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Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks

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Abstract
Compared to isometric activities, the neural basis of fatigue induced by repetitive tasks has been scarcely studied. Recently, we showed that during short-lasting repetitive tasks at the maximal possible rate (finger tapping for 10 and 30 s), tapping rate and maximal voluntary contraction (MVC) force decrease at the end of finger tapping. We also observed larger silent periods (SP) induced by transcranial magnetic stimulation during MVC *post* finger tapping. However, if SP were induced by cervicomedullary stimulation (CMS) they remained unchanged. This suggested a supraspinal origin of fatigue for repetitive tasks. Nevertheless, CMS SP only partially explore spinal excitability; therefore, to evaluate a spinal origin of fatigue it is essential to know the features of the CMS-evoked potentials (CMEP). Herein, we evaluated (n = 15) the amplitude of the CMEP during MVC executed immediately (no gap) after a short-lasting finger tapping task; we also evaluated the compound muscle action potential (CMAP) so that the amplitude of the CMEP was expressed as a function of the CMAP amplitude. Indices of fatigue obtained during finger tapping were compared with those obtained during short-lasting maximal isometric tasks. While indices of excitability increased initially in both tasks, they decreased with the isometric task only when the task was prolonged to 30 s. We suggest that the inability to maintain increased levels of spinal excitability during task execution is a neurophysiological mark of fatigue. Our results suggest that the origin of fatigue induced by brief and fast repetitive tasks is not spinal.

Abbreviations
AMT, active motor threshold; ANOVA, analysis of variance; CMAP, compound muscle action potential; CMS, cervicomedullary stimulation; MVC, maximal voluntary contraction; SP, silent periods

Key words
Fatigue; Repetitive movements; Human; Spinal cord

INTRODUCTION

The understanding of fatigue of the human motor system is of paramount importance in the fields of ergonomics, sport and neurology. The neural basis of fatigue has been studied extensively in the case of isometric contractions, either maximal or submaximal (Gandevia, 2001; Duchateau *et al*., 2002; Maluf and Enoka, 2005; Klass *et al*., 2008; Taylor and Gandevia, 2008 ; Williams *et al*., 2014), and there is strong evidence that isometric fatiguing tasks induce a reduction in the excitability of circuitry in both the spinal cord (Taylor *et al*., 1996; Duchateau *et al*., 2002; Butler *et al*., 2003 ; Klass *et al*., 2008) and motor cortex (Gandevia *et al*., 1996; Taylor *et al*., 1996 ; Di Lazzaro *et al*., 2003). The evaluation of evoked potentials in response to transcranial magnetic stimulation (TMS) and electric or magnetic cervicomedullary stimulation (CMS) has permitted the localization of the sites where excitability of the motor system has been modified during fatigue. While TMS is used to evaluate intracortical and corticospinal excitability (Hallett, 2000 ; Hallett, 2007), the potentials induced by CMS (i.e. CMEP) are adequate to explore the excitability of the spinal cord circuits (Ugawa *et al*., 1991; Taylor and Gandevia, 2004 ; McNeil *et al*., 2013).

Fatiguing isometric contractions of maximal effort increase the duration of the silent periods (SP) induced by CMS and reduce muscle force during maximal voluntary contraction (MVC) (Taylor et al., 1996), an effect already present after 10 s of MVC in the small muscles of the hand (Arias et al., 2015). This is an indication of longer lasting decreased spinal motoneuronal excitability, which is partly due to recurrent inhibition and after-hyperpolarization (Inghilleri *et al*., 1993 ; Brasil-Neto *et al*., 1995). Fatigue also induces neurophysiological changes that reveal adaptations of intracortical motor circuits: an increase of TMS-SP, mediated by GABA receptors (Taylor *et al*., 1996 ; Arias *et al*., 2015); a reduction in the descending volleys in the corticospinal tract (Di Lazzaro et al., 2003); and a increase in cortico-cortical inhibition to paired-pulse TMS (McNeil et al., 2009).

However, cortical and spinal adaptations to fatigue are known to be task dependent (Enoka and Stuart, 1992; Barry and Enoka, 2007; Enoka and Duchateau, 2008 ; Enoka *et al*., 2011). While neural mechanisms related to fatigue during isometric tasks have been thoroughly studied, those related to fatigue during repetitive movements have been much less studied. One fundamental point during the study of the neural basis of fatigue is to control (or to avoid) the recovery of the system at the time of testing (Taylor et al., 2000). This is relatively simple in the case of isometric activities where the stimulation of the brain can be performed during the task without stopping the activity. On the other hand, the study of fatigue induced by repetitive movements has been traditionally performed at rest, after the fatiguing activity (Brasil-Neto *et al*., 1993; Arias *et al*., 2012 ; Teo *et al*., 2012), which would not allow for the assessment of fast recovering forms of fatigue. This could be the case with fatigue developed during short-lasting repetitive movements executed at maximal possible rate. Recently, we have developed a protocol that allowed us to evaluate cortical and spinal adaptations to muscle fatigue when performing a repetitive finger tapping task (*ft*) with no time for recovery (Arias et al., 2015). The study involved the evaluation of TMS and CMS SPs during brief (2 s) episodes of MVC, which were executed immediately after maximal rate *ft* (10 or 30 s). When fatigued, subjects were unable to maintain the maximal tapping rate and their force was decreased during MVC, this was accompanied by an increase SP induced by TMS but not by CMS (Arias et al., 2015); those results advocate for a cortical locus of fatigue for fast rate *ft*. On the contrary, the reduction in force after short isometric MVC was accompanied by an increase in both CMS-SP and TMS-SP (Arias et al., 2015).

Therefore, the findings only partially ruled out the development of fatigue at spinal circuitry during maximal rate *ft* because we only evaluated CMS-SP duration but not the CMEP amplitude. A meaningful interpretation of the CMEP amplitude can only be achieved if considered in relation to the amplitude of the compound muscle action potential (CMAP) in response to supramaximal stimulation of the corresponding nerve (at the same time of testing the CMEP). This is fundamental because the CMAP reflects the efficiency of transmission in the periphery (Rich, 2006).

In the present work, we have modified our previous protocol to examine the spinal mechanisms of fatigue during maximal rate *ft* by mean of CMEP amplitude evaluation. We have been careful in exploring spinal and neuromuscular transmission at the time of fatigue, without allowing time for recovery. For comparison, we also explored the responses induced by a fatiguing isometric (*iso*) task. In all cases the tasks were short lasting, 10 or 30 s. We predict that spinal motoneurons will behave differently depending on the task employed.

EXPERIMENTAL PROCEDURES

Experimental protocols conformed to the Helsinki declaration and were approved by our institution Ethics Committee. All subjects were screened for incompatibility with brain stimulation protocols. All were medication free during the week preceding testing and signed a voluntary informed consent.

Subjects

The experiment included 15 healthy subjects (all men, age range 18–40 years). In all subjects the spinal excitability was evaluated with stimulation at the level of the cervicomedullary junction during several 2.5-s MVCs. Electrical stimulation was used in seven subjects, while magnetic stimulation using a double cone coil was used in the remaining eight subjects (who refused to participate if the stimulation was electrical due the produced discomfort). All subjects underwent both *ft* and *iso* fatigue testing sessions, 15 days apart.

Protocol

The two sessions were identical except for the type of *task* executed. In one of the session, participants were asked to perform index *ft*. In the other session they executed continuous index finger *iso* against a force sensor; the force direction was "toward" *flexion* of the first metacarpophalangeal joint. In all cases participants wore a small and light goniometer to monitor movements of the index finger metacarpophalangeal joint; we used also a metal ring attached at the distal phalanx of the index. Participants tapped or pressed over a thin metal plate located on the force sensor.

For both *ft* and *iso* sessions, subjects performed the tasks in three modes: comfort rate-effort (*comfort* mode) for 30 s; 10 s at maximal rate-effort (*10 max* mode); and finally 30 s at maximal rate-effort (*30 max* mode). Each mode was repeated four consecutive times (i.e., sets); rest periods between sets lasted 1 min 40 s. The decrease in frequency or amplitude (for *ft*), or in force output (for *iso*) defined the presence of fatigue.

For the *comfort-ft*, subjects were asked to "*tap at their most comfortable rate without feeling fatigued*" for as long as the set lasted. *Comfort-ft* is reliable (Arias et al., 2012) and paced about 1/3 of *ft* maximal rate. During *comfort-ft* the metabolic activity in the sensorimotor cortex is lower than at faster (>3 Hz) or slower (<1 Hz) rates (Jancke *et al*., 1998 ; Lutz *et al*., 2005), showing its suitability as control condition in our protocol. Participants were asked to press ≈1/3 MVC for *comfort-iso*; and visual feedback was provided. For maximal modes, subjects were encouraged to tap/press as fast/hard as they could from the very beginning to the end of the set.

The participants also executed 2.5-s MVCs before (*pre*) and right after (no gap allowed) task-execution (*post*), either after *ft* or *iso*, for all modes and sets (Fig. 1). The magnitudes of 2.5-s MVCs were analyzed to monitor fatigue (Bigland-Ritchie and Woods, 1984). During the 2.5-s MVCs the CMS was applied (at 1.5 s), and we recorded the SP duration and CMEP amplitudes (Taylor et al., 1996). The peripheral transmission of the potentials during the same 2.5-s-MVCs

was also evaluated (at 2.2 s) with the amplitude of the CMAP (Rich, 2006). We calculated the ratio CMEP/CMAP to evaluate spinal excitability accounting for the state of the periphery; this was always performed with the CMEP and CMAP acquired in the same MVC. Thus the stimulation pulses (CMS, and 700 ms later supramaximal to the ulnar nerve) were applied during the 2.5-s MVC. A practice sessions was scheduled (Gandevia, 2001).

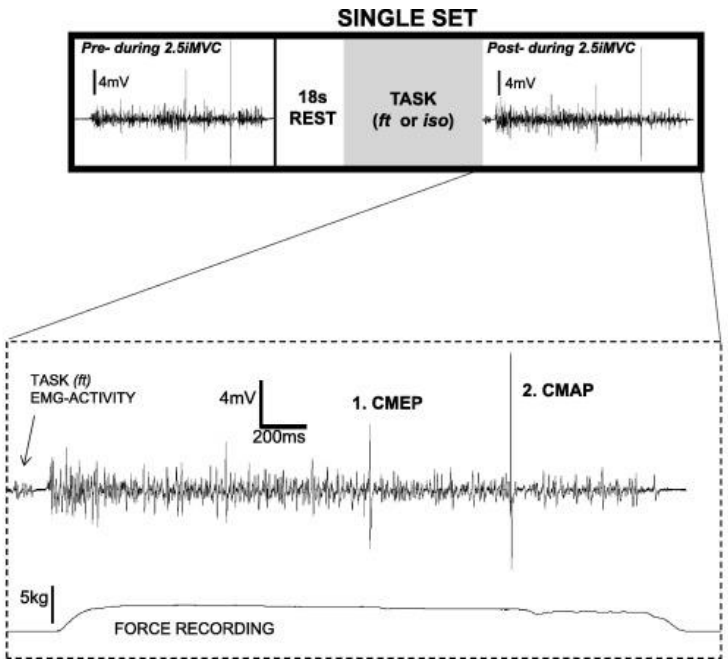

Fig. 1. The set-structure: as soon as an LED was lit, subjects performed an isometric maximum voluntary contraction (MVC) with their index against a dynamometer. The LED off (2.5 s after) served as a signal to stop the MVC. During the MVC subjects received two different types of stimulation to get: 1st CMEP, at 1.5 s after LED on; and 2nd CMAP, at 2.2 s after LED on (*pre*). After this *paired*-stimulation subjects rested for 18 s. Then, an LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In a continuation of the task, and with no resting time, the subjects performed another 2.5-s MVC in response to the LED turning on, and received stimulation (*post*; in the same way as *pre*).

Setting, recording and stimulation protocols

The subjects were sitting comfortably with the elbow flexed at 90–100°. The forearm, wrist, hand and all fingers except the index were firmly but comfortably immobilized, and fixed to a modified tablet-arm chair. The setup allowed unrestrained degrees of freedom at the metacarpophalangeal joint of the index finger, permitting *ft*. During *ft*, a Biometrics DataLink (Biometrics Ltd, Gwent, NP11 7HZ, UK) system recorded the inter-tap intervals at 0.1 kHz with a thin metal plate and a metal ring, the latter adapted to the distal phalanx of the index finger. It also recorded (at 500 Hz) the isometric force exerted during the MVC of the index flexion with a Pinch-Dynamometer (P200), which was placed flat and secured over the tablet, with the thin metal plate used to record tapping attached flat on its top. A single axis finger goniometer (F35) (sampled at 1 kHz) controlled the flexo-extension movement amplitudes of the index finger metacarpophalangeal joint. Electromyographic activity from the superficial head of the first dorsal interosseous muscle (FDI) was monitored with surface electrodes in a belly tendon arrangement, and acquired by means of D360 amplifiers (Digitimer, Welwyn Garden City, Herts), amplified

(250–1000×) and bandwidth filtered between 3 and 3000 Hz. The involvement of the muscle in the tasks has been shown elsewhere (Arias et al., 2015).

A CED 1401 mkII Power A-D converter (Cambridge Electronic Design, Cambridge, UK) sampled EMG at 10 kHz; it also controlled a red LED (indicating the task execution/rest phases) and the timing of stimulation pulses.

Electric and magnetic CMS

Electric CMS was applied using a Digitimer D180 stimulator by means of Ag–AgCl electrodes located behind the mastoid processes (anode at the right, cathode at the left).

Magnetic CMS was applied using a MagStim 200^2 (Whitland, Carmarthenshire, UK) stimulator connected to a double cone coil. The center of the coil was placed over the inion (in some subjects slightly lateral) and the direction of the current flowed down in the coil (Taylor and Gandevia, 2004).

Active motor threshold (AMT) was defined as the minimum intensity required to evoke 5 liminal responses (approximately 200 μV) in 10 consecutive pulses in the activated muscle (5–10% MVC) (Rossini et al., 1994). The stimulation intensity for the protocol was set to obtain a CMEP amplitude of approximately 50% amplitude of the CMAP during MVC in the fresh muscle. To check the absence of current spread to the spinal roots, the CMEP latency was compared to that obtained at AMT intensity, such that the amplitude of the potentials increased in size with voluntary contraction with no or liminal latency shift when stimulated at the higher level (Taylor and Gandevia, 2004).

We set 50% of CMAP as the target for baseline values of the CMEPs to be more likely away from the ceiling, to monitor the potential modulation of the CMEPs induced by fatigue; previous reports have indicated that CMEPs may reach, at least, 70% the CMAP when recorded in arm muscles (Butler et al., 2003).

Supramaximal electric stimulation of the ulnar nerve

The FDI electrodes recorded the CMAP to ulnar nerve stimulation at the elbow (Digitimer DS7A stimulator). The anode was placed lateral to the medial epicondyle along the postcondylar groove and the cathode approximately 2 cm distal to the anode, along the direction of the nerve. A 1000-μs square pulse stimulus was used at an intensity 50% above the supramaximal, and delivered during the 2.5-s-MVC.

Data reduction

The following dependent variables were analyzed:

CMEP and CMAP amplitudes: These were defined as the peak-to-peak amplitude. CMEP was normalized to the CMAP amplitude acquired at the same time point (i.e., during the same 2.5 s MVC). We analyzed the effect of fatigue on the CMEP and CMAP amplitudes.

EMG Root Mean Square amplitude at the time of CMEP testing: Amplitude of the EMG activity in the 50-ms time window prior to CMEP ($EMG\text{-}RMS_{PRIOR\text{-}CMEP}$). This value was normalized in relation to the RMS of the CMAP acquired at the same time point (i.e., during the same 2.5 s MVC).

Motor output during task execution: We considered three measures of motor output for task execution: the tapping frequency and angular amplitude for *ft*, and the force applied for *iso*. For each of the 4 Sets and 3 Modes (*comfort; 10 max* and *30 max)* we considered two time points that were embedded within task execution: the initial 3 s *(i3; pre*) and the final 3 s (*f3*; *post*). To compare data from *ft* frequency and *iso* torque, we expressed the motor output at all evaluation time points as a function of the maximum obtained at any time point for each task and subject.

For normalizing the *ft* range of motion (ROM) amplitude, we recorded the maximum (active) ROM of the index finger metacarpophalangeal joint for each subject before the protocol. The score served as divisor for the amplitude displayed at all the individual's evaluation time points during the *ft* task.

MVC (before and following task execution): We evaluated the MVC force at the time of CMS (MVC in the 50-ms period before the stimulation) either for *ft* and *iso* task, normalized to the maximum MVC obtained at any of the CMS evaluation time points for each task and subject.

SP duration (recorded during the MVC explained just above): The SP duration was defined as the time lag from the CMS pulse to the recovery of the EMG activity during the brief MVC, and determined visually by an experienced researcher blind to the conditions; under these conditions the test–retest reliability (on 100 randomly chosen SP) has been shown to be excellent for this methodology of SP determination in our previous work (Arias et al., 2015). The SP durations were normalized for their analyses. For each subject and task (*ft* or *iso*), we took as 100% the average value from all the evaluation time points of the four sets executed in the *comfort* mode, which served as divisor for all the subject's values for all execution modes. Therefore, in the figures representing the SP duration, the unit represents its average duration at *comfort* mode.

Statistical design

Statistical design to studying the effect of CMS on the amplitude of the CMAP

In our work, we recorded the CMAP 700 ms after the CMEP, during the same 2.5-s MVC. We examined if there was any influence of the preceding CMS on the amplitude of the CMAP as follows. Before each testing day (20 min before the fatigue protocol), subjects executed the 2.5-s MVC (with a 100-s rest interval) six times. In three MVCs both CMEPs and CMAPs were acquired (as in the protocol); in the other three we did not apply CMS and the order was randomized. The average of the three CMAP amplitudes (potentially) conditioned by CMS was compared to the average obtained from the unconditioned CMAPs. An ANOVA was performed with factors Conditioning (two levels, *conditioned* and *unconditioned* CMAP amplitude) and Day (levels *day-1* and *day-2*, as this was evaluated for two days). We also evaluated the intra-class correlation coefficients (ICC) considering the Conditioned vs. Unconditioned responses.

Statistical design for studying behavior during fatigue tasks

Fatigue induced by the *ft* and *iso* tasks was evaluated with repeated measures ANOVA. Previously, we checked the normality of distribution by means of Kolgomorov–Smirnov test for one sample.

For each of the variables (normalized CMEP amplitude; EMG-$RMS_{PRIOR\text{-}CMEP}$; motor output decrement -tapping rate in *ft* and force in *iso*-; SP durations; MVC before and after task execution; and CMAP amplitude) we used an independent ANOVA with repeated measures. The ANOVA included several within-subject factors: The within-subject factors were Task (*ft, iso*), execution Mode (*comfort, 10 max, 30 max*), Set (the four sets for each execution mode), and evaluation Time points (*pre, post*). The levels of the latter factor were termed *i3* and *f3* when the variable analyzed was the motor output decrease in *ft* or *iso* tasks (as it included the initial and final 3 s embedded in task execution).

For the ANOVA of the ROM amplitude, only analyzed for *ft*, factor Task was excluded.

Results are expressed as the mean and the standard error of the mean (*s.e.m*). During ANOVA execution, the degrees of freedom were corrected with Greenhouse Coefficients (ε) if sphericity could not be assumed. Significance was set at $p < 0.05$. A Bonferroni correction was used for follow-up *post hoc* comparisons involving multiple levels within the factor.

Subsequent analyses were carried out to determine if the responses obtained with magnetic and electric stimulators were different; this analysis is justified since magnetic coil geometry might increase the risk of recruiting some cortical neurons. The model was the same as above but included a between-subjects factor (Group, with two levels: electric and magnetic).

RESULTS

The mean output intensity used for magnetic CMS was 89.8% (*s.e.m* 4.3) (in 9 of the 16 sessions it was at 100%); for electric CMS was 551.0 V (*s.e.m* 30.9). Before the fatigue sessions, we observed that the CMS (magnetic and electric) did not condition CMAP amplitudes acquired at the same 2.5-s MVC ($F_{1,14} = 0.7$ $p > 0.05_{\text{CONDITIONING}}$); this happened the two testing days ($F_{1,14} = 0.7$ $p > 0.05_{\text{DAY}}$; and $F_{1,14} = 0.1$ $p > 0.05_{\text{DAY} \times \text{CONDITIONING}}$). The ICC were excellent when comparing the conditioned vs. the unconditioned CMAP amplitudes (0.98 [0.96–0.99, CI95%]) mean of the two days pooling electric and magnetic CMS, as well as in isolation: 0.98 [0.96–0.99, CI95%] for electric and 0.98 [0.95–0.99, CI95%] for magnetic stimulation. Therefore, CMS did not influence the CMAP amplitude acquired at the same MVC.

The Table 1 shows the normalizing values for the fatigue protocol, equivalent to the units depicted in the graphs.

Table 1. Mean score including all subjects. The score corresponds with the *y*-axis unit in graphs.

Task	*Max output in task at any evaluation time-point	*Max active full ROM	*MVC at any stimulation time-point	Mean CMS-SP duration at comfort
ft	7.4 Hz (*s.e.m* 0.3)	33.0° (*s.e.m* 1.9)	4.2 kg (*s.e.m* 0.2)	61.1 ms (*s.e.m* 2.7)
iso	3.7 kg (*s.e.m* 0.1)	n.a.	4.1 kg (*s.e.m* 0.2)	67.0 ms (*s.e.m* 4.0)

n.a: not applicable.
* The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects.

The Table 2 indicates the main effects and significant interactions for all variables, one column for each of the evaluated variables. For each variable (column), the first row corresponds to the ANOVA model with the two tasks (*ft* and *iso*), the three execution modes, the four sets and the two testing time points. In some variables, the interactions of Task with other factors were significant and indicated a different response for *ft* and *iso*; in those cases a follow-up ANOVA was executed by task type (indicated in subsequent rows). We proceeded likewise if the responses differed for execution mode (i.e., ANOVA by pair of modes and, eventually, with just one mode). *Post-hoc* analyses are shown in the figures.

Table 2. Summary of main effects and interactions for the different variables

CMEP amplitude	EMG-RMS$_{PRIOR-CMEP}$	Task motor execution	MVC force at CMS	SP duration	CMAP amplitude	Row
$F_{1,14}$ = 12.4 p < 0.01$_{TASK\ x\ MODE}$ $F_{2,28}$ = 3.9 p < 0.05$_{MODE\ x\ TIME}$	$F_{2,28}$ = 9.6 p = 0.001$_{TASK\ x\ MODE\ x\ TIME}$	$F_{2,28}$ = 90.9 p < 0.001$_{MODE\ x\ TIME}$ $F_{2,28}$ = 8.6 p = 0.001$_{TASK\ x\ MODE\ x\ TIME}$	$F_{1,14}$ = 12.8 p < 0.01$_{TASK\ x\ TIME}$	$F_{1,14}$ = 14.7 p < 0.01$_{TIME\ x\ TASK}$	$F_{2,28}$ = 5.4 p < 0.05$_{TASK\ x\ MODE}$	1st
FINGER TAPPING		***FINGER TAPPING***	***FINGER TAPPING***	***FINGER TAPPING***	***FINGER TAPPING***	
$F_{2,28}$ = 6.8 p < 0.01$_{MODE\ x\ TIME}$	$F_{2,28}$ = 9.7$_{\varepsilon=0.6}$ p < 0.01$_{MODE\ x\ TIME}$	$F_{1,14}$ = 101.1 p < 0.001$_{TIME}$ $F_{2,28}$ = 45.4 p < 0.001$_{MODE\ x\ TIME}$	$F_{1,14}$ = 5.8 p < 0.05$_{TIME}$ $F_{2,28}$ = 10.2 $_{\varepsilon=0.6}$ p < 0.01$_{MODE}$ $F_{3,42}$ = 4.0 p < 0.05$_{SET}$	p > 0.05 for factors and interactions	p > 0.05 for factors and interactions	2nd
Comfort	***Comfort***	***Comfort***	N.A	N.A	N.A	
$F_{1,14}$ = 2.6 p > 0.05$_{TIME}$	$F_{1,14}$ = 5.6 p < 0.05$_{TIME}$	p > 0.05				3rd
10max & 30max	***10max***	***10max***	N.A	N.A	N.A	
$F_{1,14}$ = 5.5 p < 0.05$_{TIME}$ p > 0.05 for interactions	$F_{1,14}$ = 1.1 p > 0.05$_{TIME}$	$F_{1,14}$ = 39.3 p < 0.001$_{TIME}$				4th
N.A	***30max***	***30max***	N.A	N.A	N.A	
	$F_{1,14}$ = 5.6 p < 0.01$_{TIME}$	$F_{1,14}$ = 81.8 p < 0.001$_{TIME}$ $F_{3,42}$ = 3.6 p < 0.05$_{SET}$				5th
ISOMETRIC		***ISOMETRIC***	***ISOMETRIC***	***ISOMETRIC***	***ISOMETRIC***	
$F_{1,14}$ = 4.8 p < 0.05$_{TIME}$ $F_{2,28}$ = 5.4 p < 0.05$_{MODE}$	p > 0.05 for factors and interactions	$F_{2,28}$ = 90.9 p < 0.001$_{MODE\ x\ TIME}$ $F_{6,84}$ = 5.4 $_{\varepsilon=0.5}$ p < 0.01$_{MODE\ x\ SET}$	$F_{2,28}$ = 25.8 p < 0.001$_{TIME\ x\ MODE}$	$F_{1,14}$ = 13.9 p < 0.01$_{TIME}$ $F_{2,28}$ = 3.6 p < 0.05$_{TIME\ x\ MODE}$	$F_{2,28}$ = 3.8 p = 0.051$_{MODE}$ $F_{2,28}$ = 3.8 p < 0.05$_{TIME\ x\ MODE}$	6th
Comfort		***Comfort***	***Comfort***	***Comfort***	***Comfort***	
p > 0.05 for factors and interactions		$F_{1,14}$ = 17.2 p < 0.001$_{TIME}$	$F_{1,14}$ = 15.9 p < 0.01$_{TIME}$ $F_{3,42}$ = 4.6 p < 0.05 $_{SET}$	$F_{1,14}$ = 12.4 p < 0.01$_{TIME}$	p > 0.05 for factors and interactions	7th
10max		***10max***	***10max***	***10max***	***10max***	
$F_{1,14}$ = 6.4 p < 0.05$_{TIME}$		$F_{1,14}$ = 23.8 p < 0.001$_{TIME}$ $F_{3,42}$ = 5.2 $_{\varepsilon=0.6}$ p < 0.05$_{SET}$	$F_{1,14}$ = 17.6 p < 0.001$_{TIME}$ $F_{3,42}$ = 6.0 p < 0.01 $_{SET}$	$F_{1,14}$ = 7.6 p < 0.05$_{TIME}$	p > 0.05 for factors and interactions	8th
30max		***30max***	***30max***	***30max***	***30max***	
p > 0.05 for factors and interactions		$F_{1,14}$ = 119.9 p < 0.001$_{TIME}$ $F_{3,42}$ = 25.3 p < 0.001$_{SET}$	$F_{1,14}$ = 114.0 p < 0.001$_{TIME}$ $F_{3,42}$ = 25.5 p < 0.01 $_{SET}$	$F_{1,14}$ = 10.7 p < 0.01$_{TIME}$	p > 0.05 (p = 0.09)	9th

The first (un-shaded) row of statistical values corresponds to the ANOVA model with the two tasks (ft & iso), the three execution modes, the four sets and the two testing time points. For some variables, the interactions of Task with other factor/s were significant and indicated a different response of those variables for *ft* and *iso*; in these cases follow-up ANOVA were executed by task type (in subsequent rows). We proceeded likewise with subsequent ANOVA if the responses differed for execution modes (i.e., ANOVA were executed by pair of modes and, eventually, with just one mode). Those spaces left empty are shown when subsequent ANOVA were not applicable (N.A), because interactions were not significant.

CMEP amplitude modulation during fatiguing ft and iso tasks

CMEP amplitudes (relative to CMAP) behaved differently in the three modes of execution for *ft* and *iso* tasks (ANOVA $p < 0.05$), and also differently at *pre*/*post* in each of the different modes (ANOVA $p < 0.01$). The responses can be observed in Fig. 2 (representative individual examples during isometric maximal modes) and Fig. 3, means for all subjects in all modes and tasks.

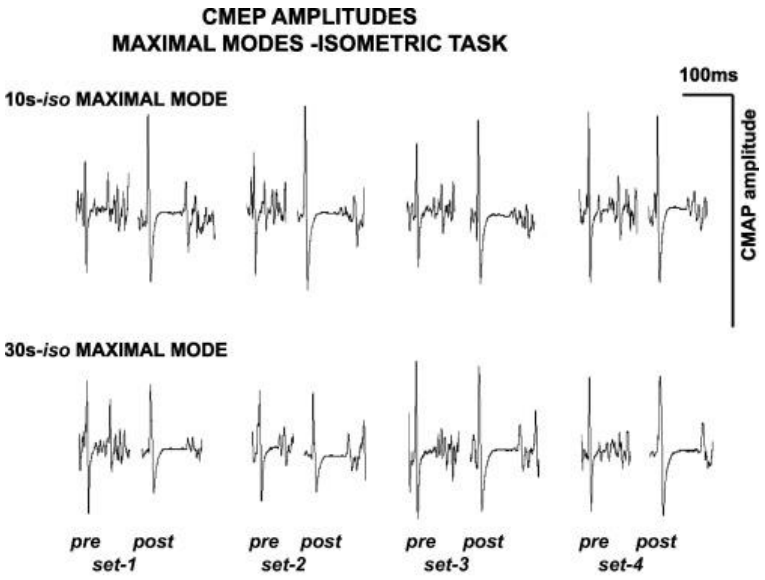

Fig. 2. Examples taken from a representative subject for *iso* maximal modes. The amplitudes are scaled so that the amplitude of the vertical line at the right corresponds to the size of the corresponding CMAP.

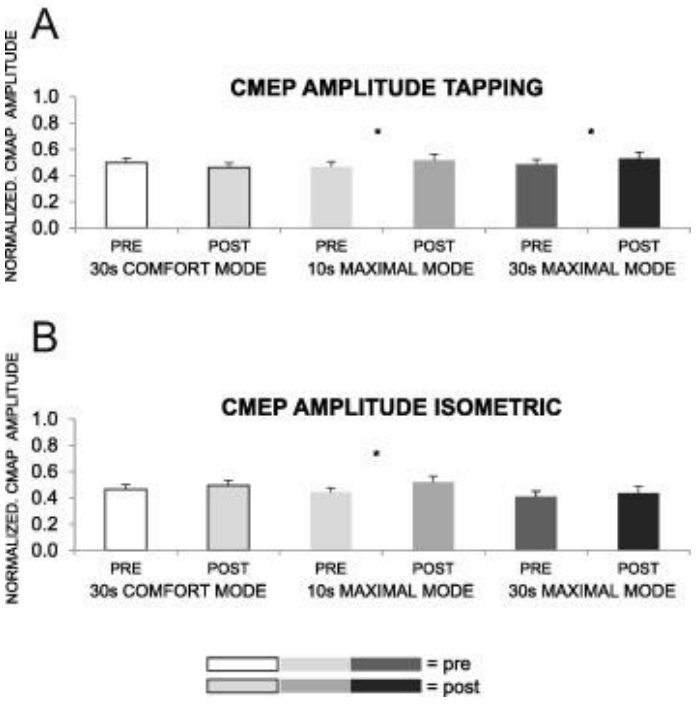

Fig. 3. CMEP amplitude changes in both tasks. CMEP amplitudes are normalized to the amplitude of the corresponding CMAP. (A) For *ft* the amplitudes were significantly increased at the end of both maximal execution modes. (B) For *iso* the amplitudes were significantly increased only at the end of *10 max*, but not for *30 max.* $^{*}p < 0.05$.

CMEP amplitude after fatiguing ft tasks

During *ft*, CMEP at *pre* and *post* also behaved differently for the three execution modes (ANOVA $p < 0.01$); thus we performed follow-up ANOVA by pair of modes and modes in isolation. For *comfort-ft* the CMEP did not change after tapping (ANOVA $p > 0.05$). Conversely, ANOVA including both maximal modes indicated that the amplitude of the potentials increased right at the end of maximal *ft* (ANOVA $p < 0.05$); the effect of TIME did not interact significantly with any other factor (i.e., mode *−10 max* and *30 max*; or set), thus a small (5%) but significant increase in the spinal excitability was shown after maximal *ft*.

CMEP amplitude after fatiguing iso tasks

For the *iso* task, the CMEP amplitudes changed with the three execution modes (ANOVA $p < 0.05$), and also at the end of the tasks (ANOVA $p < 0.05$); a similar pattern was observed if the analysis was performed by pairs of modes. When analyzing the effects mode by mode and in the case of *comfort*, main effects of TIME or SET were not significant, nor their interaction ($p > 0.05$ in all cases). For *10 max* CMEP increased significantly at the end of execution (ANOVA $p < 0.05$), from *pre*-values of 44.2% to *post*-values of 52.1% of the CMAP-amplitude (increment of 17.9%); the effect was observed in all sets. For *30 max*, main effects and interactions were always $p > 0.15$; thus CMEP *post 30 max* were similar to those observed at *pre*.

EMG-RMS$_{PRIOR\text{-}CMEP}$

The EMG-RMS$_{PRIOR\text{-}CMEP}$ differed for the two tasks and modes at *pre* and *post* (Fig. 4, ANOVA $p < 0.001$). This was observed since it remained stable at all times for *iso* ($p = 0.115$ was the smallest *p*-value for main effects and interactions), but not for *ft*. For *ft*, EMG-RMS$_{PRIOR\text{-}CMEP}$ changed from *pre* to *post*, differently for the three tapping modes (ANOVA $p < 0.01$). It was reduced after *comfort* tapping (ANOVA $p < 0.05$) and increased after *30max* (ANOVA $p < 0.01$), with no set effects. Before and after *10max* the EMG-RMS$_{PRIOR\text{-}CMEP}$ remained unchanged.

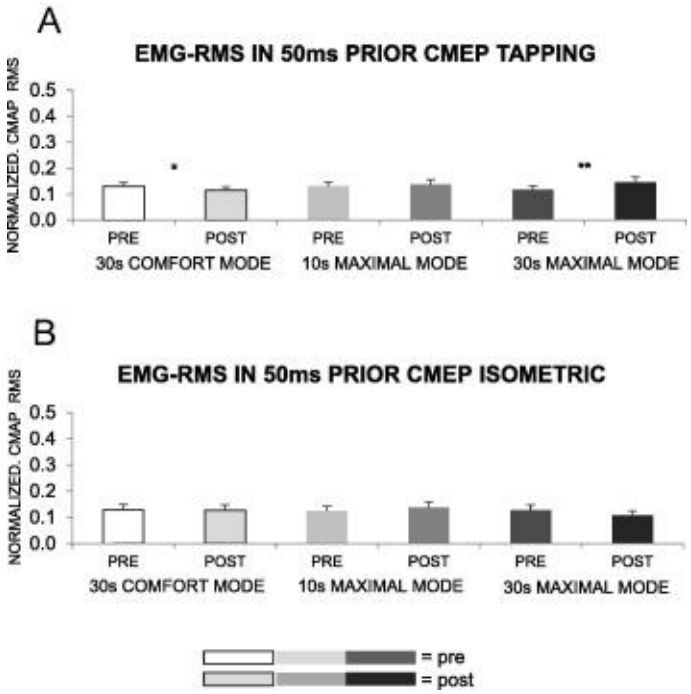

Fig. 4. EMG-RMS during MVCs at the time of CMS. EMG-RMS are normalized to the RMS of the corresponding CMAP. (A) Right after *ft* the EMG-RMS decreased for the *30 comfort*, and increased for the *30 max* modes. (B) For *iso* EMG-RMS remained unchanged after execution for all modes. $^{*}p < 0.05;^{**}p < 0.01$.

Task motor execution. Frequency/force decrement during execution in ft/iso tasks

The observed motor output decrements along the studied tasks (initial vs. final 3 s of each set) differed for *ft* and *iso*, and three execution modes (*comfort*, *10 max* and *30 max*) (ANOVA $p = 0.001$). Subsequently, we performed the analyses for each type of task.

Task motor execution during finger tapping

For *ft*, the frequency of tapping dropped along execution, but the three modes of execution were affected differently (ANOVA $p < 0.001$). *Ft* frequency at *comfort* was unchanged, but it dropped after *10 max* and *30 max* (ANOVA $p < 0.001$, Fig. 5A); in this latter case there was also an effect on set progression (ANOVA $p < 0.05$), which affected similarly the initial and final seconds of execution.

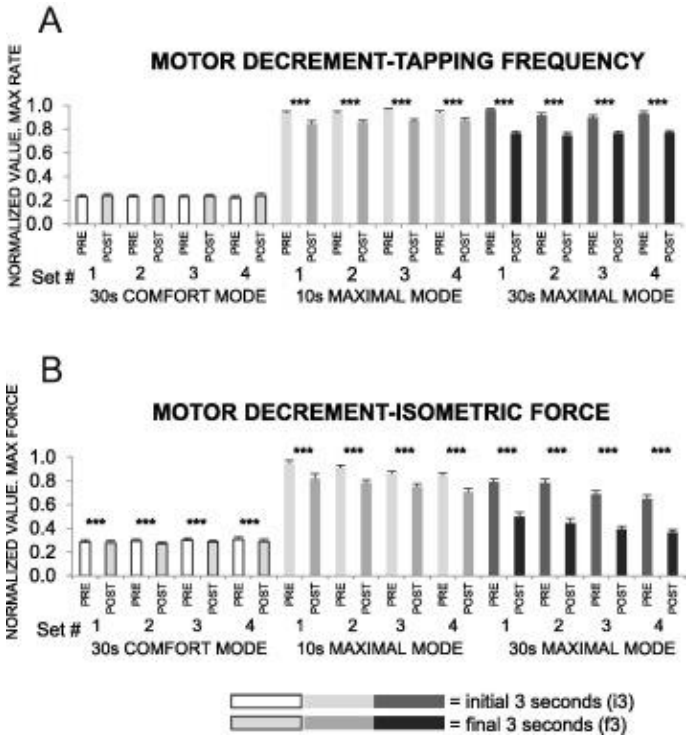

Fig. 5. Motor execution decrement induced by the tasks. (A) The frequency of *ft* decreased significantly after the maximal modes. For *30 max* a set effect was present. (B) Set by set motor decrement along the *max iso* tasks was more evident. The force reduced at *post* after *maximal* modes and also after *comfort*. $^{***}p < 0.001$.

Task motor execution during isometric contraction

The Fig. 5B shows the force drop in the *i3* and *f3 s* periods of the *iso*-task. Force dropped differently at the end of the *iso*-task for the three different modes (ANOVA $p < 0.001$). Additionally, a set effect was present with different expressions in the three execution modes (ANOVA $p < 0.001$). Follow-up ANOVA by modes indicated that for *10 max-iso post* the force dropped significantly (ANOVA $p < 0.001$), and set after set (ANOVA $p < 0.05$). A similar pattern was observed for *30 max-iso*, but more significantly expressed (ANOVA $p < 0.001$ for TIME and SET). Conversely, for *comfort* there was no set effect ($p > 0.05$), though the force at the end of the 30 s of *comfort* was significantly lower that at the beginning (ANOVA $p < 0.001$, Fig. 6), the effect was small. This means that, at the end of execution, subjects remained slightly below (1.5%) *pre*, when trying to maintained the target proposed (i.e., 30% of their MVC).

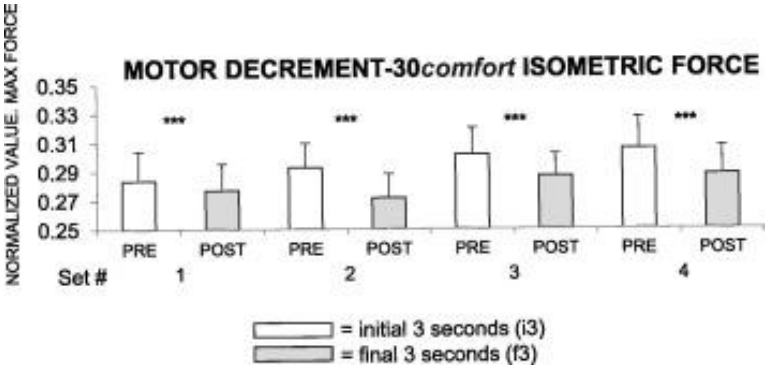

Fig. 6. Enlarged view to observe the size of the motor decrement for *comfort-iso.* $^{***}p < 0.001$.

Angular amplitude during ft

ROM amplitudes remained constant from *pre* to *post* ($F_{1,14} = 0.2$ $p > 0.05_{\text{TIME}}$) but were different in the three modes ($F_{2,28} = 13.2$ $_{\varepsilon=0.6}p < 0.01_{\text{MODE}}$). For *10max* it was 13.1% (*s.e.m* 2.9) of the maximal active ROM, and 12.6% (*s.e.m* 2.3) for *30max*. In both cases smaller than *comfort* (23.8%, *s.e.m* 2.9); *post hoc* $p < 0.01$.

MVC force at the time of CMS

The MVC force at the time of CMS waned at post; this was expressed differently in the two tasks, ft and iso (ANOVA $p < 0.01$).

MVC at the time of stimulation after finger tapping

For the *ft* task, MVC force reduced at *post* (ANOVA $p < 0.05$), from mode to mode (ANOVA $p < 0.01$), and set to set (ANOVA $p < 0.05$). Thus, *post* MVC waned always after tapping (compared to *pre*). A reduction in MVC with the progression of the testing protocol was also observed in all cases (modes and sets) (Fig. 7A).

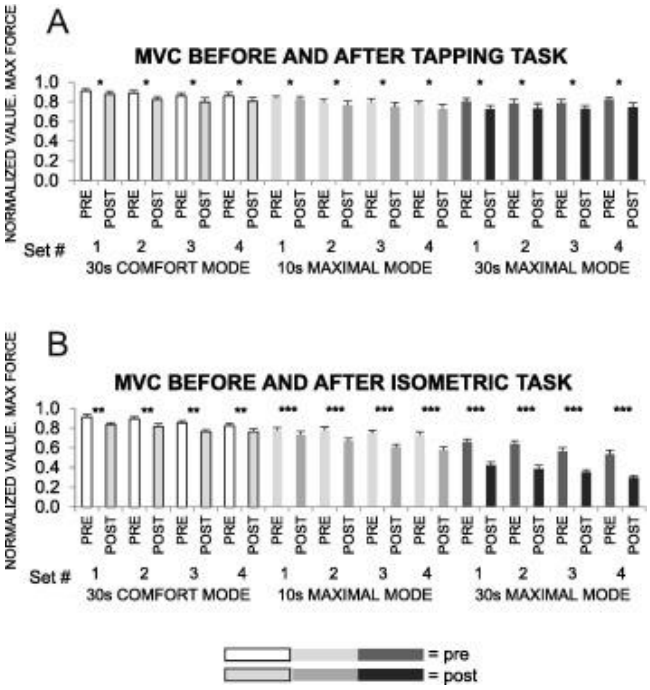

Fig. 7. MVC force at the time of CMS in both tasks. (A) MVC force was reduced at *post-ft* compared to *pre* in all cases, and also from mode to mode and set to set. (B) MVC force reduction with *iso* task resembled the pattern of *ft* but the effect was larger and more significantly expressed. $^{*}p < 0.05; ^{**}p < 0.01; ^{***}p < 0.001$.

MVC at the time of stimulation after isometric contraction

The case of *iso* was different, as the MVC dropping from *pre* to *post* was differently expressed for the three modes (ANOVA $p < 0.001$; Fig. 7B).

Analyses by modes indicated, however, that in all modes MVC dropped from *pre* to *post* (ANOVA $p < 0.01$ for *Comfort*; and $p < 0.001$ for *10max* and *30max*), and set after set (ANOVA $p < 0.05$ for *Comfort*; and $p < 0.01$ for *10max* and *30max*).

SP after ft and iso tasks

At *post* the SP change was significantly different for the two tasks (ANOVA $p < 0.01$), this is clearly observable in the representative individual recordings shown in Fig. 8.

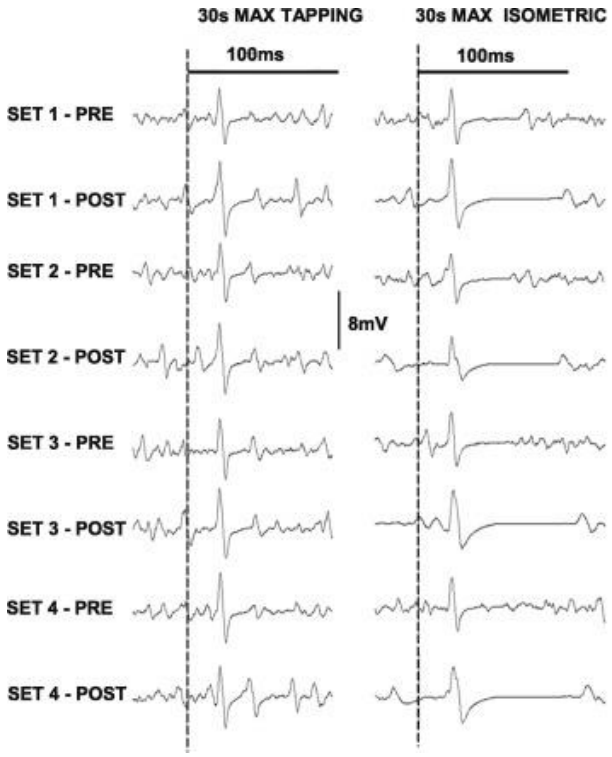

Fig. 8. Examples of silent periods acquired during the 30-s *maximal mode* before and after each set for *ft* and *iso* tasks (for the same representative subject). The increase in the SP duration *post* activity in the case of *iso* is very clear, with no set effect.

SP after ft tasks

The SPs were not modified after *ft*, regardless of the mode of execution or set progression ($p > 0.05$ for all main effects and interactions). Thus, clearly, the fatiguing *ft* activity had no reflection in SPs, Fig. 9A.

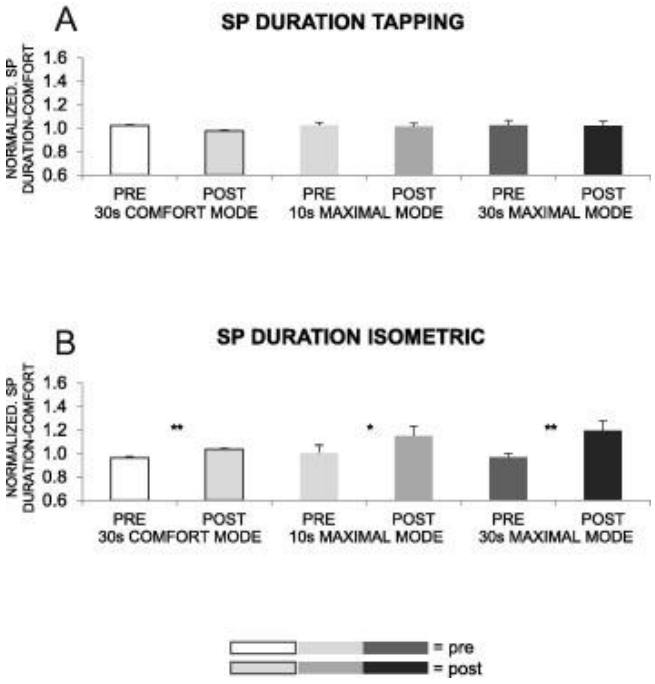

Fig. 9. Silent period changes observed in both tasks in all subjects. (A) For *ft*, SP were never modulated by the execution. (B) SP increased after *iso* tasks for all modes. $^{*}p < 0.05; ^{**}p < 0.01$.

SP after iso tasks

SP increased after *iso* execution, and such an increment was different in magnitude for the three execution modes (ANOVA $p < 0.05$). It turned out to be significant in all the cases (Fig. 9B). For *comfort-iso* SP increased by 7% (ANOVA $p < 0.01$), for *10 max-iso* the increment reached 15% (ANOVA $p < 0.01$), and for *30 max-iso* the increase in SP at *post was* 23% (ANOVA $p < 0.01$; such effects were present in all sets.

CMAP-amplitude modulation during fatiguing ft and iso tasks

The CMAP-amplitude responses were modified differently in the different tasks for the different modes (ANOVA $p < 0.05$).

CMAP-amplitude modulation during fatiguing ft tasks

For *ft*, all main effects and interactions between factors were non-significant ($p > 0.8$ was the smallest p value; Fig. 10A). Thus, CMAPs were not modified at any moment of testing in the *ft* tasks.

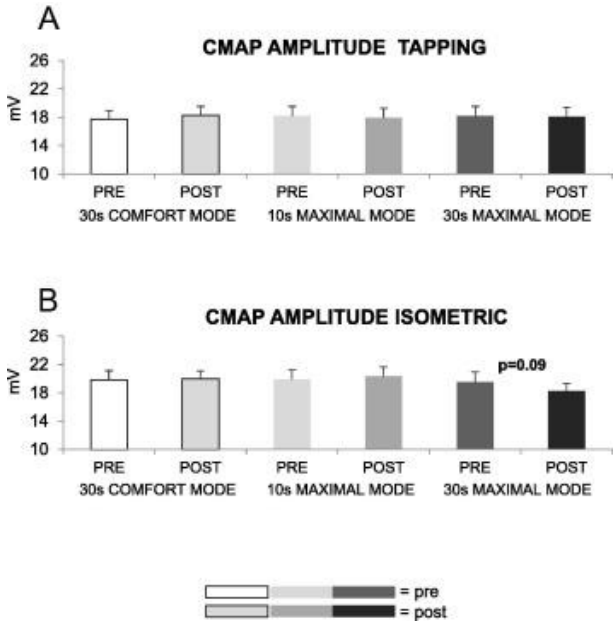

Fig. 10. CMAP amplitude changes achieved in both tasks were different. (A) The *ft* did not change the CMAP amplitudes. (B) The change in the CMAP amplitudes at the *post iso-30 max* did not reach significance (see main text for changes other than those reported in *pre-post* conditions).

CMAP-amplitude modulation during fatiguing iso tasks

For the *iso* tasks (Fig. 10B), the CMAP amplitude changed from mode to mode (ANOVA $p = 0.051$); the amplitude was always smaller at *30 max* than *10 max* (both *pre* and *post; post hoc* $p < 0.05$). It was also observed that the change from *pre* to *post* was significantly different for the three modes (ANOVA $p < 0.05$), although *post hoc* analysis indicated that the smallest *p*-value was $p = 0.09$, for *30 max*.

Responses induced by electric vs. magnetic CMS

Finally, we reproduced the above-mentioned analyses modifying the statistical design; the within-subjects factors were the same as above (*task, mode, set and time*), but a between-subjects factor (*group*) with two levels (electric-CMS, and magnetic-CMS) was added to check if the responses induced by both types of stimulation differed. This was never the case.

DISCUSSION

In a previous study, we suggested a supraspinal but not spinal origin of fatigue induced by short-lasting maximal *ft* tasks. This was based on the waning of *ft* frequency with task progression, a reduction of the MVC executed right after *ft*, and the increasing of SP if induced by TMS but not by CMS during such MVC *post* task execution (Arias et al., 2015). The present work reinforces the idea that the origin of the fatigue leading to *ft* frequency reduction (with no effect on amplitude) is not located at the spinal cord because the CMS-SPs were not modulated by fatigue and the amplitude of the CMEP did not wane with motor output reduction. Different effects were observed for maximal *iso* tasks. Because CMEPs obtained in this study were analyzed relative to the amplitude of the CMAP, the possible confounding factors affecting the neuromuscular junction, which can bias the interpretation of CMEP amplitude values, were controlled for. CMS (either magnetic or electric) is a technique that generates large short-latency responses with a main motoneuronal monosynaptic component by the stimulation of the axons at the corticospinal tract level (Taylor and Gandevia, 2004). For such reasons it provides a direct way to evaluate the behavior of spinal motoneurons at rest and in different tasks in human participants. Because presynaptic inhibition has not been described in the corticospinal axons (Nielsen and Petersen, 1994), the use of CMS is optimum for testing spinal motoneuronal excitability compared to other techniques (McNeil et al., 2013).

Fatigue task-dependency was manifested also in the EMG just preceding CMS, during the MVC. Stephen & Taylor reported (1972) that EMG activity decreases with MVC of long duration; a drop that in our 30 max *iso* did not reach significance, perhaps due to the shorter effort inherent to our task in comparison with the one used by Stephen & Taylor. Conversely, we have observed that 30 s of tapping at maximal rate increased the RMS. The profile of the RMS resembles, somehow, the responses of the CMEPs. This was expectable since the CMEPs amplitude depends, in part, on the preceding motoneuronal activity just prior stimulation. However, CMEPs are optimal to evaluate motoneuronal spinal excitability because they arise from a monosynaptic response (Ugawa *et al*., 1991 ; McNeil *et al*., 2013), while the origin of ongoing EMG activity is heterogeneous and includes: The supra-spinal descending drive; the afferent input to the spinal cord resulting from the stimulation of mechanoreceptors or nociceptors of hand fingers (Leis *et al*., 2000; McNulty and Macefield, 2001 ; Pierrot-Deseilligny and Burke, 2005); or the pre-synaptic inhibition of Ia fibers arising from tendon afferents (Priori et al., 1994), a form of inhibition not reflected in CMEPs (Nielsen and Petersen, 1994). Ongoing EMG is also influenced by the transmission in the periphery (Stephens and Taylor, 1972), for which we normalized the ongoing EMG-RMS relative to the CMAP-RMS, both acquired at a same time. Some of these mechanisms may account for different responses of CMEPs and its immediately preceding EMG-RMS activity obtained in our study.

Responses during fatiguing ft tasks

Earlier, we showed that 10 and 30 s of *ft* at maximal rate induced an effect compatible with an excitability increment of inhibitory GABAb interneurons located at the motor cortex, in parallel with the inability to maintain maximal rates of *ft* frequency and a loss in muscle force. In addition, Renshaw recurrent inhibition and axonal after hyperpolarization, which are known to be responsible for SP induced by CMS at spinal levels (Inghilleri et al., 1993), remained unchanged (Arias et al., 2015). We now extend our previous results by showing that motoneuron excitability, directly evaluated by CMEP amplitudes, seems not to be responsible for the tapping rate reduction during maximal tapping rates, up to 30 s. The spinal excitability increased at the end of both maximal activities (10 and 30 s), but *ft* rate decreased (15% at *10 max post*, and 25% *30 max post*). Therefore, the increment of excitability can hardly be considered a sign of motoneuronal fatigue but perhaps a neuronal substrate to facilitate maximal effort tasks.

Responses during fatiguing iso tasks

The pattern of fatigue observed for *iso* tasks is in agreement with a good deal of scientific research showing a reduction of spinal excitability during fatiguing isometric muscular activities (Taylor *et al*., 1996; Duchateau *et al*., 2002; Butler *et al*., 2003; Klass *et al*., 2008 ; McNeil *et al*., 2009). A characteristic finding was the increase in the SP induced by CMS during the MVC after maximal *iso* modes (Taylor *et al*., 1996 ; McNeil *et al*., 2009), which was absent for the *ft* task (Arias et al., 2015).

Considering the amplitudes of the responses, some previous work has reported that during 2-min MVC the CMAP area wanes in the last ≈30 s of the task, compared to its maximum value at the initial part of the task (Butler et al., 2003). In our work the *iso* protocol modified the CMAP amplitudes significantly, not in the comparison of *pre* vs. *post* within sets, but in how the modification of CMAP from *pre* to *post* progressed along the modes, perhaps showing that the changes were cumulative and slowly developed over time. Such effects on CMAP during *iso* might condition the interpretation of CMEP amplitudes, and the evaluation of task-dependent effects (Enoka and Stuart, 1992 ; Barry and Enoka, 2007). However, when analyzed relative to the CMAP amplitude at the same time of testing, we observed that the CMEP amplitude presented a different profile for *10 s* and *30 s iso-max*. In both cases, set after set effects were absent, but CMEP amplitudes *post 10 s iso-max* were larger than *pre* values (in agreement with both maximal *ft* modes). We suggest that the increased excitability is an adaptation of the motoneurons that contributes and permits the execution during maximal activity. However, at the end of the *30 s iso-max* the increased level of spinal excitability (compared to *pre*) could not be maintained; for this reason we suggest that the inability to maintain an increased level of spinal excitability is a hallmark of spinal fatigue. Our results complement those by Butler et al. (2003) obtained in elbow flexors. We observed increased levels of spinal excitability 10 s after task initiation, an effect possibly not observable with different testing time courses, or differentially expressed in small hand muscles (responsible for fine movements and perhaps less adapted to sustained MVC) compared to larger muscles (Butler et al., 2003). In agreement with this idea, it has been shown that motor units of intrinsic hand muscles, and those of larger muscles in the arm, present different firing rates and recruitment patterns. Small muscles increase recruitment of units up to a relatively small level of force (≈50% of MVC), with a need to increase firing rate to produce larger forces; conversely, larger muscles increase recruitment up to higher force levels (≈90% of MVC) and their maximum firing rates are lower (Masakado, 1994). This might account for a different modulatory time course of spinal excitability in our study compared to other works evaluating some other muscles (Butler et al., 2003).

Study limitations

In this study the fatigue induced by *ft* was tested during the course of a MVC. We adopted this methodology because it permits the evaluation of spinal excitability and a direct comparison on how it was influenced by immediately preceding activities of different nature (*ft* and *iso*). It also makes possible the recording of standard variables which are commonly used in this field. We recognize, however, that powerful activations during MVC might overwhelm weak expressions of spinal fatigue induced by *ft*.

CONCLUSIONS

Our works suggest that the development of fatigue induced by short-lasting repetitive tasks emerges in sites other than the spinal cord. The supraspinal origin of fatigue for this type of task (Arias et al., 2015) gains support with the present study. We have provided neurophysiological evidence for different fatigue mechanisms in *ft* and *iso*; however the mechanisms behind the changes in excitability remain unknown, for which further studies are required. Fatigue is one of the main signs in diseases affecting the spinal cord and supraspinal centers, our results may help to improve the design of the clinical tests aimed at the evaluation of fatigue in physiological and neurological conditions.

DISCLOSURE

The authors declare no conflict of interests.

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