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Influence of stimulus intensity on electromechanical delay and its mechanisms

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ABSTRACT

Electromechanical delay (EMD) is the time lag between muscle activation and force development. Using very high frame rate ultrasound, both electrochemical and mechanical processes involved in EMD can be assessed. Percutaneous electrical stimulations at submaximal intensity are often used to stimulate a specific target muscle. The aim of this study was to determine whether stimulus intensity alters the delay between stimulation and the onset of muscle fascicles motion (Dm), the onset of myotendinous junction motion (Dt), and force production (EMD). Ten participants underwent two electrically evoked contractions, with the probe maintained either the biceps brachii muscle belly or the distal myotendinous junction of the biceps brachii, for six stimulus intensities (30%, 50%, 70%, 90%, 110% and 130% of the lowest intensity inducing the maximal involuntary force production, I_{max}). In addition, inter-day reliability was tested in nine participants at both 70% and 90% of I_{max}. Dm, Dt and EMD were significantly longer ($p < 0.001$) at very low (30% and 50% of I_{max}) compared to higher intensities (70%, 90%, 110% and 130% of I_{max}). Inter-day reliability of EMD, Dm, and Dt was good (coefficient of variation ranged from 6.8% to 12.5%, i.e. SEM lower than 0.79 ms). These results indicate that the stimulus intensity needs to be standardized to perform longitudinal evaluation and/or to make between-subject comparisons.

1. Introduction

Electromechanical delay (EMD) is the time lag between muscle activation and force development (Cavanagh and Komi, 1979) and is influenced by both electrochemical processes (e.g., synaptic transmission, excitation–contraction coupling) and mechanical processes (force transmission along the active and passive fraction of the series elastic component, SEC) (Cavanagh and Komi, 1979; Sasaki et al., 2011). Using very high frame rate ultrasound (4 kHz), Nordez et al. (2009) recently determined the relative contribution of these processes to EMD during electrically evoked contractions. More precisely, by measuring the onset of motion for the muscle fascicles and myotendinous junctions of the gastrocnemius medialis they concluded that 47.5% of the total EMD was due to propagation of force along the passive part of the series elastic component ($\approx 20.3\%$ for aponeurosis and $\approx 27.6\%$ for tendon) (Nordez et al., 2009). Since EMD is modified in case of pathology [e.g., neuropathy (Granata et al., 2000), myopathy (Orizio et al., 1997)] or by training regime (Linford et al., 2006; Grosset et al., 2009), this innovative non-invasive methodology has been proposed to be useful for evaluating the effects of neuromuscular disorders or training/rehabilitation protocols (Hug et al., 2011a).

Because quantification of EMD during voluntary contraction presents some drawbacks associated with the difficulty in precisely detecting the beginning of muscle activation (Hug et al., 2011b), EMD is often quantified during involuntary muscle contractions such as tendon reflex (Häkkinen and Komi, 1983; Zhou et al., 1995; Moore et al., 2002), electrical nerve stimulation (Muro and Nagata, 1985; Grosset et al., 2009; Hopkins et al., 2007; Yavuz et al., 2010), or percutaneous muscle electrical stimulation (Zhou et al., 1995; Muraoka, 2004; Nordez et al., 2009; Hug et al., 2011a; Sasaki et al., 2011). Among them, percutaneous stimulation is preferable because it allows the clinician/researcher to study the EMD of a specific target muscle (Muraoka, 2004; Nordez et al., 2009; Sasaki et al., 2011). However, it is unclear if the stimulus intensity alters EMD. This information is of great interest because performing experiments at submaximal intensities would both limit the discomfort associated with the electrical stimulation and limit activation of adjacent muscles.

Focusing on these potential outcomes, the purpose of the present experiment was to determine whether stimulus intensity alters electromechanical delay in biceps brachii. Using very high frame rate ultrasound, we measured the delay between muscle stimulation and (i) the onset of muscle fascicles motion (Dm), (ii) the onset of myotendinous junction motion (Dt), and (iii) force production (i.e., EMD). It allowed us to isolate the putative effect of intensity on the main structures/mechanisms of EMD. As percutaneous electrical stimulation activates muscles with random and non-selective muscle recruitment in terms of both fiber type (Gregory

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and Bickel, 2005) and spatial organization (Adams et al., 1993), we hypothesised that electrochemical processes are not affected by the stimulation intensity. On the other hand, it seems unclear whether muscle force transmission velocity is influenced by stimulation intensity.

2. Materials and methods

2.1. Participants

Ten active males volunteered to participate in the present study (age: 22.9 ± 2.2 years, height: 181 ± 7.7 cm, body mass: 75.8 ± 8.4 kg). They were informed of the possible risk and discomfort associated with the experimental procedures prior to giving their written consent to participate. This study was conducted according to the Declaration of Helsinki (last modified 2004) and has been approved by the local ethics committee.

2.2. Instrumentation

2.2.1. Ergometer

A schematic representation of the experimental set-up is depicted in Fig. 1. Participants sat on an isokinetic dynamometer (Biodex System 3 Research, Biodex Medical, Shirley, USA) with shoulder abducted at 90° and forearm placed in a 90° flexed position with the wrist in a neutral position. Because of the lack of sensitivity of the isokinetic ergometer to precisely detect the onset of elbow flexion force, a force transducer (SML-50, Interface, Arizona, USA) was incorporated in the ergometer and connected with Velcro straps to the wrist to ensure constant contact (Fig. 1). Isometric elbow flexion force was digitized at a sampling rate of 5 kHz (MP36, BIOPAC, Goleta, California).

2.2.2. Electrical stimulation

Elbow flexion was initiated by means of percutaneous electrical stimulation over the biceps brachii. A constant current stimulator

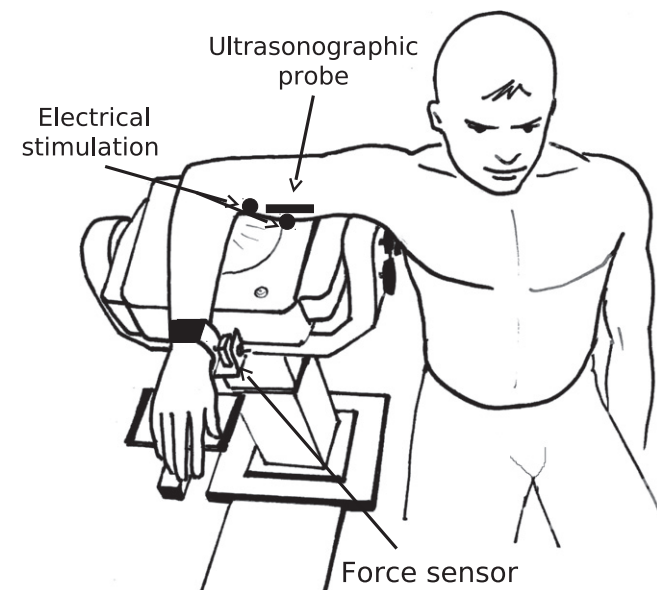


Fig. 1. Schematic representation of the experimental setup. Positioning of the subject with shoulder abducted 90° and forearm placed in a 90° flexed position. The wrist was directly in contact with a force sensor and velcro straps ensured constant contact. Elbow flexion was initiated by percutaneous electrical stimulation over the biceps brachii using two electrodes placed on the motor point and proximal portion of biceps brachii. Each subject underwent two bouts composed of two electrically evoked contractions with the echographic probe maintained over either the biceps brachii muscle belly or the distal myotendinous junction of the biceps brachii muscle.

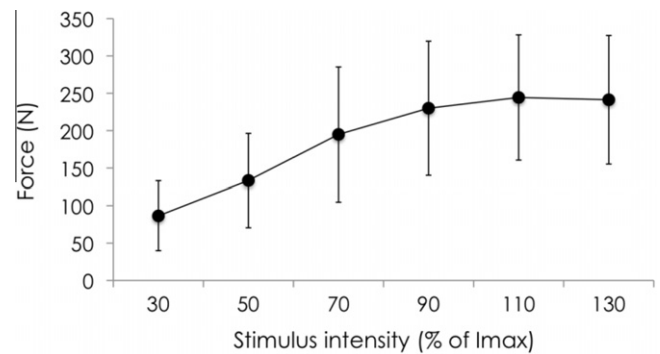


Fig. 2. Dependence of peak twitch force on the stimulus intensity. Values are means $\pm$ SD. Relationship between force (Newtons, N) and stimulus intensity (% I_{max}).

(Digitimer DS7A, Digitimer, Letchworth Garden City, UK) delivered a single electrical pulse (pulse duration = $500 \mu\text{s}$, 400 V) through two electrodes (2×1.5 cm, Compex, Annecy-le-vieux, France) placed on the main motor point and proximal portion of biceps brachii (Hug et al., 2011a). The motor point was considered as the location inducing the strongest twitch with the lowest electrical stimulation. To determine the minimal stimulation intensity required to induce the maximal elbow flexion force (I_{max}), the output current was incrementally increased (incremental step of 5 mA) until a maximum force output was reached (Fig. 2). The mean I_{max} was 98.5 ± 11.3 mA.

2.2.3. Ultrasonography

A very high frame rate ultrasound scanner (Aixplorer, version 4.2, Supersonic Imagine, Aix en Provence, France) coupled with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, Tours, France) was used in « research » mode to acquire raw radio frequency (RF) signals at 4 kHz.

2.2.4. Synchronisation

At the start of each ultrasound acquisition, the scanner sent a transistor–transistor logic (i.e., TTL) pulse to a train/delay generator (Digitimer Ltd, DG2A, Welwyn Garden City, England) which generated a TTL pulse to the electrical stimulator with a 48.00-ms delay to have a sufficient baseline to detect the onset of tissue motion. To check the absence of desynchronization throughout the experiments, TTL pulses from both the ultrasound scanner and the train/delay generator were recorded using the same device as for the force measurements (MP36, Biopac, Goleta, California).

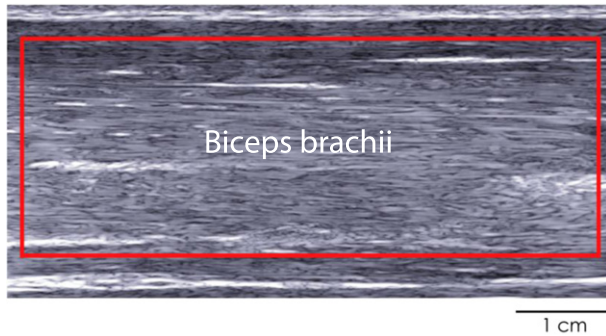
2.3. Protocol

After the previously described recruitment ramp, six electrically evoked contractions were performed at six intensities (30%, 50%, 70%, 90%, 110%, and 130% of I_{max}). They were applied in a randomized order with 1-min rest between each and two trials were performed for each stimulation intensity (designated as muscle trials and tendon trials). During the muscle and tendon trials, the echographic probe was maintained parallel to the muscle fascicles and on the previously localized distal myotendinous junction of the biceps brachii, respectively. Participants were instructed to be fully relaxed prior to each stimulation.

2.4. Data processing

The data processing was performed using standardized Matlab scripts (The Mathworks, Natick, USA). First, ultrasonic raw data (i.e., RF signals) were used to create echographic images by apply-

A. Muscle trial



B. Tendon trial

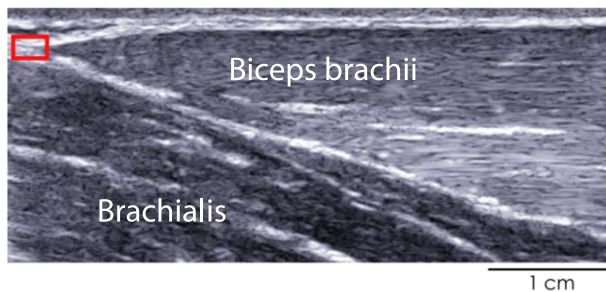


Fig. 3. Typical ultrasound images of the muscle belly (A) and the distal myotendinous junction (B). The region of interest used to calculate particle velocity is indicated by the red rectangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ing a conventional beam formation, i.e., applying a time-delay operation to compensate for the travel time differences. These ultrasound images were used to determine the region of interest (ROI; cf. Fig. 3) for each contraction (i.e., between the two aponeurosis of the biceps brachii muscle for muscle trials and on the biceps brachii myotendinous junction for tendon trials). Using one-dimensional cross correlation of windows of consecutive RF signals, the displacements along the ultrasound beam axis (i.e., y-axis in Fig. 3) were calculated (Catheline et al., 1999; Deffieux et al., 2006, 2008). Thus, the tissue motion between the two consecutive images (i.e., particle velocity) was measured with a micrometric precision.

Displacements were then averaged over the previously determined ROI, and these averaged signals were used to detect the onset of motion. As visual detection has been shown to be highly reliable (Hodges and Bui, 1996), the onset of motion for both muscle and myotendinous junction was defined visually by an experienced examiner. The same method was used to detect the onset of force production. We defined the EMD as the time lag between the onset of the electrical stimulation (i.e., artefact of stimulation) and the onset of force production. Delays between the onset of electrical stimulation and the onset of muscle fascicles motion (Dm, for muscle trials) and between the onset of electrical stimulation and the onset of myotendinous junction motion (Dt, for tendon trials) were calculated. The mechanical processes involved in EMD were calculated as the delay between the onset of muscle fascicles motion and the onset of force production (Tm) and delay between the onset of myotendinous junction motion and the onset of force production (Tt).

2.5. Statistical analysis

Due to a technical problem during the experimentation leading to the loss of some data, one subject was not included in the anal-

ysis and statistics were thus performed on nine subjects. Normality testing (Kolmogorov–Smirnov) was consistently passed and so values are reported as mean $\pm$ SD. A two-way analysis of variance with repeated measures [factors = four locations (Dm and EMD for muscle trials, Dt and EMD for tendon trials) and six stimulus intensities (30%, 50%, 70%, 90%, 110%, and 130% of I_{max})] was used to test whether the stimulation intensity altered Dm, Dt and EMD. Another two-way analysis of variance with repeated measures [factors = two locations (Dm and Dt) and six stimulus intensities (30%, 50%, 70%, 90%, 110%, and 130% of I_{max})] was used to test the effect of stimulus intensity on the relative values of Dm and Dt (i.e., expressed in % of EMD). Finally, the effect of stimulus intensity on mechanical processes involved in EMD (i.e., Tm and Tt) was tested by a two-way analysis of variance [factors = two mechanical processes (Tm and Tt) and six stimulus intensities]. Post hoc analyses were performed when appropriate using Scheffe's method. The statistical significance was set at $p < 0.05$.

2.6. Inter-day reliability

An additional experiment was performed to test the inter-day reliability (reproducibility) of EMD, Dm and Dt at both 70% and 90% of I_{max}. Briefly, the methodology described above was used in nine participants. Seven of them were tested 3 months after the first session. Two new participants were tested on two separate days. Both the coefficient of variation (CV) and the standard error of measurements (SEM) were calculated between the two sessions to assess the reliability (Hopkins, 2000).

3. Results

Fig. 4 depicts the results obtained for both muscle and tendon trials. ANOVA revealed a significant main effect ($p < 0.001$) of location. More precisely, Dm was significantly shorter than EMD for muscle trials (4.1 ± 0.3 ms vs. 8.6 ± 0.5 ms; $p < 0.001$) and Dt was significantly shorter than EMD for tendon trials (4.0 ± 0.5 ms vs. 8.6 ± 0.6 ms; $p < 0.001$). No significant difference was found either between Dm and Dt ($p = 1$) nor between EMD measured during muscle trials and tendon trials ($p = 0.98$). In addition, a main effect of stimulus intensity was found ($p < 0.001$). Post hoc analysis showed differences between extreme values. More precisely, 30% of I_{max} induced significant longer delays compared to 70% ($p = 0.043$), 90% ($p = 0.009$), 110% ($p < 0.001$), and 130% of I_{max} ($p < 0.001$). A significant difference was also found between 50% and 110% of I_{max} ($p = 0.023$). However, no significant interaction location $\times$ stimulus intensity was found ($p = 0.50$) indicating that Dm, Dt, and EMD were similarly altered by the stimulation intensity.

No significant difference between Dm and Dt was found when expressed as a percentage of total EMD ($p = 0.910$) (Dm: $47.3 \pm 1.7\%$ and Dt: $47.1 \pm 2.4\%$). Similarly, no significant effect of intensity was found for relative values of Dm and Dt ($p = 0.058$). The interaction location $\times$ stimulus intensity was also no significant effect ($p = 0.772$).

Although the ANOVA did not revealed significant difference between Tm and Tt ($p = 0.667$) a significant main effect ($p = 0.013$) of stimulus intensity was found. The post hoc analysis revealed no significant difference. Also, no significant interaction was found between mechanical process (Tm and Tt) $\times$ stimulus intensity ($p = 0.954$).

The inter-day reliability was good for both 70% and 90% of I_{max}. For EMD, SEM was 0.66 and 0.75 ms for 70% and 90% of I_{max}, respectively (corresponding to a CV of 6.8% and 8.0%). For Dm, SEM was 0.51 and 0.43 ms for 70% and 90% of I_{max}, respectively (corresponding to a CV of 12.4% and 11.0%). Finally, for Dt, SEM was 0.34 and 0.39 ms for 70% and 90% of I_{max}, respectively (corresponding to a CV of 8.2% and 10.8%).

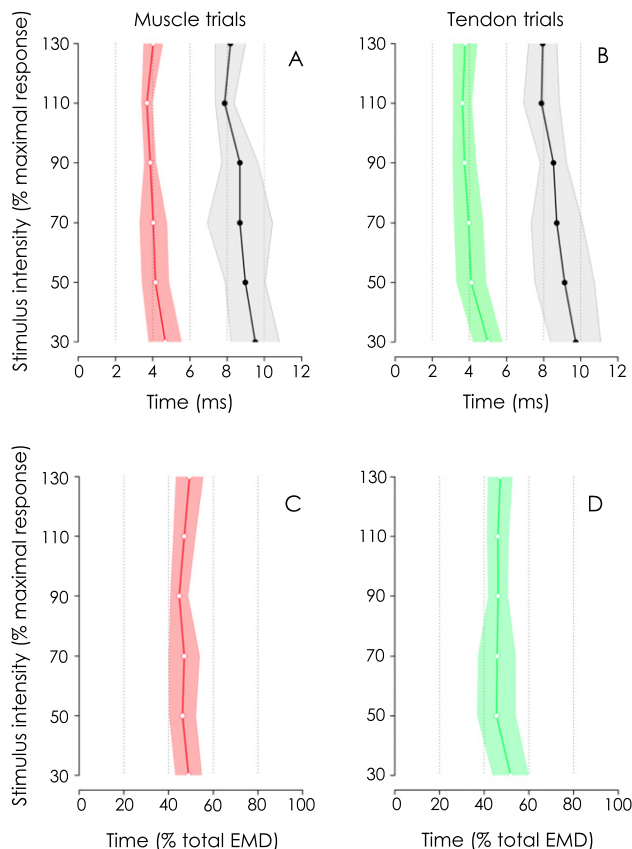


Fig. 4. Influence of stimulus intensity on onset times for muscle and tendon trials. Values are means $\pm$ SD. Stimulus intensity (% of I_{max} , the minimal stimulation intensity required to induce maximal elbow-flexion force) related to the onset times (force in black and tissue motion) for muscle (A in red) and tendon trials (B in green). Relationship between stimulus intensity and the relative part of Dm (%) and Dt (%) on EMD appears respectively in red (C) and green (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The aim of the present study was to determine the effects of the electrical stimulation intensity on the different processes of EMD using very high frame rate ultrasound (Nordez et al., 2009). The results showed that the overall delays were significantly longer at very low (30% and 50% of I_{max}) compared to higher intensities. However, there were no differences among stimulus intensities from 70% to 130% I_{max} .

The I_{max} value obtained in this study has to be interpreted with caution because the potential activation of adjacent muscles (e.g., brachialis and triceps brachii) was not evaluated. Despite the use of muscle belly stimulation with small electrodes, the probability of current spread to these muscles increases with increasing stimulus intensity. For instance, the antagonist activation may limit the joint torque and influence the I_{max} value by an early occurrence of the force plateau. Nevertheless, it should be kept in mind that the aim of this study was to determine the influence of stimulus intensity on EMD and its mechanisms. Regardless of potential adjacent muscle activation we showed a significant effect of stimulus intensity that should be taken into consideration in future works.

EMD values reported herein (≈ 8.7 ms) were relatively close to those reported by Hug et al. (2011a) in the same muscle, i.e., biceps brachii (≈ 10.0 ms). However, a direct comparison between these two studies is difficult. Indeed, while the shoulder was placed in

a neutral position in Hug et al. (2011a), the shoulder was abducted at 90° in the present work (Fig. 1). This would have induced different muscle lengths and thus slightly different delays (Muraoka, 2004). The results of the present study also confirm previous results that Dm and Dt are not different for the biceps brachii (Hug et al., 2011a), while they are for the gastrocnemius medialis (Nordez et al., 2009).

The present study showed that both EMD and the onset of tissues motion (i.e., muscle fascicles and myotendinous junction) were significantly longer at very low stimulation intensities (i.e., 30% and 50% of I_{max}). This is in accordance with the results of Zhou et al. (1995) who reported a decrease in EMD as stimulus current increased (i.e., 22, 18, and 17.2 ms for 90, 120, and 150 V, respectively). However, they provided no information about the relative intensity (in % of I_{max}) which corresponded to these currents. In addition, the onset of motion of the muscle fascicle and the myotendinous junction were not measured. Overall, this dependency of EMD to extreme changes in stimulation intensity does not validate our initial hypothesis. Some works initially suggested that electrical muscle stimulation is associated with a specific recruitment of motor units with larger (fast) motor units over the recruitment of smaller ones (slow) (for review, see Gregory and Bickel, 2005). However, recent evidences points to the recruitment pattern of motor units being random and non selective, i.e., without obvious sequencing related to fiber type (Jubeau et al., 2007; Maffiuletti, 2010). Despite the fact that the motor nerves depolarized by percutaneous muscle stimulation (Hultman et al., 1983) innervate muscle fibers which spread throughout the muscle, electrical stimulation can recruit muscle fibers deep within the muscle as demonstrated with magnetic resonance imaging (Adams et al., 1993). Taken these elements together, we believe that the effect of stimulus intensity reported in the present study is due to other issues rather than differences in recruitment patterns. First, it has been shown that a strong electrical stimulus causes the muscle tension to fall before it begins to rise (Rauh, 1922; Hill, 1949; Goodall, 1958), a phenomenon known as “latency relaxation”. Based on the recording of the onset of a low frequency sound wave, Hufschmidt (1985) showed that “electro-mechanic latency” (that can be associated to Dm measured in the present work) decreased when the stimuli intensity increased due to the lack of latency relaxation with lower stimulation intensities. Because we determined Dm as the beginning of muscle motion in either direction, the latency relaxation may have influenced our results and could partly explain the observed effect of stimulation intensity on the Dm for lower intensities. Second, the increase in number of recruited motor units associated with an increase in stimulus intensity (Adams et al., 1993) is likely to increase force production rather than modify the onset of muscle motion. This greater force production and the higher rate of force development associated with the increase in stimulation intensity could have enhanced the signal-to-noise ratio which was likely to influence the detection of the onset force production.

Our results indicate that the stimulus intensity needs to be standardized to perform longitudinal evaluation and/or to make between-subject comparisons. The good inter-day reliability for EMD, Dm and Dt (i.e., CV ranged between 6.8% and 12.5%) opens interesting perspectives regarding the use of this methodology in the longitudinal assessment of muscle function. Indeed, EMD has been shown to be altered by training program (Grosset et al., 2009), neuromuscular disorders (Orizio et al., 1997), prolonged bed-rest (Kubo et al., 2000) or ligament reconstruction (Kaneko et al., 2002). It is thus promising to monitor the effects of these interventions/pathologies on each process of EMD by making within- and between-subjects comparisons.

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