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ORIGINAL ARTICLE

Effects of fatigue on the electromechanical delay components in *gastrocnemius medialis* muscle

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Abstract
Purpose Under electrically evoked contractions, the time interval between the onset of the stimulation pulse (Stim) and the beginning of force (F) development can be partitioned ($Delay_{TOT}$), by an electromyographic (EMG), mechanomyographic (MMG) and F combined approach, into three components each containing different parts of the electrochemical and mechanical processes underlying neuromuscular activation and contraction. The aim of the study was to evaluate inter- and intra-operator reliability of the measurements and to assess the effects of fatigue on the different $Delay_{TOT}$ components.
Methods Sixteen participants underwent two sets of tetanic stimulations of the *gastrocnemius medialis* muscle, with 10 min of rest in between. After a fatiguing protocol of 120 s, tetanic stimulations were replicated. The same protocol was repeated on a different day. Stim, EMG, MMG and F signals were recorded during contraction. $Delay_{TOT}$ and its three components (between Stim and EMG, Δt Stim-EMG; between EMG and MMG, Δt EMG-MMG and between MMG and F, Δt MMG-F) were calculated.
Results Before fatigue, $Delay_{TOT}$, Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F lasted 27.5 ± 0.9, 1.4 ± 0.1, 9.2 ± 0.5 and 16.8 ± 0.7 ms, respectively. Fatigue lengthened $Delay_{TOT}$, Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F by 18, 7, 16 and 22 %, respectively. Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F contributed to $Delay_{TOT}$ lengthening by 2, 27 and 71 %, respectively. Reliability was always from high to very high.
Conclusions The combined approach allowed a reliable calculation of the three contributors to $Delay_{TOT}$. The effects of fatigue on each $Delay_{TOT}$ component could be precisely assessed.

Keywords Electrically evoked contraction · Isometric contraction · Recovery · EMG · MMG · Reliability

Abbreviations

D_1 and D_2	Day 1 and day 2
$Delay_{TOT}$	Latency between the onset of stimulation current and force development
Δt Stim-EMG	Time interval between the onset of stimulation current and the onset of muscle electrical activation. Synaptic component
Δt EMG-MMG	Time interval between the onset of muscle electrical activation and the onset of muscle contraction. E–C coupling component
Δt MMG-F	Time interval between the onset of muscle contraction and the force development. Mechanical component
EMD	Electromechanical delay. Time interval between the onset of muscle electrical activation and force development
EMG	Electromyography
F	Force
GM	*Gastrocnemius medialis* muscle
MMG	Mechanomyography
MMG p-p	MMG peak-to-peak

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MTU	Muscle–tendon unit
Op. 1 and Op. 2	Operator 1 and operator 2
pF	Peak force
RFD	Rate of force development
S_A and S_B	Session A and session B
Stim	Stimulation current

Introduction

The electromechanical delay (EMD) is traditionally defined as the time lag between the onset of muscle electrical activation and the onset of force production (Cavanagh and Komi 1979). During this time frame, a sequence of several physiological events transduces the electrical processes of muscle activation into a mechanical phenomenon: (1) the propagation of the action potential along the sarcolemma and the T-tubule system; (2) the coupling between the dihydropyridine and ryanodine receptors and the following release of Ca^{2+} from the sarcoplasmic reticulum; (3) the interaction among Ca^{2+}, troponin and actin; (4) the cross-bridges formation; and (5) the myosin heads rotation with subsequent force transmission to the tendon insertion point through the elongation of the series elastic components (SEC). While the first four mechanisms can be considered as electrochemical in nature, the last events are mainly mechanical.

Because of the several approaches with different experimental setup and protocols, EMD values from different human skeletal muscles have been found to vary between 8 and 127 ms (Grosset et al. 2009; Hopkins et al. 2007; Nordez et al. 2009; Yavuz et al. 2010). Changes in any of the previously listed events could potentially induce EMD alterations (Cé et al. 2013; Esposito et al. 2011; Granata et al. 2000; Lacourpaille et al. 2013a; Muraoka et al. 2004; Viitasalo and Komi 1981; Yavuz et al. 2010).

To provide more insights into the sequence of events underlying muscle activation and contraction, a technique based on very high frame rate ultrasounds was utilized to determine in vivo the delay between muscle electrical activation and the onset of muscle fascicles and tendon motion (Hug et al. 2011a; Lacourpaille et al. 2013b; Nordez et al. 2009). However, this methodology requires the latest generation of echographic devices to obtain accurate measurements.

Also a combined electromyographic (EMG), mechanomyographic (MMG) and force (F) signals analysis (Cé et al. 2013; Esposito et al. 2011; Petitjean et al. 1998; Sasaki et al. 2011) can provide useful information about muscle electrical and mechanical behaviour from the same muscle area (Barry 1992; Esposito et al. 2005; Orizio et al. 1999). MMG, indeed, can be considered during contraction as the mechanical counterpart of motor unit electrical activity detected by surface EMG (Gordon and Holbourn 1948). Moreover, MMG and F characteristics during isometric contraction can be explained by a recent muscle–tendon unit mechanical model, which defines two distinct elastic elements (K_1 and K_2) with different compliance (Orizio et al. 1999) and can help to provide physiological explanation of the mechanical events included in EMD. Due to the highly compliant K_2, indeed, the shortening of the contractile elements results first in a muscle geometry change (with dimensional changes of the transverse diameter of the muscle fibres that generates pressure waves, detectable as MMG at the skin level) with low force output. Thereafter, when the slack of the elastic-connective tissue has been taken up, the tension is efficiently transmitted to the tendon with a quick raise in F and a reduction in MMG amplitude (Orizio et al. 1999, 2003).

With this approach, EMD can be, therefore, partitioned in: (1) a component that is mainly electrochemical (time lag between the onset of EMG and the onset of MMG, Δt EMG-MMG), in which the excitation–contraction (E–C) coupling, from the propagation of the motor unit action potential at the sarcolemmal level to myosin head rotation, and pressure wave transmission to the skin surface (detected by MMG) are included (Hufschmidt 1985; Petitjean et al. 1998); and (2) a mechanical component (time lag between the onset of MMG and the onset of F, Δt MMG-F), a potential index of the time required for taking up the muscle–tendon unit slack, before force transmission becomes efficient at the tendon insertion point (Cé et al. 2013; Esposito et al. 2011; Hufschmidt 1985; Sasaki et al. 2011).

Fatigue, which is one of the factors affecting the contraction process, alters the mechanisms involved in neuromuscular activation and muscle contraction, causing an EMD elongation (Cé et al. 2013; Yavuz et al. 2010; Zhou et al. 1996, 1998). In fatigued muscle, indeed, among other phenomena, a slowing of the motor unit action potential propagation along the sarcolemma, a decrement of Ca^{2+} efflux from sarcoplasmic reticulum and a reduction in cross-bridge cycling rate take place (Ament and Verkerke 2009; Fitts 1994, 2008). When partitioning EMD in Δt EMG-MMG and Δt MMG-F component, fatigue has been shown to affect both of them to a similar extent (Cé et al. 2013). However, although with some controversy related to voluntary contraction (Bigland-Ritchie et al. 1982), fatigue may alter not only the processes included in EMD, but also the mechanisms at the neuromuscular junction (Kirkendall 1990; Wieser 1915).

With electrically evoked contractions, the simultaneous detection of the stimulation current (Stim) together with the surface EMG may provide additional information on the processes at the pre-synaptic and synaptic level (Δt Stim-EMG). Indeed, the time delay between Stim, applied either on the nerve or on the muscle motor point, and the

generated M-wave reflects the latency between the origin of the action potential at the axonal level and the action potential along the sarcolemma, thus including also the synaptic latency. Consequently, in addition to the traditionally defined EMD, the latency between the beginning of Stim and force development can be calculated ($Delay_{TOT}$).

Methodological concerns about EMD measurement, though, have been recently raised by Hug et al. (2011b). In particular, EMG electrodes positioning may greatly influence onset detection of electrical muscle activation (up to 20 ms), thus affecting EMD measurement reliability. A detailed and reliable characterization of $Delay_{TOT}$ and its components may represent an important aid for the evaluation of neuromuscular activation and muscle contraction in clinical, rehabilitative and physical training fields.

Therefore, the aim of the present study was twofold: (1) to evaluate the intra- and inter-operator reliability of the measurement of each single $Delay_{TOT}$ component, both before and after fatigue; and (2) to assess the effects of fatigue on $Delay_{TOT}$ and its components, and the time course of recovery. To this purpose, participants underwent, on two different days, electrically evoked tetanic stimulations before and 1, 3 and 7 minutes after a fatiguing protocol.

Methods

Participants

After receiving a full explanation of the aim of the study and of the experimental procedures, 16 physically active males (age: 25.0 ± 3.9 years, body mass: 77.5 ± 13.8 kg; stature: 1.79 ± 0.08 m; mean ± standard deviation, SD) gave their written informed consent to volunteer in the study. Participants were all clinically healthy with no history of previous lower limb injuries. They were asked to abstain from caffeine or similar beverages in the 24 h preceding tests and to report to the laboratory without any form of physical exercise of heavy intensity of the lower limbs in the previous 48 h. The study was approved by the local University Ethical Committee and had been performed in accordance with the principles of the 1975 Declaration of Helsinki.

Experimental design

After a first visit for familiarization purposes, participants reported to the laboratory on two different days (D_1 and D_2), with at least 48 h in between. In both occasions, they were tested at about the same time of the day, to minimize possible differences induced by circadian effects, and with the same protocol to assess inter-day reliability. A schematic representation of the testing procedures is given in Fig. 1.

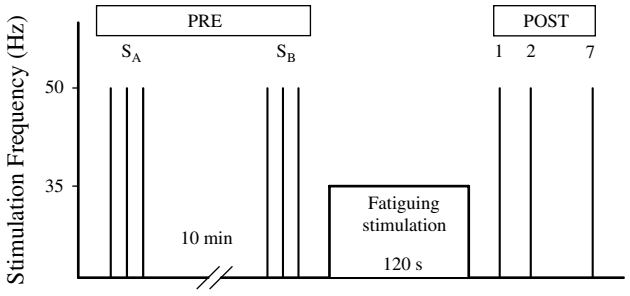

Fig. 1 Experimental design. During D_1, two sessions (S_A and S_B) of three tetanic stimulations (50 Hz for 3 s, with 5 min of rest in between) were applied on the motor point of the *gastrocnemius medialis* muscle. Between S_A and S_B, 10 min of rest was allowed. Thereafter, a fatiguing stimulation protocol (35 Hz for 120 s) was administered. To monitor the time course of the investigated parameters during recovery, the same tetanic stimulations (50 Hz for 3 s) before fatigue were delivered at minute 1, 2 and 7 from the end of the fatiguing protocol ($POST_1$, $POST_2$ and $POST_7$, respectively). Identical procedures were followed during D_2. Pooled data from S_A and S_B during D_1 and D_2 of both operators were considered as the PRE condition

Each test consisted of two sessions (S_A and S_B) of three tetanic stimulations of the *gastrocnemius medialis* muscle (GM), for the assessment of intra-day (inter-session) reliability. S_A and S_B were divided by 10 min of rest and were considered as the PRE condition. Thereafter, a fatiguing stimulation of 120 s began, after which tetanic stimulations at minute 1, 2 and 7 ($POST_1$, $POST_2$ and $POST_7$, respectively) were administered to assess the effects of fatigue and to monitor the time course of the investigated parameters during recovery.

Experimental procedures and measurements

All experiments were performed in a laboratory at constant temperature (22 ± 1 °C) and relative humidity (50 ± 5 %). During tests, all participants sat on a custom-built ergometer with a resonant frequency >200 Hz. As shown in Fig. 2 (panel A), the knee of the dominant limb was fully extended and, to minimize the elongation of the *triceps surae* muscle at the distal level and the pre-tensioning of the fibres [which could per se alter EMD duration (Muraoka et al. 2004)], the ankle joint was fixed at 20° in plantarflexion, the reference position (0°) being perpendicularity of the tibia relative to the sole. The foot was attached by Velcro® straps (Velcro Industries, Willemstad, Netherlands Antilles) to a metal plate provided with a heel support and connected to a load cell.

Neuromuscular transcutaneous electrical stimulations under isometric condition were delivered to GM in monopolar technique by an electrical stimulator (St-Pro Multichannel Programmable Neuromuscular Stimulator, LiSin, Turin, Italy). Electrical stimulation was used to activate only a target muscle (Muraoka et al. 2004), to by-pass

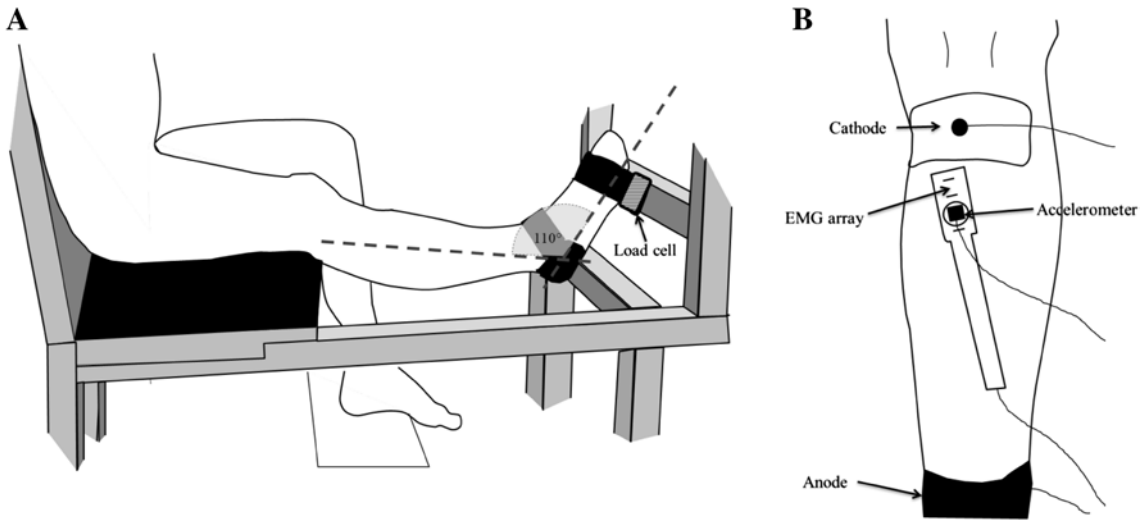

Fig. 2 Schematic representation of the experimental setup. *Panel A* Positioning of the participant on the ergometer. *Panel B* Positioning of the stimulation and EMG electrodes and of the accelerometer for MMG detection on the *gastrocnemius medialis* muscle. For stimulation, the cathode was positioned on the motor point, whereas the anode was positioned posteriorly at the third distal of the leg. The ankle joint was fixed at 20° in plantarflexion, with a total angle of 110° between the foot and the leg

central nervous system inhibitory mechanisms and to focus mainly on the peripheral mechanisms of fatigue-induced muscle alterations (Bigland-Ritchie et al. 1982; Fitts 2008). The cathode (90 × 40 mm) was placed over the most proximal motor point of GM (Gobbo et al. 2011), whereas the anode (130 × 100 mm) was positioned posteriorly at the third distal of the leg. A set of brief 2-Hz stimulations of increasing amplitude was administered to determine the maximum compound motor unit stimulus (M-wave). Once detected the stimulus that elicited the maximal peak-to-peak M-wave, participants rested for 5 min. Hence, a session of three tetanic stimulations (S_A), consisting of a train of pulses (wave shape: biphasic; pulse duration: 304 μs; stimulation frequency: 50 Hz; current amplitude: 110 % of the maximum M-wave; duration: 3 s), was delivered with 5 min of rest in between. After S_A, participants rested for 10 min while sitting on the ergometer. Then, the same set of stimulations was repeated (S_B). Thereafter, the fatiguing stimulation protocol (120 s at 35 Hz) previously utilized by Esposito et al. (2009) was administered, after which GM was stimulated tetanically at minute 1, 2 and 7 with the same train of pulses as before fatigue.

Signal acquisition

The stimulation current, the surface EMG and MMG and F signals were recorded during contraction. MMG probe and electrode positioning on the investigated muscle are shown in Fig. 2 (panel B). The stimulation current and the surface EMG and MMG signals from the GM were acquired by a multichannel amplifier (mod. EMG-USB, OtBioelettronica, Turin, Italy; input impedance: >90 MΩ; CMRR: >96 dB; EMG and MMG bandwidth: 10–750 and 0.7–100 Hz, respectively; gain: *1, *1,000 and *2 for stimulation current, EMG and MMG, respectively) with a sampling rate of 10,240 Hz. F signal was recorded by a calibrated load cell (mod. SM-1000 N, Interface, UK) operating linearly between 0 and 1,000 N, amplified (gain: *200) by a 16 bits A/D converter (mod. UM150, Biopac System, CA, USA) and driven to the auxiliary input of the EMG amplifier. EMG signal was detected in single differential modality by a linear array of four electrodes (mod. ELSCH004, OtBioelettronica, Turin, Italy; linear array 45 × 20 mm; electrode 2 × 1 mm; inter-electrode distance 10 mm) fixed to the skin by dual-adhesive foam (mod. AD004, OTBioelettronica, Turin, Italy) filled with conductive gel (Cogel, Comedical, Trento, Italy). The EMG array was oriented with the major axis parallel to the muscle fibres direction and with the EMG electrodes positioned perpendicularly to the major axes of muscle fibres, in accordance with the European recommendations about surface EMG (Hermens et al. 1999). The skin area under the electrodes was cleaned carefully with ethyl alcohol and gently abraded with a special abrasive and conductive cream (Nuprep, Weaver and Co., Aurora, USA) to achieve an inter-electrode impedance below 2,000 Ω. The third electrode of the EMG array was removed and replaced by a mono-directional accelerometer (mod. ADXL103, Analog Devices, Norwood, MA, USA; device weight: <1.0 g; sensitivity: 1,000 mV/g; measure range: ± 1.7 g) placed directly on the skin over the muscle belly for MMG detection. Consequently, the inter-electrode distance from which surface EMG was detected (between

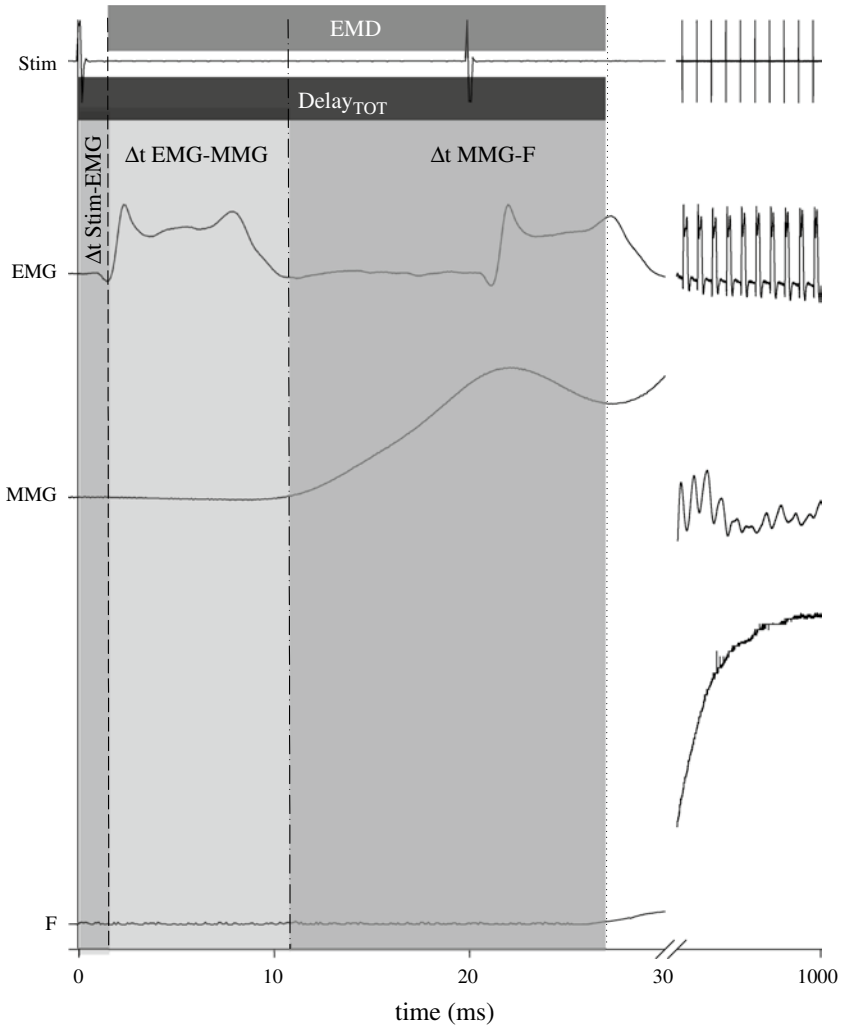

Fig. 3 Stimulation current (*Stim*), EMG, MMG and F signals in a representative participant. Continuous, *dashed*, *dashed-and-dotted*, and *dotted lines* indicate the onset of the stimulation current (*Stim*), EMG, MMG, and force (*F*) signals, respectively. *First grey area* represents the synaptic component, *light grey area* shows the E–C coupling component, *dark grey area* indicates the mechanical component, *top dark grey area* represents the traditionally computed EMD and the *very dark grey area* indicates the Delay$_{TOT}$

the second and the fourth EMG electrodes displaced across the accelerometer) became 20 mm. To minimize site-to-site variability in EMG and MMG signals during repeated measurements on different days, a map with the EMG–MMG probe and the stimulation electrodes position, together with some skin *repere* points (moles, angiomas and scars), was drawn on a transparency. EMG electrodes were also positioned over the belly of the *tibialis anterior* muscle to exclude the presence of simultaneous contractions of the GM antagonist muscle.

To evaluate any possible temperature effect on the investigated variables, skin temperature was measured in proximity of the EMG and MMG probes by an infrared thermometer with a laser beam pointer (mod. 826-T2, Testo, Lenzkierch, Germany) throughout the entire testing sessions.

Data analysis

Data analysis was performed off-line by a custom-built routine using a commercially available software (Labview 7.1, National Instruments, Austin, TX, USA). In Fig. 3, the four raw signals from a representative participant are given.

We considered the first positive peak of the recorded signal to determine Stim onset. The EMG onset corresponded to the first negative peak of the stimulation artefact (Yavuz et al. 2010). These determinations were based on automatic procedures. According to previous studies (Cé et al. 2013; Esposito et al. 2011), MMG and F signals onsets required to exceed, for three consecutive points, the three SD of the mean value obtained in a 100-ms interval of the resting condition immediately preceding the contraction. The three-SD method is a good compromise between operator visual recognition and objective thresholds assessments, even though the risk of an overestimation cannot be ruled out. Signals were visually inspected and the interval was moved when necessary, e.g. when this time frame contained spurious oscillations that would contaminate mean and SD values and, in turn, MMG and F signals onset. This procedure could potentially induce an operator-dependent variation in delays measurement. Therefore, delays calculation

was conducted by two independent and expert operators (Op. 1 and Op. 2) to assess inter-operator reliability.

The overall time delay between the onset of the stimulation current and the onset of F development, $Delay_{TOT}$, and its partitioning in the time delay between Stim and EMG (Δt Stim-EMG, which includes the processes between the action potential at the axonal level and the action potential along the sarcolemma, thus containing also the synaptic latency), between EMG and MMG (Δt EMG-MMG, which includes the E–C coupling processes, cross-bridge formation and the time for pressure waves transmission to the skin), and between MMG and F (Δt MMG-F, the mechanical component from pressure wave propagation to the skin to force transmission at the bone insertion point) were then calculated. Therefore, by adding Δt EMG-MMG to Δt MMG-F, the time delay corresponding to the classical EMD, as defined by Cavanagh and Komi (1979), can be obtained. Consequently, the addition of Δt Stim-EMG to EMD (Δt EMG-MMG + Δt MMG-F) leads to $Delay_{TOT}$.

After identifying the peak F (pF) as the highest level of F achieved, only the transient phases corresponding to muscle contraction (on-phase) between 5 and 95 % of the pF were considered for calculations (Esposito et al. 2009). Subsequently, the rate of F development (RFD, i.e. the slope of F raise during the on-phase), was determined together with MMG peak-to-peak (MMG p-p).

Force and MMG variables were computed off-line by a custom-built routine of a commercially available software (Labview 7.1, National Instruments, Austin, TX, USA).

Statistical analysis

Raw data were analysed using a statistical software package (IBM SPSS Statistics v. 19, Armonk, NY, USA). To check the normal distribution of the sampling, a Kolgomorov–Smirnov test was applied. A sample size of 16 participants was selected to ensure a statistical power higher than 0.80. A one-way analysis of variance (ANOVA) for repeated measures and an intraclass-coefficient correlation (ICC) analysis were performed to determine the repeatability of the three tetanic stimulations within each session (S_A and S_B of both D_1 and D_2) before fatigue. A three-way ANOVA for repeated measures was applied on each variable before fatigue to assess the effects of session, day and operator, and the interaction among the three factors. The effect of fatigue and the time course during recovery were determined by a one-way ANOVA for repeated measures. The location of possible differences was assessed by a Holm–Sidak post hoc test. The magnitude of the changes was assessed using effect size (ES) statistics with the lower and upper 95 % confidence interval (CI). ES was classified as trivial for ES values <0.2, small between 0.2 and 0.6, moderate between 0.6 and 1.2, large between 1.2 and 2.0, and very large when >2.0. Intra- and inter-operator reliability among sessions and days was assessed using a two-way, mixed ICC model and the standard error of measurements (SEM). ICC values were consider as very high if >0.90, high if between 0.70 and 0.89 and moderate if between 0.50 and 0.69. The sensitivity of the different delays was checked by calculating the minimum detectable change at 95 % confidence as a percentage (MDC_{95} %). The level of significance was set at $\alpha < 0.05$. Unless otherwise stated, the results are expressed as mean ± standard error (SE).

Results

PRE and POST ($POST_1$, $POST_2$ and $POST_7$) values were assessed according to the following results and considerations.

Before fatigue, the repeatability analysis among the values of the parameters was calculated from the three tetanic stimulations within each session for the two operators. ICC values ranged from high to very high for all the variables (0.874–0.996) and SEM was comprised between 0.78 and 6.61 %. Moreover, ANOVA did not disclose significant differences among the stimulations. Thus, the three values obtained for each session were averaged (S_A and S_B for the first and the second session within the same day, respectively) and used for further statistical analysis. Moreover, no significant differences were detected by the three-way ANOVA (session, day and operator) among results of each variable. All the data were then pooled together and used as PRE value for further comparisons.

After fatigue, two-way ANOVA (day and operator) disclosed no significant differences in each variable. Thus, for further comparisons, all the data were pooled together and considered as $POST_1$, $POST_2$ and $POST_7$ for the values after 1, 2 and 7 min from the end of the fatiguing stimulation, respectively.

Reliability

MDC_{95} % values were 11.0, 3.7, 21.5 and 11.1 % for $Delay_{TOT}$, Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F, respectively.

In Table 1, the mean values ($n = 16$) of $Delay_{TOT}$ and its components calculated by both operators (Op. 1 and Op. 2) for each session (S_A and S_B) in both days (D_1 and D_2) before fatigue are given. Inter-operator analysis for all the considered variables showed ICC values between 0.901 and 0.999, indicating a very high reliability. Intra-operator analysis revealed a very high intra-day reliability between sessions (S_A and S_B, from 0.919 to 0.989), while reliability was from high to very high for between-day analysis (Inter-day reliability, D_1 and D_2, from 0.781 to 0.981). SEM values ranged from 1.79 to 8.71 % of the relative mean value.

Table 1 Mean ± SE, ICC and SEM values ($n = 16$) of latencies calculated during S_A and S_B in the 2 days (D_1 and D_2)

	Op. 1					Op. 2					S_A Op. 1 vs. Op. 2			S_B Op. 1 vs. Op. 2		
	S_A	S_B	*P*	ICC	SEM	S_A	S_B	*P*	ICC	SEM	*P*	ICC	SEM	*P*	ICC	SEM
$Delay_{TOT}$ (ms)																
D_1	***26.25 ± 0.97***	*27.45 ± 0.94*	*0.351*	*0.954*	*0.968*	**26.65 ± 1.14**	27.67 ± 0.95	0.173	0.964	0.909	**0.996**	**0.921**	**1.031**	0.900	0.989	0.480
D_2	27.30 ± 1.06	28.26 ± 1.12	0.188	0.975	0.833	27.76 ± 1.03	28.75 ± 0.99	0.134	0.970	0.711	0.363	0.937	1.151	0.854	0.933	1.322
P	0.215	0.226				0.278	0.174									
ICC	0.858	0.795				0.877	0.864									
SEM	1.622	2.624				1.583	1.617									
Δt Stim-EMG (ms)																
D_1	*1.449 ± 0.030*	*1.444 ± 0.030*	*0.734*	*0.987*	*0.015*	1.449 ± 0.030	1.444 ± 0.030	0.734	0.987	0.015						
D_2	1.435 ± 0.027	1.439 ± 0.026	0.735	0.981	0.016	1.435 ± 0.027	1.439 ± 0.026	0.735	0.981	0.016						
P	0.617	0.749				0.617	0.749									
ICC	0.926	0.981				0.926	0.981									
SEM	0.033	0.017				0.033	0.017									
Δt EMG-MMG (ms)																
D_1	***8.58 ± 0.54***	*8.98 ± 0.46*	*0.348*	*0.984*	*0.265*	**8.75 ± 0.74**	9.31 ± 0.55	0.280	0.989	0.278	**0.672**	**0.981**	**0.303**	0.273	0.980	0.361
D_2	9.28 ± 0.55	9.44 ± 0.54	0.648	0.930	0.580	9.39 ± 0.60	10.10 ± 0.65	0.110	0.972	0.410	0.585	0.989	0.242	0.169	0.901	0.735
P	0.525	0.902				0.629	0.359									
ICC	0.933	0.908				0.960	0.936									
SEM	0.511	0.702				0.505	0.651									
Δt MMG-F (ms)																
D_1	***16.21 ± 0.79***	*17.02 ± 0.78*	*0.489*	*0.919*	*1.084*	**16.45 ± 0.75**	16.76 ± 0.78	0.168	0.929	0.918	**0.883**	**0.951**	**0.725**	0.448	0.987	0.438
D_2	16.59 ± 1.01	17.37 ± 0.82	0.156	0.967	0.919	16.93 ± 0.78	17.09 ± 0.64	0.223	0.946	0.677	0.787	0.931	1.042	0.964	0.973	0.593
P	0.223	0.159				0.190	0.279									
ICC	0.849	0.788				0.848	0.781									
SEM	1.543	2.256				1.275	2.042									

In Op. 1 and Op. 2 columns, data refer to the intra-operator reliability between sessions (rows) and days (columns). In S_A and S_B columns, data refer to the inter-operator reliability of the same session for both days. Inter-operator reliability was not calculated for Δt Stim-EMG (automatically determined). Examples for inter-operator (between Op. 1 and Op. 2 during S_A in D_1), inter-session (between S_A and S_B in D_1 determined by Op. 1), and inter-day reliability (between D_1 and D_2 determined in S_A by Op. 1) are in bold, italic and underlined, respectively, for each latency

Reliability analysis after fatigue is provided in Table 2. Inter-operator reliability results were between 0.847 and 0.999, indicating a high to very high reliability for all variables. Intra-operator analysis showed values between 0.863 and 0.991 for all the variables during recovery ($POST_1$, $POST_2$ and $POST_7$), evidencing a high to very high reliability of the measurement. SEM values after fatigue ranged from 1.77 to 11.29 % of the relative mean value.

Effects of fatigue

The effects of fatigue on F and MMG signals are presented in Fig. 4. After fatigue, ANOVA disclosed a pF reduction from 687 ± 51 to 639 ± 51 N ($P < 0.05$, ES = 0.9, CI 0.19–1.65). pF remained depressed during the entire recovery period ($POST_2$: 671 ± 60 N and $POST_7$: 655 ± 60 N; $P < 0.05$, ES = 0.3, CI 0.42–0.98 and ES = 0.6, CI 0.15–1.27 for $POST_2$ and $POST_7$, respectively). Fatigue reduced significantly also RFD from 860 ± 111 to 555 ± 57 N/s ($P < 0.05$, ES = 3.4, CI 2.29–4.45), which returned to baseline within 2 min from the end of the fatiguing stimulation. Lastly, MMG p-p decreased significantly after the fatiguing stimulation from 19.6 ± 1.9 to 15.9 ± 1.6 m/s^2 ($P < 0.05$, ES = 2.0, CI 1.20–2.91), then remaining significantly lower for the entire recovery period (17.4 ± 1.7 m/s^2 at minute 7 of recovery; ES = 1.2, CI 0.44–1.94, $P < 0.05$).

In Fig. 5, the effects of fatigue on $Delay_{TOT}$ and its components are shown.

After the fatiguing stimulation, ANOVA revealed a significant increase in $Delay_{TOT}$ by 4.7 ± 0.7 ms ($P < 0.05$, ES = 1.5 and CI 0.73–2.31). All $Delay_{TOT}$ components were significantly affected by fatigue, with an increase in Δt Stim-EMG, in Δt EMG-MMG and in Δt MMG-F by 0.106 ± 0.028, 1.30 ± 0.31 and 3.35 ± 0.54 ms, respectively ($P < 0.05$, ES = 0.8 and CI 0.06–1.50, ES = 0.6 and CI 0.06–1.36, ES = 1.4 and CI 0.60–2.13 for Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F, respectively). When partitioning each contribution to the overall fatigue-induced increase in $Delay_{TOT}$, Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F contributed by about 2.2, 27.3 and 70.5 %, respectively. During recovery, Δt Stim-EMG and Δt EMG-MMG recovered within 2 min from the fatiguing stimulation, while $Delay_{TOT}$ returned to baseline within 7 min. On the contrary, Δt MMG-F was still elongated after 7 min of recovery.

Discussion

The novel finding of the present study was that intra- and inter-operator reliability of the measurement of each single $Delay_{TOT}$ component was from high to very high, When assessing reliability after the effects of fatigue, reliability was still from high to very high. Collectively, these data indicate that when delays are assessed under a controlled and standardized condition, very reliable and sensible data can be obtained. Fatigue lengthened $Delay_{TOT}$ by about 18 %, which recovered within 7 min. $Delay_{TOT}$ components were all altered by fatigue, with a larger effect on the mechanical aspect. Moreover, Δt Stim-EMG and Δt EMG-MMG recovered within 2 min, while mechanical events took longer to restore.

Reliability

The electromechanical latency reliability has been already evaluated only for EMD (Almosnino et al. 2009; Cé et al. 2013; Hopkins et al. 2007; Howatson et al. 2009; Lacourpaille et al. 2013b; Sasaki et al. 2011), while reliability assessment for $Delay_{TOT}$ and its components is still missing in the literature. Moreover, only few studies tested EMD reliability over different days (Almosnino et al. 2009; Cé et al. 2013; Lacourpaille et al. 2013b). Therefore, the present study is the first to assess the consistency of the measurement of each $Delay_{TOT}$ component when data refer to different sessions, different days and also different operators. As previously mentioned (see "Data analysis"), indeed, an operator-dependent variation in MMG and F signal onset detection could exist. Intra-operator reliability analysis compared the data of the different sessions and the different days within each operator, and showed values from high to very high for all $Delay_{TOT}$ components, both between different sessions within the same day (inter-session reliability, S_A vs. S_B) and between different days (inter-day reliability, D_1 vs. D_2). Δt Stim-EMG and Δt EMG-MMG showed the highest ICC values for inter-session and inter-day reliability. Inter-operator reliability assessment (Op. 1 vs. Op. 2) showed a high consistency of the measurement for the delays calculated by the operators, both between sessions and days. Therefore, this approach can provide repeatable measurements, even when performed by different operators on different days.

Lastly, the significant changes of the different components above the MDC_{95} found after fatigue indicate that this approach can afford adequate levels of sensitivity under this experimental condition.

Electrochemical and mechanical contribution to $Delay_{TOT}$

Δt Stim-EMG in the present study was about 1.44 ms, a period of time compatible with the duration of the mechanisms included (see "Introduction"). Indeed, the neurotransmitter release by the motor nerve impulse starts after 210 μs (Hubbard and Schmidt 1963) and lasts 200 μs (Martyn et al. 2009). The time to depolarize the end-plate region takes less than 150 μs (Katz and Miledi 1965) and

Table 2 Mean ± SE, ICC and SEM ($n = 16$) of latencies measured before (PRE) and during recovery ($POST_1$, $POST_2$ and $POST_7$) by both operators in the 2 days

	Op. 1					Op. 2					D_1 Op. 1 vs. Op. 2			D_2 Op. 1 vs. Op. 2			Op. 1 vs. Op. 2 D_1 and D_2		
	D_1	D_2	*P*	ICC	SEM	D_1	D_2	*P*	ICC	SEM	*P*	ICC	SEM	*P*	ICC	SEM	*P*	ICC	SEM
$Delay_{TOT}$ (ms)																			
PRE	26.85 ± 0.89	27.78 ± 1.04	0.126	0.856	1.808	27.16 ± 0.99	28.25 ± 0.98	0.169	0.889	1.394	0.615	0.968	0.778	0.915	0.939	1.137	0.159	0.853	1.601
$POST_1$	31.74 ± 0.69	31.15 ± 0.77	0.429	0.989	0.321	33.54 ± 0.73	32.37 ± 0.99	0.772	0.895	1.001	0.210	0.944	0.701	0.148	0.868	1.160	0.586	0.958	0.540
$POST_2$	29.67 ± 1.31	29.07 ± 0.92	0.142	0.879	1.312	30.31 ± 1.03	30.21 ± 1.09	0.805	0.952	0.826	0.170	0.961	0.750	0.190	0.905	1.154	0.209	0.983	0.432
$POST_7$	27.56 ± 1.55	28.40 ± 0.98	0.207	0.871	1.540	27.90 ± 1.26	28.69 ± 0.94	0.642	0.889	1.493	0.150	0.939	1.074	0.165	0.948	1.007	0.310	0.982	0.546
Δt Stim-EMG (ms)																			
PRE	1.446 ± 0.029	1.437 ± 0.026	0.659	0.959	0.024	1.446 ± 0.029	1.437 ± 0.026	0.659	0.959	0.024									
$POST_1$	1.555 ± 0.040	1.541 ± 0.039	0.333	0.991	0.016	1.555 ± 0.040	1.541 ± 0.039	0.333	0.991	0.016									
$POST_2$	1.486 ± 0.037	1.479 ± 0.031	0.722	0.978	0.022	1.486 ± 0.037	1.479 ± 0.031	0.722	0.978	0.022									
$POST_7$	1.493 ± 0.030	1.472 ± 0.026	0.263	0.943	0.028	1.493 ± 0.030	1.472 ± 0.026	0.263	0.943	0.028									
Δt EMG-MMG (ms)																			
PRE	8.78 ± 0.49	9.36 ± 0.50	0.683	0.943	0.462	9.03 ± 0.63	9.72 ± 0.59	0.390	0.966	0.449	0.501	0.961	0.433	0.204	0.963	0.421	0.523	0.954	0.450
$POST_1$	9.80 ± 0.62	10.37 ± 0.52	0.228	0.977	0.315	11.19 ± 0.59	10.70 ± 0.59	0.547	0.863	0.883	0.116	0.977	0.347	0.220	0.884	0.743	0.782	0.975	0.308
$POST_2$	8.12 ± 0.91	9.44 ± 0.54	0.254	0.913	0.587	9.58 ± 0.56	9.74 ± 0.64	0.690	0.937	0.624	0.125	0.960	0.425	0.238	0.883	0.803	0.568	0.986	0.265
$POST_7$	8.11 ± 0.92	9.49 ± 0.61	0.116	0.951	0.443	8.41 ± 0.92	9.66 ± 0.61	0.422	0.954	0.533	0.135	0.894	0.672	0.231	0.915	0.706	0.225	0.993	0.169
Δt MMG-F (ms)																			
PRE	16.62 ± 0.71	16.98 ± 0.87	0.136	0.850	1.617	16.53 ± 0.69	17.01 ± 0.67	0.159	0.858	1.087	0.806	0.983	0.439	0.630	0.958	0.756	0.139	0.842	1.349
$POST_1$	20.38 ± 0.56	19.24 ± 0.73	0.517	0.977	0.513	20.80 ± 0.78	20.12 ± 0.71	0.517	0.962	0.445	0.117	0.847	0.924	0.104	0.955	0.557	0.608	0.978	0.360
$POST_2$	20.06 ± 0.90	18.16 ± 0.86	0.883	0.877	0.948	19.24 ± 1.04	18.99 ± 0.79	0.833	0.939	0.705	0.131	0.938	0.738	0.105	0.934	0.814	0.388	0.980	0.399
$POST_7$	17.95 ± 1.15	17.53 ± 0.91	0.552	0.880	1.136	18.00 ± 1.07	17.65 ± 0.67	0.670	0.873	1.203	0.383	0.933	0.918	0.833	0.956	0.822	0.563	0.975	0.616

PRE values were obtained by pooling S_A and S_B data. The intra-operator reliability between D_1 and D_2 is listed in Op. 1 and Op. 2 columns. The inter-operator reliability of each single day is listed in D_1 and D_2 columns. The inter-operator reliability after combining D_1 and D_2 is listed in Op. 1 vs. Op. 2 columns. Inter-operator reliability has not been calculated for Δt Stim-EMG as this latency has been determined by automatic procedures for both operators

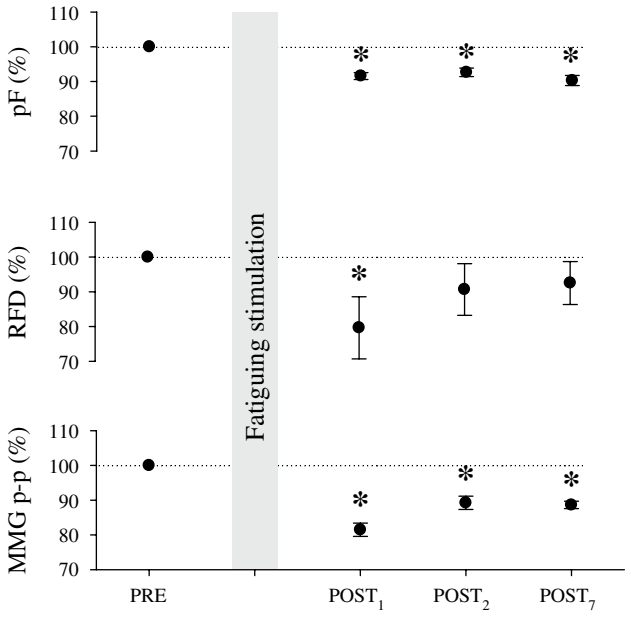

Fig. 4 Main force and MMG parameters. Peak force (*pF*), rate of force development (*RFD*) and MMG peak-to-peak (*MMG p-p*) values before (PRE) and 1, 2 and 7 min after the fatiguing protocol ($POST_1$, $POST_2$ and $POST_7$, respectively), normalized to the value before fatigue. PRE values were pooled from each session (S_A and S_B) of both days (D_1 and D_2). POST values were pooled from results of both days (D_1 and D_2). The *vertical grey bar* represents the fatiguing protocol. Data are expressed as mean ± SE. $^*P < 0.05$

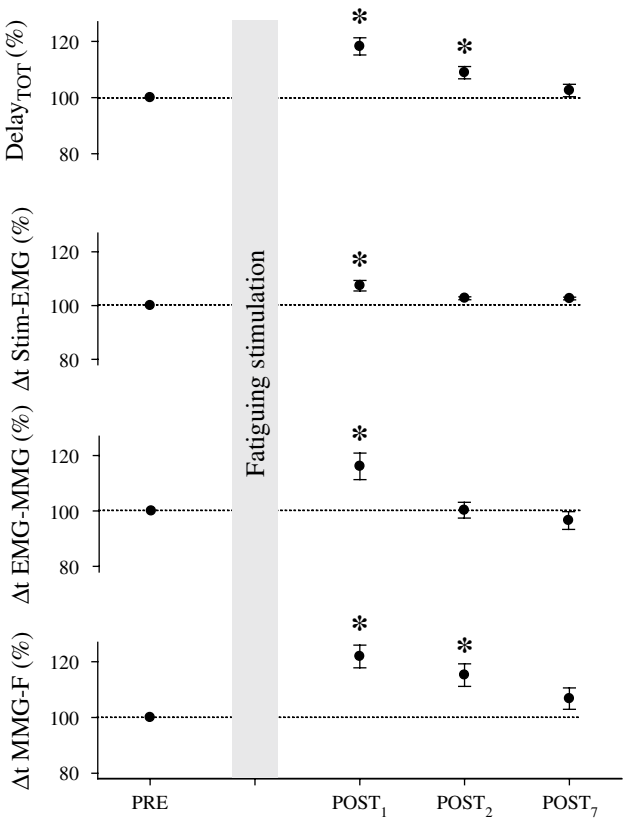

Fig. 5 $Delay_{TOT}$ and its subcomponents. $Delay_{TOT}$, Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F values before (PRE) and 1, 2 and 7 min after the fatiguing protocol ($POST_1$, $POST_2$ and $POST_7$, respectively), normalized to the value before fatigue. PRE values were pooled from the data measured by the two operators in each session (S_A and S_B) of both days (D_1 and D_2). POST values were pooled from results of both operators in the 2 days (D_1 and D_2). The *vertical grey bar* represents the fatiguing protocol. Data are expressed as mean ± SE. $^*P < 0.05$

the delay between the excitatory post-synaptic potential and the firing of the postsynaptic cell have been shown to range between 210 and 650 µs, respectively (Sabatini and Regehr 1999).

Although slightly longer than in previous reports (Esposito et al. 2011; Hufschmidt 1985; Nordez et al. 2009; Petitjean et al. 1998; Sasaki et al. 2011), also Δt EMG-MMG (about 9 ms) lasted for a period of time that can reasonably include all the processes involved in this latency. Indeed, after sarcolemmal depolarization, dihydropyridine and ryanodine receptors interaction occurs within 2 ms (Fill and Copello 2002), Ca^{2+} release by sarcoplasmic reticulum has a time to peak between 1.6 and 4.0 ms (Baylor and Hollingworth 2007), and the time for troponin activation of about 2.7 ms (Solzin et al. 2007). Lastly, the time for the pressure wave generated by myosin heads rotation to propagate toward the skin should also be included, even though its duration has not been measured yet.

The slight discrepancy between Δt EMG-MMG duration in the present study and that previously reported (Esposito et al. 2011; Hufschmidt 1985; Petitjean et al. 1998; Sasaki et al. 2011) could be possibly explained by differences in the reference point adopted for EMG onset detection, in the criteria adopted for signal onset detection, in the transducers utilized for monitoring muscle mechanical activity, in the transducer position with respect to EMG electrodes location, in fibre typing of the investigated muscles, in the intensities and durations of stimulation and in the joint angles investigated (Hug et al. 2011b; Lacourpaille et al. 2013a; Muraoka et al. 2004; Nilsson et al. 1977; Viitasalo and Komi 1981; Yavuz et al. 2010).

Lastly, the duration of Δt MMG-F (about 17 ms) is within the range of previous studies reporting values between 8 and 42 ms (Cé et al. 2013; Esposito et al. 2011; Nordez et al. 2009; Petitjean et al. 1998; Sasaki et al. 2011). Differences in muscle fibre type composition, muscle temperature, subject positioning on the ergometer, joint angle, site of stimulation, frequency and amplitude of the current delivered to the muscle, and criteria for detecting signals onset could explain also in this case the difference.

Overall, $Delay_{TOT}$ was about 27.5 ms. When partitioning $Delay_{TOT}$ in its three sub-components, the relative contribution of Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F

was about 5, 34 and 61 %, respectively. When extracting EMD (Δt EMG-MMG + Δt MMG-F) from $Delay_{TOT}$, this latency was about 26 ms, which is within the range of the previously reported values between 11.6 and 45 ms (Esposito et al. 2011; Hufschmidt 1985; Nordez et al. 2009; Petitjean et al. 1998; Sasaki et al. 2011). The relative contribution of Δt EMG-MMG and Δt MMG-F to EMD was about 35 and 65 %, respectively, indicating that the relative weight of the mechanical component was predominant. This observation is qualitatively in line with previous findings, in which the electrochemical contribution to EMD ranged from 17 to 40 % (Hufschmidt 1985; Petitjean et al. 1998; Sasaki et al. 2011).

Noticeably, Nordez et al. (2009) found by a high frame rate ultrasound technique that the delay between the electrical stimulation and the onset of fascicle motion and between the onset of fascicle motion and the onset of F production contributed to EMD by 52 and 48 %, respectively. The first delay had an absolute duration relatively similar to that found in the present study (about 6.0 and 9.2 ms for Nordez et al. and the present study, respectively). The slightly shorter duration in Nordez et al. can be reasonably ascribed to the ultrasound technique, which permits to detect the first mechanical event, fascicle motion, before pressure wave transmission to the skin, where the accelerometer can detect it. On the contrary, the absolute time required for the processes included in the second delay differs markedly between these two studies (about 5.5 and 16.8 ms for Nordez et al. and the present study, respectively). This discrepancy may be attributed to the different criteria utilized for force onset detection (first point with a negative derivative in the reverse direction of time and 3 SD above baseline, for Nordez et al. and the present study, respectively) and the ankle joint angle utilized during isometric contraction. Indeed, the 10° angle in plantarflexion adopted by Nordez et al. compared to the 20° in the present study likely induced a higher level of passive pre-tensioning of the muscle–tendon unit, thus shortening force transmission to the tendon insertion point. Moreover, inter-subject variability of the mechanical properties of the passive elements involved in tension transmission along the muscle and the tendon should not be disregarded (Nordez et al. 2009).

Acute effects of fatigue on delays

In the present study, $Delay_{TOT}$ and its three subcomponents all lengthened after fatigue.

The elongation of Δt Stim-EMG suggests a fatigue-induced effect on several mechanisms, among which a propagation failure at axonal level (Sieck and Prakash 1995), an alteration of synapsis functionality (Sieck and Prakash 1995; Wieser 1915), a desensitization of the post-synaptic end plate (Sieck and Prakash 1995) and an impairment of the propagation of the action potential at the sarcolemmal level (Juel 1988). Given some controversial findings, whether this lengthening occurs also during voluntary contraction is still a matter of debate (Kirkendall 1990) and requires further investigation.

Similarly, also Δt EMG-MMG elongated after fatigue. Such an increase depends on the well-known alterations induced by fatigue on the several factors included in this latency, such as a decrease in sarcolemmal propagation velocity (Edwards et al. 2012), an impairment in Ca^{2+} release by sarcoplasmic reticulum (Ament and Verkerke 2009; Fitts 2008), an alteration of the ryanodine receptor functionality (Debold 2012), and of the actin–myosin cross-bridges cycle rate (Ament and Verkerke 2009; Fitts 2008).

Lastly, a significant and large Δt MMG-F elongation occurred after fatigue, suggesting modifications of the MTU mechanical properties, such as a decrease in stiffness (Jaskolski et al. 2007; Taylor et al. 1997; Zhang and Rymer 2001). A reduced MTU stiffness, indeed, causes an elongation of the time necessary to tension the SEC and, consequently, to transfer F to the insertion point (Yavuz et al. 2010).

Overall, the distribution of the effects of fatigue appeared to affect mainly the mechanical factors. Indeed, $Delay_{TOT}$ elongation after the fatiguing stimulation was distributed among the three sub-components by 2, 27 and 71 % for Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F, respectively.

Taking into account that before fatigue Δt Stim-EMG represents only 5 % of $Delay_{TOT}$, its variations may have paltry effects on the overall delay.

The present study is the first that stepped forward to evaluate the time course of recovery of $Delay_{TOT}$ and its sub-components, showing that during recovery, $Delay_{TOT}$ returned to baseline within 7 min from the end of the fatiguing stimulation. This was the case also for Δt MMG-F, but not for Δt Stim-EMG and Δt EMG-MMG, which recovered earlier (within 2 min), indicating that the mechanical events occurring after myosin head rotation took longer to restore than those involved in synaptic transmission, action potential propagation, and E–C coupling processes. Only one previous study evaluated the time course of recovery of the electromechanical latency (EMD, not $Delay_{TOT}$) changes after a fatiguing protocol and found that the fatigue-induced elongation recovered within 5 min (Zhou 1996). However, the mechanisms responsible for this behaviour could not be identified, as EMD partitioning was not applied.

Lastly, a final consideration should be taken into account. EMG onset assessment may influence markedly the exact duration of the first two components of $Delay_{TOT}$

(Δt Stim-EMG and Δt EMG-MMG). In the present study, we decided not to remove the stimulation artefact to avoid partial EMG signal cancellation due to artefact suppression (O'Keeffe et al. 2001). Consequently, the beginning of M-wave was fused with the stimulation artefact. Therefore, the choice of the stimulation artefact as the beginning of muscle electrical activation, which was adopted also in many other investigations (Lacourpaille et al. 2013a, b; Muraoka et al. 2004; Nordez et al. 2009), may slightly anticipate the EMG onset and, therefore, introduce the risk of a bias in Δt Stim-EMG (underestimation) and Δt EMG-MMG (overestimation) measurement.

Conclusion

The combined EMG, MMG and F approach allowed the partitioning of the time interval between neuromuscular activation and force production ($Delay_{TOT}$). The three components, (Δt Stim-EMG, Δt EMG-MMG and Δt MMG-F) contributed to the total delay by 5, 34 and 61 %, respectively. After the administration of a fatiguing protocol, our approach was sufficiently sensitive to detect fatigue-induced elongations of each sub-component. In spite of methodological issues raised on previous investigations, the present study indicates that the measurement of $Delay_{TOT}$ and its components presents a very high intra- and inter-operator reliability within the same session and between different days, both before and after fatigue. Collectively, these findings indicate that, when the measurement is performed under controlled and standardized conditions, sensitive and reliable data on the investigated latencies can be obtained. Consequently, this combined approach could be a valid means to detect changes induced by physiological and/or pathological processes, such as the effects of training, fatigue, muscle temperature as well as myotonic phenomena, even when the evolution of the changes has to be monitored in different laboratories over a long period of time.

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Conflict of interest Authors declare no conflict of interest.

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