremely short delays. Regardless of the mechanism, this delay appears unsuitable for the purposes of VA testing, and the 2.5 s delay seems to be more suitable than any other delay tested herein to improve ITT sensitivity. This result emphasizes the need of stimulating carefully and automatically at controlled timings after the MVC to avoid biases during VA evaluation.

Conversely, delaying the stimulation from the start of the force plateau of the MVC did not influence the SIT amplitudes, most likely because the MVC force remained stable along the contraction after reaching the force peak. Nevertheless, two points require attention as follows:

i) We optimised our setup to test mainly the SOL (type I muscle fibres). Previously, testing the hand first dorsal interosseous muscle (mostly composed of type II fibres) induced a fast MVC force decrement from its plateau (Madrid et al., 2018). Therefore, our results may not be generalised across muscles, especially in those with different histo-chemical properties (Moore and Stull, 1984). Therefore, shorter delays after MVC peak plateau might be preferable during SIT testing (Madrid et al., 2018).

ii) Previous studies found an effect of stimulation timing during the MVC on SIT amplitude in the absence of fatigue (Folland and Williams, 2007). By contrast, we did not observe such effect, but the difference is that we tested different SIT delays during different MVCs (with 5 min rest), not different delays during the same MVC (Folland and Williams, 2007). In the latter case, muscle potentiation induced by the series of stimuli remains a possibility.

Finally, the levels of perceived fatigue and amplitude of the resting twitches indicated fatigue accumulation with set progression. Thus, executing the MVC for VA testing has an impact on fatigue itself (even when allowing 5 min rest between MVCs during testing), but only few studies have considered this issue when designing experiments (Madrid et al., 2018). Peripheral fatigue can be present without reductions in MVC muscle force because physiological processes depending on different factors do not behave in arithmetic addition/reduction of their components. Thus, we believe reduction in resting twitch amplitude with set progression is still possible with a no significant reduction in MVC force and stable levels of SITs.

Understanding the key physiological mechanisms underlying our observations is beyond the scope of this study. Notwithstanding, the contractile properties of the muscle (as tested by the half relaxation time) appear not to be amongst the key mechanisms, as these remained unchanged in the present study. We could not confirm if the neuromuscular transmission and propagation of potentials through the sarcolemma are mechanisms involved in this process: We used doublet stimulation at 100 Hz to evoke twitches, a standard way of using the ITT (Kennedy et al., 2014); therefore, no CMAP is available because the stimulation artefacts (doublet stimulation) alter its shape.

5. Conclusion

The stimulation time delay after the MVC must be < 5 s and carefully controlled for the greatest ITT sensitivity during VA testing. Repeated short MVC efforts may generate fatigue despite considerably long resting periods; this can be a confounding factor in fatigue testing and must be controlled. Our suggestion is to reduce the number of MVCs to a minimum, allow longer resting periods between them, and include the reporting of practice trials.

Declarations

Author contribution statement

Antonio Madrid, Pablo Arias: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Elena Madinabeitia-Mancebo, Verónica Robles-García, Marcelo Chouza-Insaú: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Javier Cudeiro: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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