

## Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of silent periods evoked at spinal and supraspinal levels

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### Abstract

The neural substrates of fatigue induced by muscular activity have been addressed in depth in relation to isometric tasks. For these activities, when fatigue develops, it has been noted that the duration of the silent periods (SPs) increases in response to both transcranial magnetic stimulation (TMS) of primary motor cortex or electric cervicomedullary stimulation (CMS). However, fatigue is known to be task-dependent and the mechanisms giving rise to a decrease in motor performance during brief, fast repetitive tasks have been less studied. We hypothesized that fatigue induced by repetitive fast finger tapping may have physiological mechanisms different from those accounting for fatigue during an isometric contraction, even in cases of matched effort durations. In these tasks, we examined the contribution of spinal and supraspinal motor circuits to the production of fatigue. The tapping rate and maximal voluntary contractions (MVC), and TMS- and CMS-evoked SPs were obtained at the time of fatigue, and while subjects maintained maximal muscle activation after fast finger-tapping (or isometric activity) of different durations (10 or 30 s). Results showed different mechanisms of fatigue triggered by isometric contraction and repetitive movements, even of short duration. Short-lasting repetitive movements induce fatigue within intracortical inhibitory circuits. They increased TMS-SPs, but not CMS-SPs. On the other hand, isometric contraction had a clear impact on spinal circuits. The consideration of these differences might help to optimize the study of fatigue in physiological conditions and neurological disorders.

### Abbreviations

10max-30max, 10 or 30 s of maximal mode execution; CF, central fatigue; CMAP, compound muscle action potential; CMS, cervicomedullary stimulation; *comfort*, 30 s of comfort mode execution; FDI, first dorsal interosseous muscle; *ft*, finger tapping task; *iso*, isometric task; MVC, (isometric) maximal voluntary contraction; ROM, range of motion; SP, silent period; TMS, transcranial magnetic stimulation

### Key words

Central fatigue; repetitive movements; human

### Introduction

The finger tapping (*ft*) test is a reliable procedure used world-wide to evaluate physiological and pathological mechanisms of repetitive movements (Shimoyama et al., 1990 and Arias et al., 2012). However, when *ft* is performed at the fastest rate its frequency drops in very few seconds, suggesting the development of muscle fatigue during the task (Arias et al., 2012 and Teo et al., 2012). Remarkably, muscle fatigue has been chiefly studied when induced by isometric tasks, but the underlying neurophysiological mechanisms of fatigue during fast repetitive movements have been hardly explored.

Muscle fatigue is characterized by a reduction in maximal voluntary contraction (MVC) muscle force (Bigland-Ritchie and Woods, 1984). The progressive failure of muscle-force generating capacity, and failure of impulse conduction through the neuromuscular junction, are known peripheral causes of fatigue (Allen et al., 2008). However, fatigue also involves circuits of the spinal cord, cerebral cortex and subcortical structures (i.e. central fatigue, CF) (Gandevia, 2001), which is of interest in neurological conditions (Zwarts et al., 2008 and Kluger et al., 2013).

In order to evaluate the contribution of supra-spinal and spinal motor circuits to CF, transcranial magnetic stimulation (TMS) permits the study of the motor-evoked potentials resulting from activation of

M1 and the corticospinal tract. Likewise, electrical cervicomedullary stimulation (CMS) induces motor responses by depolarization of the axons in the corticospinal tract at the cervicomedullary junction (Ugawa et al., 1991).

When a single TMS pulse is applied to the motor cortex during an active contralateral muscle contraction, the electromyographic activity is arrested for a few hundred milliseconds after the motor-evoked potential. This period of electromyographic suppression is referred to as a silent period (SP) and can be induced by either TMS or CMS. Therefore, the SP is usually studied while the target muscle maintains a certain level of isometric (*iso*) contraction. TMS-SP duration increases with stimulus intensity and can reach durations of 200–300 ms in hand muscles ( Cantello et al., 1992 and Inghilleri et al., 1993). However, TMS-SP has two components: the early part corresponds to the first  $\approx 75$  ms and is due to spinal inhibition ( Fuhr et al., 1991, Cantello et al., 1992, Inghilleri et al., 1993, Brasil-Neto et al., 1995 and Butler et al., 2012) and involves mechanisms following on from motoneuron excitation, like recurrent (Renshaw) inhibition and axonal after-hyperpolarization. These processes are known to be dependent on the level of preceding motoneuron activity ( Hultborn et al., 1979 and Ziemann et al., 1993).

The second, late part is supraspinal; it is cortical in origin (Fuhr et al., 1991, Cantello et al., 1992, Inghilleri et al., 1993, Ziemann et al., 1993, Brasil-Neto et al., 1995, Butler et al., 2012 and Ziemann, 2013) and is linked to intracortical inhibitory circuits operating via GABA<sub>B</sub> receptors (Ziemann et al., 1993 and Ziemann, 2013). Thus, it is considered a marker of motor cortical inhibition.

On the other hand, the CMS-SP is induced by spinal mechanisms exclusively (Fuhr et al., 1991, Inghilleri et al., 1993 and Brasil-Neto et al., 1995), sharing the mechanisms of the early part of the TMS-SP.

The development of CF is task-dependent and each task may involve different circuits and mechanisms within the central nervous system (Enoka and Stuart, 1992 and Enoka and Duchateau, 2008). For instance, fatiguing isometric activity (i.e., during a 2 min *iso*-MVC) increases SP duration in response to TMS and CMS ( Taylor et al., 1996). This suggests that fatigue induced by *iso*-MVC is generated both at the spinal cord level ( Butler et al., 2003), and, potentially, at a supraspinal level ( Taylor et al., 1996, Di Lazzaro et al., 2003 and Hilty et al., 2011a). Interestingly, repetitive isometric activity has been shown to increase TMS-SP ( Taylor et al., 2000 and Hilty et al., 2011b), and part of this increase seems to be caused by processing of afferent (opioid) signaling from the fatigued muscle to the brain (Hilty et al., 2011b). Conversely, CF induced by non-isometric tasks (1 Hz resisted concentric-eccentric contractions to reaching task-failure) has been attributed to supraspinal mechanisms (Brasil-Neto et al., 1993) after testing the motor-evoked potentials and H-reflex at rest; however a possible contribution of fast-recovery forms of spinal fatigue could not be totally ruled out. This was also the case with CF evaluation after short-lasting and maximal-rate *ft* ( Arias et al., 2012 and Teo et al., 2012). To avoid this drawback, it is recommended that evaluation of CF be made during maximal effort, without allowing recovery of the nervous system after the fatiguing activity (Taylor et al., 2000).

In this study, we have evaluated the SPs evoked by TMS and CMS to explore the origin of CF underlying the reduction in motor-output that is expected during a *ft* task, performed at the fastest possible rate for 10 and 30 s. We compared neurophysiological signs of fatigue induced by *ft* with those induced by *iso*-MVC, both of the highest intensity and matched in durations of the effort. In all cases, we evaluated fatigue with no gap in muscle activation to avoid any possibility of recovery of the motor system. Based on the “task-dependency” of fatigue signs ( Enoka and Stuart, 1992, Enoka and Duchateau, 2008 and Enoka et al., 2011), our hypothesis is twofold: Firstly, maximal *ft* compared to the same duration of maximal *iso* effort would produce a different reduction of motor output; secondly, changes in excitability of the cortical and spinal circuits under investigation would be different for short-lasting maximal *ft* and *iso* tasks.

## Experimental procedures

Experimental protocols complied with the Helsinki declaration and were approved by the University of A Coruña Ethics Committee. Subjects were screened for incompatibility with brain stimulation techniques and were medication-free during the week preceding testing. All subjects consented to participate.

### Subjects

The experiment included two groups of subjects: the TMS-group composed of nine right-handed healthy subjects (eight males and one female; age range 22–38 years), and the CMS-group composed of

12 right-handed healthy subjects (all males; age range 18–41 years), each group underwent both *ft* and *iso* fatigue protocols.

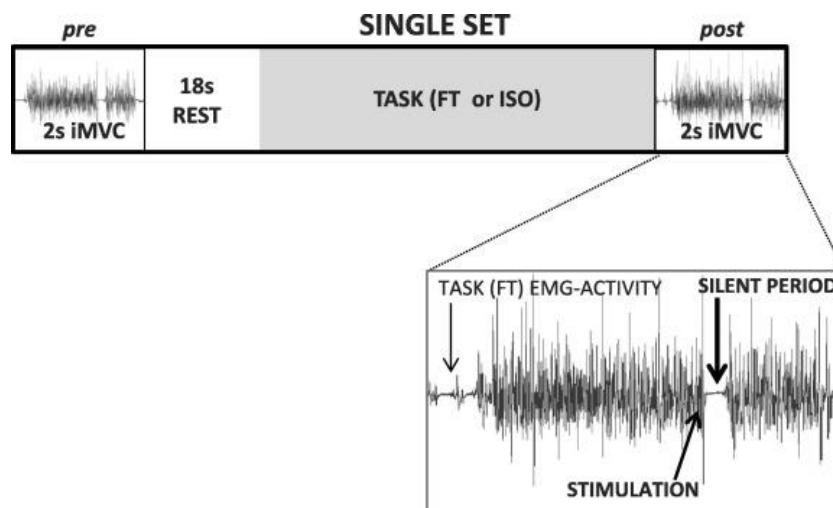
### Protocol

Each subject underwent two experimental sessions, at least one week apart, in randomized order. Sessions were identical but for the type of *task* executed. In one session, subjects were requested to perform index *ft*, and, in the other session, continuous index finger *iso* against a force sensor, with the direction of the force “toward” *flexion* of the first metacarpophalangeal joint of the index finger. Subjects always wore a small goniometer on the index finger metacarpophalangeal joint, and a metal ring at the distal phalanx of the same finger. Subjects pressed or tapped over a thin metal plate placed on the force sensor.

For both *ft* and *iso* sessions, subjects executed the tasks in three different modes, and each mode was executed four times (four *sets*). Therefore, the subjects performed four sets at comfort rate/effort (*comfort* mode) for 30 s; then four sets of 10 s at maximal rate/effort (*10max* mode); and finally four sets of 30 s at maximal rate/effort (*30max* mode), always in this order. In all cases there was an inter-set rest period of 1 min 40 s.

For the *comfort-ft* subjects were asked to “tap at their most comfortable rate without feeling fatigued” for as long as the set lasted. In a previous paper ( Arias et al., 2012), we observed that this *ft* mode is reliable, and performed at a pace of about 1/3 of the maximal rate. Because *comfort ft* is linked to lower metabolic activity in the sensorimotor cortex compared to faster (>3 Hz) and slower (<1 Hz) rates ( Jancke et al., 1998 and Lutz et al., 2005), its use seems to be adequate as a control condition to evaluate the fatigue induced by maximum *ft*. For the *comfort-iso* participants were asked to press  $\approx 1/3$  MVC with visual feed-back provided by means of online isometric force display. For maximal modes subjects were requested to tap/press as fast/hard as they could from the very beginning to the end of the set.

Fatigue was assessed as either the decrease in frequency or amplitude (for *ft*), or in force output (for *iso*). CF, either supraspinal or spinal, was evaluated by recording the changes in the SP duration in response to TMS or CMS ( Taylor et al., 1996). The stimulation pulses were delivered during brief isometric MVCs (2s-iMVC). The 2s-iMVCs were performed before (*pre*) and immediately after (with no gap inbetween) task-execution (*post*), either for *ft* or *iso*, for all modes and sets ( Fig. 1). The magnitude of the brief 2s-iMVCs was also an analyzed variable for fatigue monitoring (Bigland-Ritchie and Woods, 1984). An initial session was scheduled to allow some practice and answer all the subjects’ questions about the experimental methods (Gandevia, 2001).



**Fig. 1.** The set-structure: as soon as the LED was lit subjects performed an isometric maximum voluntary contraction (iMVC) with their index against the dynamometer. The LED off (2 s after) served as a signal to stop the iMVC. During the iMVC (1.5 s after LED on) subjects received an initial test stimulation (*pre*) (TMS in one group ( $n = 9$ ); electric CMS in the other ( $n = 12$ )). Stimulation induced subsequent silent periods, as shown in the enlarged area. After resting for 18 s, a LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In continuation of the task, and with no resting time, the subjects performed another 2s-iMVC 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 seated comfortably with the elbow flexed at 90–100°. The forearm, wrist, hand and all fingers except the index were firmly but comfortably fixed to a modified tablet-arm chair, allowing unrestrained degrees of freedom at the metacarpophalangeal joint of the index finger, permitting *ft*. During *ft*, a Biometrics DataLink system (Biometrics Ltd., Gwent, NP11 7HZ, UK) 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 iMVC of the index flexion with a Pinch-Dynamometer (P200), which was placed flat and secured over the table, 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 ( $\times 250$ –1000) and band-width filtered between 3–3000 Hz.

EMG were sampled at 10 kHz and stored in a computer by means of a CED 1401 mkII Power A–D converter (Cambridge Electronic Design, Cambridge, UK). This device also controlled the on/off state of LED's (indicating the different phases of execution/rest within the sets) and timing of TMS/CMS pulses.

In separate sessions, in six subjects we recorded the level of EMG activation of the FDI and different intrinsic and extrinsic hand muscles during the *ft* and different *iso* tasks; i.e., index iMVC, and iMVC of the 3rd, 4th and 5th fingers working together. Also, we recorded the FDI activation during different index-iMVC tasks applying force against immovable resistances in different directions, toward flexion (the same as above), abduction, adduction and extension. This was done using the same setting, hand position and fixation as during fatigue testing; and adapting immovable resistances to permit iMVC in the different planes. For this purpose the subjects executed two 3s-trials/task, with task presentation order randomized. The inter-trial rest period was one minute.

The trial started with the recording of the compound muscle action potential (CMAP) of each muscle, which was acquired by supramaximal electrical stimulation at the Erb's point (1 ms electric pulses; EBNeuro Stimulator, Italy; cathode lateral, anode medial) with the muscle at rest; 10 s after stimulation the onset of a LED light indicated the start of 3s-task. The root mean square (*r.m.s.*) of the CMAP was computed, which served as the divisor of *r.m.s.* activity during task execution.

For *ft*, we computed the *r.m.s.* within the tapping cycles (lasting about 150 ms, one per trial) with the highest frequency (shortest inter-tap interval). For the *iso* tasks, we identified the peaks (one per trial) in the force recordings, then the muscle activity was computed in the 150 ms around the peaks (75 ms before/after the corresponding peak in each trial).

### *TMS*

During the sessions of fatigue evaluation, and for TMS-SP generation, a Magstim 200<sup>2</sup> stimulator delivered monophasic wave-form pulses through a 70 mm figure of eight coil. The coil was positioned (and marked for reference) to induce currents in a posterior-anterior direction, and placed over the hot-spot for the FDI muscle of the executing hand.

The intensity was set to evoke a SP duration  $\approx 150$  ms during a 2s-iMVC in the non-fatigued muscle (this produced TMS motor evoked potentials of about 50% amplitude of the maximal CMAP); TMS intensity was expressed relative to the individual's active motor threshold, defined as the minimum intensity required to evoke five liminal responses (about 200  $\mu$ V) in 10 consecutive pulses, in the activated muscle (5–10% MVC) (Rossini et al., 1994).

### *CMS*

CMS was applied using a Digitimer D180 stimulator connected to a pair of Ag–AgCl electrodes. Electrodes were placed behind the mastoid processes with the anode at the right and the cathode at the left. Active motor threshold was defined as described above for TMS (Rossini et al., 1994); then the stimulation intensity for the protocol was set (about 10% above this threshold) to have a SP of  $\approx 70$  ms in the un-fatigued muscle (this produced CMS motor evoked potentials of about 50% amplitude of the CMAP). To make sure this intensity did not produce current spread to the spinal roots, the CMS motor-evoked potential latency was compared to that obtained at threshold 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).

Baseline un-fatigued SP's durations for TMS and CMS setting were  $\approx 150$  and 70 ms respectively, (CMS-SP is much shorter than TMS-SP; (Inghilleri et al., 1993). We set these values away from their

ceiling to allow a potential modulation induced by fatigue. The mean TMS intensity applied was 17.9% (*s.e.m.* 1.0) above the AMT. The mean voltage used in the CMS experiments was 543.4V (*s.e.m.* 27.1).

### Data reduction

The following dependent variables were analyzed:

Level of muscle activation in intrinsic and extrinsic hand muscles: This is expressed as the percentage of the *r.m.s.* of the CMAP, and defined as  $100 (\text{TASK}_{r.m.s.}/\text{CMAP}_{r.m.s.})$ .

In the fatigue sessions we studied the following variables:

Motor output during tasks execution: We considered three measures of motor output for task execution: the tapping frequency, angular amplitude for *ft* and the force applied for *iso*. For each of the four Sets and three Modes (*comfort*; *10max* and *30max*) we considered two time points which were embedded within task execution: the initial 3 s (*pre*) and the final 3 s (*post*). To make data from *ft* frequency and *iso* torque comparable, 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 at the time of stimulation (MVC in the 50-ms period before the stimulation) either for *ft* and *iso* task, normalized to the maximum obtained at any evaluation time-point 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 TMS/CMS pulse to the recovery of the EMG activity during the brief-iMVC, and determined visually by an experienced researcher blind to the conditions (in two separated sessions intra-rater reliability was checked on 124 random-chosen SPs, 62 each technique, TMS-CMS). Because we evaluated SP for both TMS and CMS, whose durations are not directly comparable (Inghilleri et al., 1993), the SP durations were normalized. 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 for studying FDI activation during the tasks

To study the level of the FDI activation in comparison with other muscles in each task, we first analyzed whether activation of each muscle was different in the two trials executed. After checking normality (one sample KS test) a paired Student *t*-test was used. Since activation in the two trials was never different ( $p > 0.05$ ), we averaged the values to compare the level of activation of the different muscles and tasks using different models of one-factor ANOVA (with repeated-measures). One model evaluated FDI activation compared to *opponens pollicis*, *abductor digiti minimi*, *extensor digitorum*, and *flexor digitorum superficialis*. This model was applied independently to the tasks: *ft*, *index iso-flexion*, and *3–5th fingers iso-flexion*. Another one-factor ANOVA model had four levels, and it was used to compare the activation of the FDI during *iso* in the four planes; “toward *flexion*” vs. “*extension*”, “*abduction*” and “*adduction*”.

#### Statistical design for studying intra-rater reliability in determining SP durations

To study intra-rater reliability during SP duration determination (session 1 vs. 2, on the same random-chosen SPs) the intra-class correlation coefficient, and its 95% confidence interval (95% CI) were evaluated. For TMS-SP we obtained an ICC = 0.97 (0.94–0.98; 95% CI); and for CMS-SP the ICC = 0.97 (0.96–0.98; 95% CI).

#### Statistical design for studying behavior during fatigue tasks

To study fatigue various repeated measures ANOVA's models were used, after checking the normality of distributions.

For the variables Motor Output (tapping rate-*ft*; or force-*iso*) decrement, SP duration, and MVC before and after task execution, we used an ANOVA with repeated measures. The ANOVA included one between-subject factor Group with two levels (the *TMS-group* and the *CMS-group*) and several within-subject factors. In the specific case of the SP, if the factor Group interacted significantly with any of the

within-subject factors, it means that there was a significant different effect of within-subject factors on the response to spinal or corticospinal stimulation.

The within subject factors were Task (*ft, iso*), execution Mode (*comfort, 10max, 30max*), 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 ( $\epsilon$ ), 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.

## Results

The Table 1 indicates the level of activation of the FDI obtained in *ft* and *iso* tasks in all subjects ( Fig. 2 shows activations in a representative participant). FDI had a main role in the tasks used in the fatigue protocols (index *ft* or *iso*). Table 2 shows the normalizing values equivalent to the unit in graphs during the fatigue testing sessions.

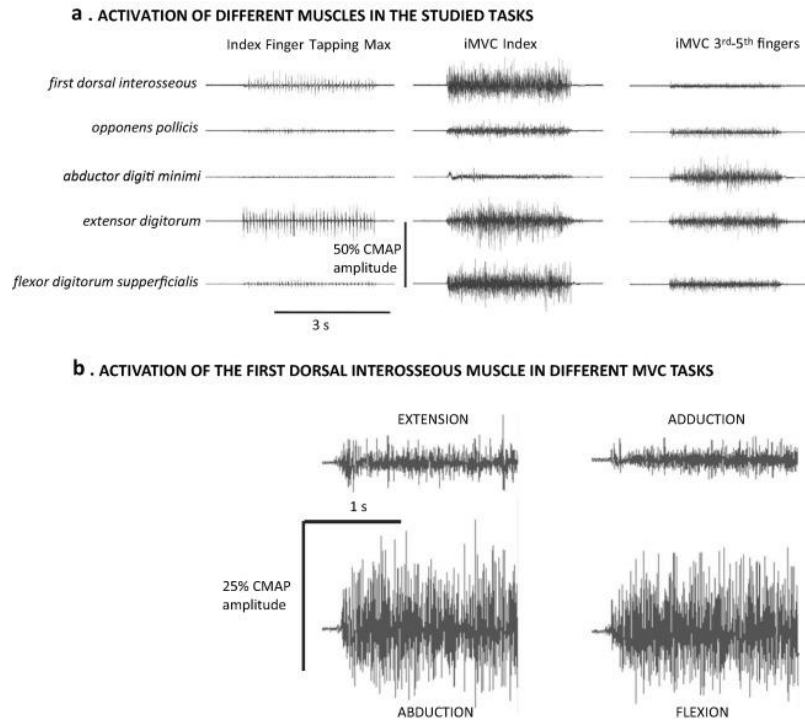
**Table 1.** First dorsal interosseous activation in the tasks of the fatigue protocol (bold fonts) compared to other tasks and muscles

FDI activation compared to activation of other muscles in three different tasks						
<i>Task</i>	ANOVA task-effect	<b>FDI activation</b>	<i>vs. opp (post hoc)</i>	<i>vs. adm (post hoc)</i>	<i>vs. ext (post hoc)</i>	<i>vs. fds (post hoc)</i>
<i>Index ft task</i>	$F_{4,20} = 5.6$ $p < 0.05$	<b>6.7%</b> ( <i>s.e.m.</i> <b>1.0</b> )	n.s.	n.s.	n.s.	n.s.
<i>Index iso-MVC task</i>	$F_{4,20} = 10.5$ $p < 0.001$	<b>13.3%</b> ( <i>s.e.m.</i> <b>1.1</b> )	5.8%* ( <i>s.e.m.</i> 1.3)	4.4%* ( <i>s.e.m.</i> 0.5)	n.s.	n.s.
<i>3-5th fingers iso-MVC task</i>	$F_{4,20} = 21.9$ $p < 0.001$	<b>5.6%</b> ( <i>s.e.m.</i> <b>1.1</b> )	n.s.	16.3%* ( <i>s.e.m.</i> 1.7)	n.s.	14.4%** ( <i>s.e.m.</i> 0.9)
FDI activation during different index iso MVC tasks						
	ANOVA direction-effect	<b>toward flexion</b>	<i>vs. abduction (post hoc)</i>	<i>vs. extension (post hoc)</i>	<i>vs. adduction (post hoc)</i>	
<i>Index iso-MVC</i>	$F_{3,15} = 37.1$ $p < 0.001$	<b>13.3%</b> ( <i>s.e.m.</i> <b>1.1</b> )	n.s.	5.2%* ( <i>s.e.m.</i> 0.6)	4.0%* ( <i>s.e.m.</i> 0.9)	

Units (%) = 100 ( $r.m.s_{TASK}/r.m.s_{CMAP}$ ). *opp*: oponens pollicis; *adm*: abductor digiti minimi; *ext*: extensor digitorum; *fds*: flexor digitorum superpericialis. Asterisks indicate Bonferroni post hoc after ANOVA evaluation of main effects. n.s.: not significant.

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .



**Fig. 2.** (a) Activation of the *first dorsal interosseous* in the *ft* and *iso* tasks, for comparison with another task (iMVC flexion of the 3rd, 4th and 5th fingers). Example of individual recordings in one subject (on a separate session) with the settings as describe in Fig. 1. Execution of *ft* at maximal rate (left recordings); *iso* MVC with the index finger, as in the protocols (central recordings); and *iso* MVC flexion with 3–5th fingers together with no index finger pressing (right recordings). The EMG-recordings of the *first dorsal interosseous*, *opponens pollicis*, *abductor digiti minimi*, *extensor digitorum* and *flexor digitorum superficialis* are shown in the three tasks. The EMG-amplitudes are scaled to the vertical bar to allow raw-data representation, showing the 50% of each muscle CMAP amplitude; statistical comparisons were applied for *r.m.s.* activation; the involvement of the FDI compared to the rest of the muscles is clear and more specific in the task evaluated. (b) Similar maximal EMG-recruitment of the FDI during index finger flexion and abduction, larger than during adduction and extension, all iMVC against unmovable loads.

**Table 2.** Mean score including all subjects. The score is the y-axis unit in graphs

Task	*Max output in task at any evaluation time-point	*Max active full ROM <sup>1</sup>	Mean (% <sup>1</sup> ) <i>ft</i> amplitude along the task	*MVC at any stimulation time-point	Mean TMS-SP duration at comfort	Mean CMS-SP duration at comfort
<i>ft</i>	7.1 Hz ( <i>s.e.m.</i> 0.1)	24.7 ° ( <i>s.e.m.</i> 2.0)	28.3% ( <i>s.e.m.</i> 3.9)	4.7 kg ( <i>s.e.m.</i> 0.3)	145.1 ms ( <i>s.e.m.</i> 4.1)	67.0 ms ( <i>s.e.m.</i> 4.0)
<i>iso</i>	3.8 kg ( <i>s.e.m.</i> 0.2)	n.a.	n.a.	4.5 kg ( <i>s.e.m.</i> 0.2)	158.9 ms ( <i>s.e.m.</i> 5.9)	71.5 ms ( <i>s.e.m.</i> 4.6)

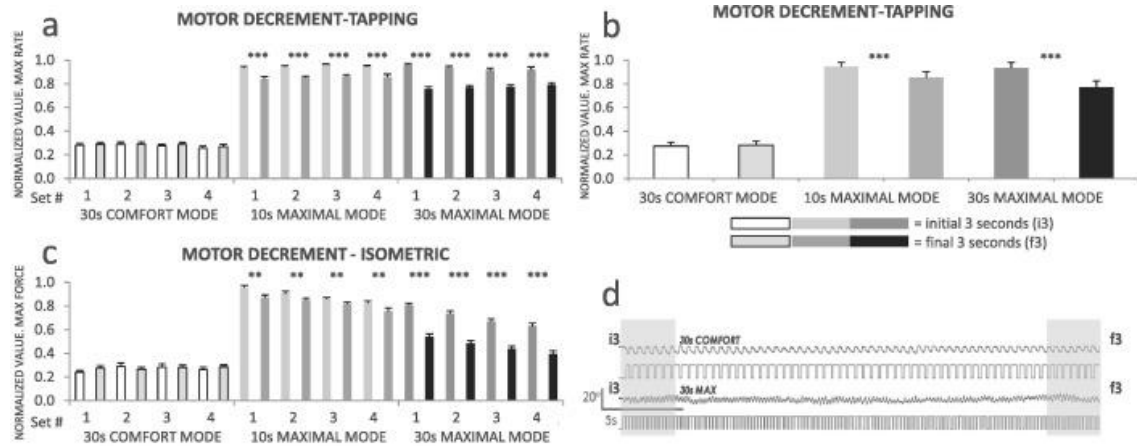
\* The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects. n.a.: not applicable.

### Frequency/force decrement during execution in *ft/iso* tasks

The motor output decrements during the task execution (comparing motor output in the initial and final 3 s of the tasks) were different for the tasks and execution modes ( $F_{2,38} = 8.6$ ,  $p < 0.01_{\text{TASK} \times \text{MODE} \times \text{TIME}}$ ). Therefore, we split the analyses for each kind of task.

For *ft*, the frequency reduced over execution ( $F_{1,19} = 86.4$ ,  $p < 0.001_{\text{TIME}}$ ), but differently on the three modes ( $F_{2,38} = 43.7$ ,  $p < 0.001_{\text{MODE} \times \text{TIME}}$ ); this effect was not differently observed in the two groups

(subjects stimulated with TMS or CMS;  $F_{2,38} = 0.4$ ,  $p > 0.05_{\text{MODE} \times \text{TIME} \times \text{GROUP}}$  - note that the execution protocol was the same for the two groups of subjects). The frequency of  $ft$  at *comfort* was unchanged, conversely to *10max* and *30max* (both reduced in the last 3 s of task execution (i.e., *post*) compared to the initial 3 s of task execution (*pre*), post hoc  $p < 0.001$ . Set progression had no effect ( $F_{3,57} = 0.3$ ,  $p > 0.05_{\text{SET}}$ ), as clearly observed in Fig. 3a, and for this reason the effects are shown pooled at Fig. 3b.



**Fig. 3.** Motor decrement induced by the tasks. (a) The frequency of  $ft$  decreased significantly after the maximal modes; there was no set effect. (b) Same effect seen pooling sets as there was no set effect. (c) Set by set motor decrement along the *iso* tasks. The force reduced at *post* after maximal modes and set after set. (d) Representative example in one subject,  $ft$  amplitude for *comfort* and *30max*. The amplitude never decreased during the tasks. The unit in the y-axis represents the normalized value with respect to the maximal motor output (maximal  $ft$  rate, or maximal *iso* torque obtained at any evaluation time of the corresponding session). In all figures, light colors are the initial 3s of execution (*pre*), dark colors the final 3 s (*post*); asterisks denote statistical significance between *pre* and *post*, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Fig. 3c illustrates the force developed during *iso* in the initial and final 3s of the task in the different modes and sets. It was evident that the force dropped at the end of the task for the different modes ( $F_{2,38} = 109.0$ ,  $p < 0.001_{\text{MODE} \times \text{TIME}}$ ); and also there was a set effect for some modes impacting the level of the dropping of force at the end of the *iso* task ( $F_{6,114} = 11.0$ ,  $p < 0.001_{\text{MODE} \times \text{SET}}$ ). Again, the execution protocol was identical for the two groups of subjects, those stimulated with TMS and CMS; likely, this is the reason why the dropping of force was not significantly different for the two groups of subjects ( $p > 0.05$  for  $\text{MODE} \times \text{SET} \times \text{GROUP}$  OR  $\text{MODE} \times \text{TIME} \times \text{GROUP}$  interactions). Then, we followed-up ANOVAs by modes of execution, this was done to understand the before-mentioned differential effects of the modes of execution on the dropping of force at the end of the *iso*-tasks. We observed no effects of *comfort-iso* on force drop at *post* ( $p > 0.05$  for any main effect or interaction). For *10max-iso*, the execution dropped significantly at *post* ( $F_{1,19} = 13.4$ ,  $p < 0.01_{\text{TIME}}$ ), and set after set ( $F_{3,57} = 13.7$ ,  $p < 0.001_{\text{SET}}$ ). A similar pattern of force-drop was observed for *30max-iso* ( $F_{1,19} = 174.5$ ,  $p < 0.001_{\text{TIME}}$  and  $F_{3,57} = 39.6$ ,  $p < 0.001_{\text{SET}}$ ).

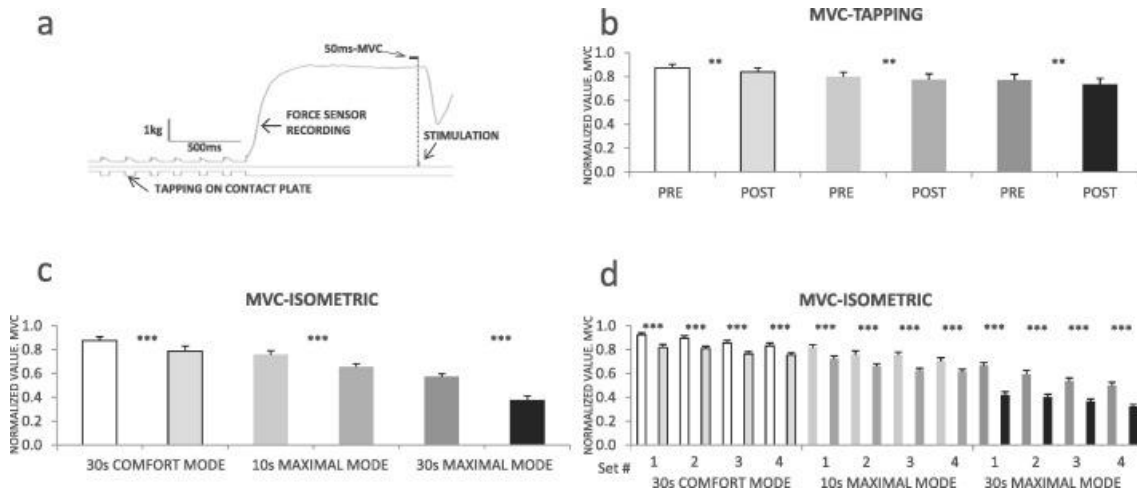
#### Finger angular amplitude during tapping

During  $ft$ , the expressions of fatigue might affect not only the tapping-frequency, but also the tapping amplitude. For this reason, we analyzed the ROM amplitude at the first ( $i3 = \text{pre}$ ) and last ( $f3 = \text{post}$ ) 3 s of the task. Remarkably, during  $ft$  the ROM amplitude was not significantly different at *pre* vs. *post* ( $i3$  vs.  $f3$ ;  $F_{1,19} = 1.7$ ,  $p > 0.05_{\text{TIME}}$ ; Table 2). Likewise, it is worth mentioning, that in all sets, the mean ROM amplitude was not different for the three tapping modes either ( $F_{2,38} = 2.3$ ,  $p > 0.05_{\text{MODE}}$ ; Fig. 3d shows an example of the ROM amplitude in one subject). These effects on motor execution were not differently expressed for the subjects stimulated with TMS or CMS (identical task for both groups).



### MVC at the time of stimulation

The MVC at the time of stimulation (Fig. 4a) was reduced at *post* differently for the *ft* and *iso* tasks ( $F_{1,19} = 30.1, p < 0.001_{\text{TASK} \times \text{TIME}}$ ; Fig. 4b–d). We followed by splitting the analyses by task-type.



**Fig. 4.** (a) Example of force recording at *post-ft*. MVCs shown in 4(b–d) sections are acquired in the 50 ms prior to stimulation. (b) MVC decreased after *ft* (*post*) in all modes and from mode to mode. (c–d) MVC decrease after *iso* (*post*) in all modes and also from mode to mode and set to set.

For *ft* tasks, MVC force was reduced at *post* (i.e., in the MVC performed right at the end of tapping;  $F_{1,13} = 12.3, p < 0.01_{\text{TIME}}$ ) and differed also for the three modes of tapping ( $F_{2,38} = 18.7, p < 0.001_{\text{MODE}}$ ). Thus, *post* MVC was weaker after tapping (*vs. pre*), at all modes and sets ( Fig. 4b), and it also was reduced from mode to mode.

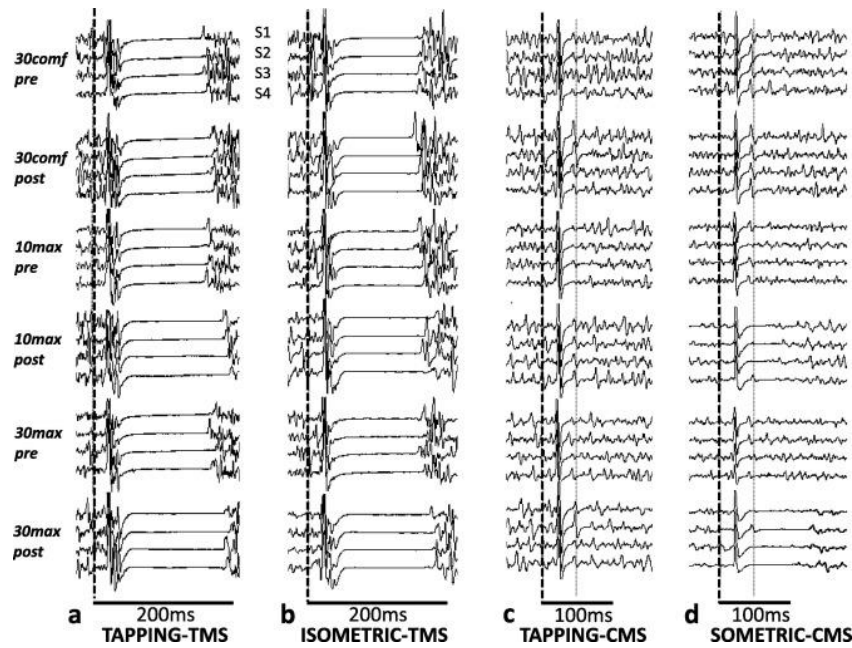
A different matter happened for *iso*; we initially observed that the MVC decrease at *post* was different for the three modes ( $F_{2,38} = 15.9, p < 0.001_{\text{TIME} \times \text{MODE}}$ ; Fig. 4c, d). Then, we followed-up the analyses by modes. The analyses indicated that for *comfort* and *10max* the MVC dropped at *post* and set after set (i.e., factors TIME and SET for these to modes were always significant ( $p < 0.001$ )). This was also the case of *30max*, but in addition, the responses at *pre* and *post* also differed for the different sets ( $F_{3,57} = 4.9, \epsilon = 0.7, p < 0.01_{\text{SET} \times \text{TIME}}$ ) notwithstanding the fact that post hoc *pre vs. post* were always significant ( $p < 0.001$ , see Fig. 4c, d).

Again, in agreement with the other variables described thus far, these responses were not significantly different for the subjects stimulated with TMS or CMS (i.e., factor group did not interact significantly in any case), and comparable levels of fatigue were shown in the two groups for each task.

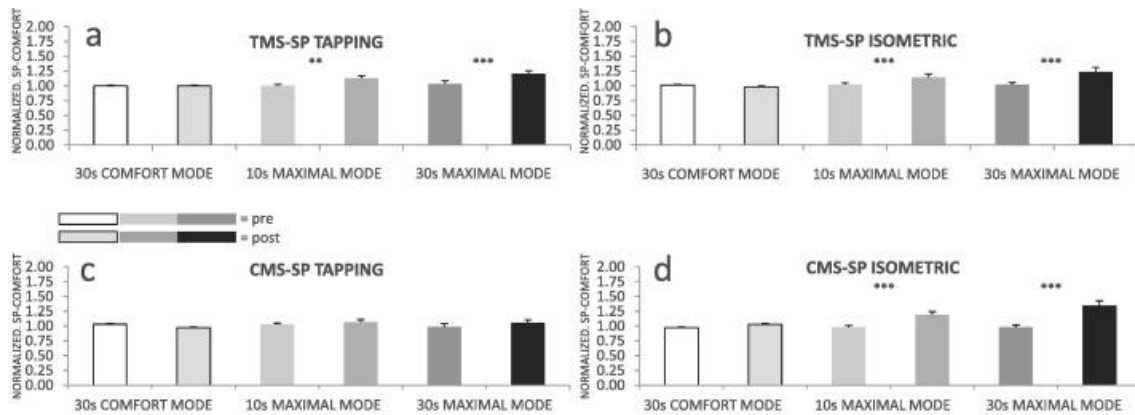
### SP adaptation to maximal *ft* and *iso* tasks

As described in the introduction, the SP duration increases with CF. Therefore, we analyzed if SP duration changed after execution (at *post*); and also if the putative change was different for *ft* and *iso*. In one group of subjects the SP was induced by TMS in the two different task-sessions (*ft* and *iso*); in the other it was induced by CMS in the two sessions. Therefore, in all cases, the task-dependency (*ft vs. iso*) changes in SP were evaluated for the two kinds of stimulation (i.e., in the two groups). As mentioned above, fatigue did not affect differently the motor execution in the two groups and tasks; thus we compared SP's duration evoked by TMS and CMS in the two tasks; to make their baseline duration comparable the durations were normalized. For this purpose the SP duration at *comfort* was considered the reference for each kind of stimulation.

The normalized SP durations increased after execution (at *post*), in a significant different manner for the two task, and for TMS and CMS groups ( $F_{1,19} = 9.4, p < 0.01_{\text{GROUP} \times \text{TASK} \times \text{TIME}}$ ). See Fig. 5 (individual's raw-examples), and Fig. 6. We split the analyses to evaluate the task-dependency effect on the two groups.



**Fig. 5.** Individual's recordings of the SP *pre* and *post* execution. S1...S4 are the sequential recordings of the four sets for a given time of evaluation. TMS-SP increased at *post* after *ft* (a) and after *iso* (b), only at maximal modes. Conversely CMS-SP was unchanged after *ft* in all modes (c), but increased after the maximal modes for the *iso* task, see (d) section. The vertical thick dashed lines indicate the time of stimulation. The vertical thin dashed line lies at the end of the transcortical reflex potential, evoked by CMS. The representation occludes part of the motor evoked potentials, in order to optimize the representation of the SPs.



**Fig. 6.** SPs in response to TMS or CMS during fatiguing motor activities in all subjects. (a) and (b) show that the TMS-SP significantly increased after executing at maximal modes, either 10 or 30 s for *ft* and *iso*. (c) However in no case did the CMS-SP increase after *ft*. (d) CMS-SP significantly increased after 10*max* and 30*max iso*. A significant effect of set never appeared for SP – durations, therefore the four sets are shown pooled. The y-axis expresses the mean duration of the SP in *comfort* mode (pooling all sets and *pre* and *post* values).

For *ft*-task, the SP increase at *post* was significantly different for the CMS and TMS groups ( $F_{1,19} = 11.0$ ,  $p < 0.01_{\text{GROUP} \times \text{TIME}}$ ). Subsequently, the analyses of the TMS-SPs showed a significant increase at the end of execution, which was also different for the execution modes ( $F_{2,16} = 9.1$ ,  $p < 0.01_{\text{MODE} \times \text{TIME}}$ ). We observed that the increase of the TMS-SP was only significant after 10*max* ( $p < 0.01$  post hoc) and 30*max* ( $p < 0.001$  post hoc), not for *comfort*. Conversely the CMS-SP never increased at the end of the *ft* ( $F_{1,11} = 0.7$ ,  $p > 0.05_{\text{TIME}}$ ).

For *iso*-tasks the responses to fatigue of the SP induced by TMS and CMS were never significantly different. For both types of stimulation, the normalized SP increase at the end of the execution was

different in the three modes ( $F_{2,38} = 25.0$ ,  $p < 0.001_{\text{MODE} \times \text{TIME}}$ ); and the analyses by modes of execution indicated that for both the TMS and CMS groups, the SP increased significantly only after *10max* and *30max* (Post-hoc;  $p < 0.001$ , in two cases) (Fig. 5b, d).

Set sequence never had a significant effect on SP ( $p > 0.05$  for main effects or interaction; i.e., in all modes and task).

## Discussion

The main goal of this study was to determine the contribution of some spinal and supraspinal motor circuits to the generation of fatigue during short-lasting repetitive movements (finger tapping), if performed at the fastest possible rate. The site of CF was assessed by means of SPs evoked by TMS and electrical CMS at the time of fatigue within the FDI, a muscle with a more specific role in the tasks of our protocol than the other muscles explored in this work. Then during fatigue protocols, we evaluate SPs allowing no recovery of the neural system after the fatiguing activity (Taylor et al., 2000). While the SP generated by TMS has cortical and spinal components, the one generated by CMS has a purely spinal origin (Fuhr et al., 1991, Inghilleri et al., 1993 and Brasil-Neto et al., 1995). To our knowledge, this is the first study attributing fatigue induced by short-lasting repetitive *ft* activity at the maximal rate to supraspinal structures, while controlling fast forms of fatigue-recovery from the task. Moreover, our results are consistent with a prominent contribution of spinal circuits to CF during isometric MVC (Butler et al., 2003 and Klass et al., 2008), which does not rule out the additional contribution of a supraspinal component (Gandevia et al., 1996, Di Lazzaro et al., 2003, Maruyama et al., 2006, Taylor and Gandevia, 2008 and McNeil et al., 2009).

The decrement in motor output was expressed as a reduction in *ft* frequency, and in force for *iso*, in both cases at the end of *10max* and *30max*; this effect was observed in the two groups of subjects (one group stimulated with TMS, and the other with CMS), without significant differences in their task executions. We consider these changes in motor output as signs of fatigue, as they paralleled known fatigue behavioral markers like the reduction in MVC output right at the end (in continuation) of the fatiguing-tasks. There was also an increase in SP-durations evoked by TMS in both tasks after maximal modes (in a same group of subjects) (Gandevia, 2001 and Taylor and Gandevia, 2001). Remarkably, CMS-SP (tested in a same group of subjects during *ft* and *iso* tasks) increased only after maximal *iso* tasks, and not after *ft* tasks; this is a clear sign of different contribution of spinal cord mechanisms to the development of fatigue during maximal *iso* and *ft*.

For fatigue-induced SP increase, there seems to be a ceiling effect at about 30 s (Taylor et al., 1996 and Taylor and Gandevia, 2001). In line with this, we never detected significant effects of set on SP, although it was present in behavior (MVC) in the case of *iso*.

The analyses of the SP-durations contribute to the understanding of the different origin of fatigue and task dependency (Enoka and Stuart, 1992) for *ft* and *iso* at *maximal* modes. SP to TMS increased immediately at the end of both maximal *ft* and *iso*. However, when in a same group of subjects we evaluated the task-dependency response of SP to CMS, it only increased after *maximal iso* activity, and not after maximal *ft activity*, despite the fact that in these subjects maximal *ft* induced reduction of tapping rate and MVC at the end of the task. CMS-SP emerges from spinal inhibitory mechanisms following motoneuronal excitation, such as after-hyperpolarization and recurrent inhibition (Inghilleri et al., 1993 and Brasil-Neto et al., 1995), whereas the late part of the TMS-SP arises from the activation of inhibitory cortical interneurons projecting to the pyramidal cortico-spinal neurons (Inghilleri et al., 1993), acting via GABA<sub>B</sub> receptors (Ziemann et al., 1993 and Ziemann, 2013). This suggests that fatigue induced by *ft* in our study is not induced by the spinal mechanisms mentioned above, but takes place at supraspinal levels. On the other hand a spinal origin of fatigue seems to be clear for *iso maximal* (Duchateau and Hainaut, 1993, Gandevia et al., 1999, Taylor et al., 1999, Butler et al., 2003 and McNeil et al., 2009), but likely to be present also at a supraspinal level (Di Lazzaro et al., 2003).

### *Maximal voluntary contractions at fatigue time*

At the time of testing, our subjects exhibited a reduction of MVC as an expression of fatigue (Bigland-Ritchie and Woods, 1984 and Gandevia, 2001). The *ft* and *iso* tasks were matched for efforts (*comfort* or *maximal*) and durations (10–30 s). However, it was observed that the different combinations of efforts, durations, and tasks had different impacts on the MVC at the time of fatigue testing, reflecting the fatigue tasks dependencies and suggesting different mechanisms of fatigue. For this reason, it is work for the future to design a protocol to match the drop of MVC after the different tasks (note that this was present in some sets of our protocol).

### *Fatigue accumulation after iso maximal tasks*

For *iso* tasks we observed an accumulation of fatigue set after set expressed in the MVC dropping of force, which was not different for subjects belonging to TMS or CMS groups. However, this effect was not reflected in a set effect for SPs (regardless TMS or CMS); this is in agreement with a previously described ceiling-effect for this variable ( Taylor et al., 1996 and Taylor and Gandevia, 2001). It is possible that some other circuits different to the ones explored in this study, or even peripheral expressions of fatigue, might explain this effect. A sign of peripheral fatigue, such the reduction on the CMAP (a marker of action potential sarcoplasmic propagation), should not be expectable for 30 s of maximal *iso* accordingly to previous studies ( Butler et al., 2003); but the effect of set after set accumulation on the CMAP response is unknown. Admittedly, the objective of this study (SP exploration) does not permit the recording of CMAP at the same time of testing. Notwithstanding that, although the CMAP is essential to normalized motor evoked potential amplitudes in fatiguing conditions, it does not condition the SP durations explained in this study.

### *MVC drop during comfort mode evaluation at the time of fatigue*

MVC testing right at the end of the tasks (*post*) showed that the force dropped in all cases, including *comfort* modes for *ft* and *iso* tasks. This suggests fatigue development even after *comfort* execution. However, the SP durations were not modified *post* for *comfort* modes. The presence of fatigue after *ft* *comfort* execution has been suggested previously ( Teo et al., 2012), but the evaluation was done with the fatigued muscle at rest and using TMS paired-pulse protocols. It is possible that fatigue after *ft* and *iso* *comfort* develops first in cortical circuits un-explored in our protocol. For instance, intracortical inhibitory gabaergic mechanisms acting via GABA<sub>A</sub> receptors ( Werhahn et al., 1999, Di Lazzaro et al., 2000, Hallett, 2007 and Ziemann, 2013), or excitatory glutamatergic circuits responsible for motor evoked potential amplitude. In fact, an involvement of intracortical circuits (GABA<sub>A</sub> receptor mediated) has been suggested previously for the development of fatigue in *ft* tasks ( Teo et al., 2012).

### *Fatigue expressions on rate but not on amplitude for maximal ft tasks*

The subjects were asked to tap “as fast as possible”, with no instructions about angular movement amplitude. In our study, fatigue never induced changes in angular amplitude during the task, and the movement amplitude did not change across execution modes (Rodrigues et al., 2009). This supports the view that the expression of supraspinal fatigue observed during maximal *ft* was directly related to the tapping-rate, i.e. to the number of changes in the finger directions (sequences) per unit of time, and not to their amplitude. Interestingly, we observed that the level of activation of the FDI during maximal rate *ft* is much lower than during *iso* MVC. This reinforces the view that the control of alternating fast sequences is demanding for supraspinal centers even when the level of muscle activation is not very high.

## **Conclusion**

In conclusion, early forms of fatigue during short-lasting repetitive movements at the fastest rate do not originate at the circuits responsible for CMS-SP generation at spinal level. In contrast, short-lasting isometric MVC tasks induce spinal inhibitory mechanisms following motoneuronal excitation, likely explained by recurrent inhibition and after-hyperpolarization in response to the sustained maximal contraction. The fatigue induced by fast-rate repetitive movements possibly involves intracortical inhibitory circuits operating via GABA<sub>B</sub> interneurons, and its main expression is the decrease in tapping frequency.

Fatigue is one of the main signs in diseases of the spinal cord and supraspinal centers. We have provided neurophysiological evidence for different physiological mechanisms in *ft* and *iso*. Our results, if replicated in the future with larger samples, may help to improve the design of the clinical tests aiming at the evaluation of CF in pathological and physiological conditions.

## **Disclosure**

The authors declare no conflict of interests.

## Authors contributions

1. Conception and design of the experiments: P.A., V.R.G., J.C.
2. Collection, analysis and interpretation of data: P.A., V.R.G., Y.C.B., A.M., N.E., J.V., K.G., A.O., J.C.
3. Drafting the article or revising it critically for important intellectual content: P.A., V.R.G., Y.C.B., A.M., N.E., J.V., K.G., A.O., J.C.

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