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Short title: Cortical motor output after electrically induced fatigue

Abstract

Aim: Neuromuscular electrical stimulation (NMES) causes early onset of neuromuscular fatigue. Peripheral electrophysiological explorations suggest that supra-spinal alterations are involved through sensitive afferent pathways. As sensory input is projected over the primary somatosensory cortex (S1), S1 area involvement in inhibiting the central motor drive can be hypothesized. This study assessed cortical activity under a fatiguing NMES protocol at low frequency.

Methods: Twenty healthy males performed five NMES sequences of 17 trains over the plantar flexors (30 Hz, 4s on/6s off). Before and after each sequence, neuromuscular tests composed of maximal voluntary contractions (MVCs) were carried out. Cortical activity was assessed during MVCs with functional near-infrared spectroscopy over S1 and primary motor (M1) areas, through oxy- [HbO] and deoxy-hemoglobin [HbR] variation. Electrophysiological data (H-reflex during MVC, EMG activity and level of voluntary activation) were also recorded.

Results: MVC torque significantly decreased after the first 17 NMES trains ($p<0.001$). The electrophysiological data were consistent with supra-spinal alterations. In addition, [HbO] declined significantly during the protocol over the S1 and M1 areas from the first 17 NMES trains ($p<0.01$ and $p<0.001$, respectively), while [HbR] increased ($p<0.05$ and $p<0.01$, respectively), indicating early decline in cortical activity over both primary cortical areas.

Conclusions: The declining cortical activity over the M1 area is highly consistent with the electrophysiological findings and supports motor cortex involvement in the loss of force after a fatiguing NMES protocol. In addition, the declining cortical activity over the S1 area indicates that the decreased motor output from M1 is not due to increased S1 inhibitory activity.

Key words: Neuromuscular electrical stimulation, supra-spinal fatigue, central motor drive, fNIRS

1.Introduction

Neuromuscular electrical stimulation (NMES) is a peripheral type of solicitation that consists of setting off a muscular contraction electrically by activating intramuscular nerve branches (Hultman et al., 1983). Despite widespread clinical use, NMES presents a strong limitation: it provokes rapid and excessive neuromuscular fatigue (Doucet et al., 2012, Downey et al., 2011). In rehabilitation, low stimulation frequencies are often preferred as they delay the development of neuromuscular fatigue compared with high stimulation frequencies (Gorgey et al., 2009). Therefore, previous investigations of NMES-induced neuromuscular fatigue were conducted at low frequencies, notably over the plantar flexors (Papaiordanidou et al., 2010b) and the thumb abductor muscle (Papaiordanidou et al., 2010a). Opposite effects were reported, depending on the nature of the stimulated muscle: whereas NMES applied over the high fatigue-resistant plantar flexor muscles mainly induced supra-spinal alterations (Papaiordanidou et al., 2010b), the authors failed to report the same effects over the low fatigue-resistant thumb abductor muscle (Papaiordanidou et al., 2010a). In both studies, given the preservation of spinal excitability, the presence or absence of supra-spinal alterations were deduced at the peripheral level through (i) the level of voluntary activation (LOA) and (ii) the root mean square (RMS) of the electromyographic (EMG) activity normalized by the maximal M-wave (RMS_{EMG}/M), two markers that may lead to misinterpretation. Indeed, LOA sometimes overestimates the neural drive (Kooistra et al., 2007), while RMS_{EMG} may underestimate it because of the amplitude cancellation phenomenon (Keenan et al., 2005). Therefore, if based only on the use of peripheral markers, the existence of supra-spinal alterations in electrically induced fatigue remains mainly speculative.

The complementary use of neuroimaging techniques like functional near infrared spectroscopy (fNIRS) or functional magnetic resonance imaging (fMRI) could resolve this issue. Previous studies have reported a decline in cortical activity during volitional fatigue

using fNIRS (Shibuya & Tachi, 2006) and fMRI (Liu et al., 2002), suggesting the relevance of such techniques to explore the neurophysiological mechanisms associated with the fatigue phenomenon.

One might therefore wonder which brain areas would be of interest in a neuroimaging-based investigation of electrically induced neuromuscular fatigue. The primary motor area (M1) is incontestably of relevance in this context as it represents the main motor output from the cortex to the spinal cord. Second, the primary somatosensory cortex (S1) is a candidate as it houses projections from the group III and IV afferents (Almeida et al. 2004; Chen et al. 2009; Kalliomaki et al. 1998; Schouenborg et al. 1986), which are strongly suspected of involvement in the neuromuscular fatigue under NMES (Boerio et al. 2005; Papaiordanidou et al. 2010b). The potential involvement of the S1 area in central motor drive inhibition is supported by several studies reporting increased activity in this area during electrical nerve stimulation (Forss et al., 1994, Hlushchuk & Hari, 2006). Also, the increase in S1 activity was found to occur in parallel to a decrease in primary motor cortex (M1) activity (Chen et al., 1999, Hlushchuk & Hari, 2006) and excitability (Chen et al., 1999, Tokimura et al., 2000). Accordingly, an inhibitory influence exerted by the S1 area toward the M1 area has been hypothesized (Sutherland, 2006).

Besides the potential involvement of the S1 area in decreasing motor output, many studies have reported prefrontal cortex (PFC) deactivation during volitional fatigue just before task failure (Shibuya et al., 2004, Rupp & Perrey, 2008). As the PFC area is the main facilitation structure of the brain (Tanaka & Watanabe, 2012), it seems likely that it would be implicated in non-volitional fatigue, although this has not yet been investigated.

To contribute to a better understanding of the neural substrates of NMES-induced neuromuscular fatigue, the present study aimed to (i) attest the supra-spinal alterations by cortical measurements and (ii) test for S1 area involvement in the decrease in motor output

after a fatiguing NMES task at low frequencies over the plantar flexor muscles. We hypothesized an increase in S1 area activity, a decrease in M1 activity and central motor drive failure after electrically induced neuromuscular fatigue.

2. Materials and methods

2.1. Participants

Twenty healthy males (mean age 23.9 ± 2.4 years) participated in the study. They were all physically active, with no history of lower limb surgery or recent injury and no neurological, endocrine, respiratory, or cardiovascular disease or medication use that could affect brain function or perfusion. After being informed about the objectives and potential risks of participation in the study, they all gave written consent. They were asked to avoid any violent effort during the two days before the experimental session. All procedures were approved by the local Ethics Committee and complied with the principles of the Declaration of Helsinki for human experimentation.

2.2. Pre-experiment

Nine participants were tested in a control session 7 to 10 days prior to the experiment. During this control session, the settings and the protocol were strictly the same as for the experimental session (as detailed below), except that the NMES sequences (fatigue protocol) were replaced by resting periods that lasted 3 minutes.

2.3. Experimental design

The experimental session began with a standard warm-up consisting of 12 submaximal plantar flexions for 2 s every 5 s, and then six maximal plantar flexions every 30 s. The

participants were also familiarized with superimposed evoked potentials at this time. Familiarization to maximal plantar flexions was assumed to be sufficient when task criteria were reached (quick rise in force, maintenance of a plateau, absence of compensatory movements). The protocol began with a 3-min resting period to stabilize the participant's physiological variables. The fatigue protocol consisted of five sequences of 17 stimulation trains of the plantar flexor muscles, each lasting 3 min. Neuromuscular tests were performed before and after each stimulation sequence in order to obtain a follow-up before (pre), during (post17, post34, post51, post68), and after (post85) the protocol. The design is presented in Fig. 1A.

2.4. Fatigue protocol

The plantar flexor muscles of the dominant leg, formed by the gastrocnemii medialis and lateralis and the soleus muscles, were electrically stimulated by a constant-current stimulator (Cefar Physio 4, Cefar Medical, Lund, Sweden) delivering rectangular symmetric, biphasic pulses at maximal tolerated intensity. The two rectangular 50-cm² surface electrodes (Medicompex, Ecublens, Switzerland) were respectively placed 5 cm under the popliteal fossa and at the level of the insertion point of the two gastrocnemii muscles on the Achilles tendon. Train characteristics were the following: frequency 30 Hz, pulse duration 450 μs, duty cycle 40% (4 s on, 6 s off), and intensity: maximal tolerated, readjusted at the beginning of each sequence to reach the maximal tolerated threshold continuously (Maffiuletti, 2010). The mean intensity was 33.83 ± 7.7 mA. The mean percentage of maximal voluntary torque evoked by the stimulation trains was 44.06 ± 13.82%.

2.5. Measurements

2.5.1. Mechanical recording

Plantar flexion torque during voluntary and electrically induced contractions of the dominant leg was recorded by a Biodex System 3 isokinetic dynamometer coupled with the Biodex Advantage Software Package (Medical System Inc., Shirley, NY, USA). The dominant leg was determined using the balance recovery test (Hoffman et al., 1998). The participant was comfortably seated on the Biodex chair in a semi-lying position, with a 30° back inclination. Chair adjustments were made to line up the foot, the patella and the coxofemoral articulation in the same axis. The ankle and knee angles were respectively set to 90° and 110° (180° full extension) and the dynamometer axis was aligned with the anatomical ankle plantar- and dorsi-flexion axis. The pelvis and the proximal extremity of the patella were securely attached to the chair to minimize the movements of adjacent muscles.

2.5.2. EMG recording

The surface EMG signals of the soleus and antagonist tibialis anterior muscles were recorded using bipolar, silver chloride, square surface electrodes with a 9-mm diameter (Contrôle Graphique Médical, Brie-Compte-Robert, France). In order to minimize impedance (<5 kΩ), the skin was shaved, abraded, and cleaned with alcohol. After verification of an appropriate M-wave acquisition (single response and highest amplitude), electrodes were placed over the muscle belly with an interelectrode distance of 20 mm. The reference electrode was placed on the opposite wrist. The EMG signal was amplified (gain × 1000), bandpass-filtered (10-500 Hz) and recorded at a sampling frequency of 4096 Hz (Biopac MP100, Biopac Systems, Santa Barbara, CA, USA).

126 2.5.3. Electrically evoked potentials

127 Muscle excitability was evaluated by the superimposed M-wave (Msup), spinal excitability by
128 the superimposed H-reflex (Hsup), and spinal and supra-spinal adaptations by the volitional
129 wave (V-wave). These three evoked potentials were assessed during plantar flexor maximal
130 voluntary contraction (MVCs) by stimulation of the tibial nerve with a constant-current, high
131 voltage stimulator (DS7AH, Digitimer, Hertforshire, UK). Rectangular monophasic pulses of
132 200 μs were used (Papaiordanidou et al., 2010b). The cathode (a silver chloride square
133 surface electrode) was fixed on the popliteal fossa. A piece of foam, sustained by a strap, was
134 applied over the cathode to optimize contact with the tibial nerve, situated in depth. The anode
135 (a rectangular 50 cm² surface electrode) was placed beneath the patella. After localization of
136 the optimal site for tibial nerve stimulation, where no response could be observed on the
137 antagonist tibialis anterior, the M-wave and H-reflex recruitment curves were performed at
138 rest. One pulse was delivered on the tibial nerve every 10 s with the intensity increasing from
139 10 mA until no further increase in twitch mechanical response and M-wave amplitude
140 occurred. To ensure that M-wave was maximal during MVCs, the maximal intensity at which
141 maximal M-wave amplitude was reached was increased by 20% (supramaximal intensity,
142 I_{Mmax}) to account for activity-dependent changes in motor axonal excitability (Racinais et al.,
143 2013). This intensity was used during MVCs to obtain Msup and V-wave. Subsequently to
144 I_{Mmax} determination, the intensity where the maximum H-reflex was observed was carefully
145 sought (I_{Hmax}) using 2-mA increments. I_{Hmax} was used during MVCs to obtain Hsup.
146
147 2.5.4. Cortical activity recording

148 Cortical activity was assessed with a continuous wave multichannel functional near-infrared
149 spectroscopy (fNIRS) system (Oxymon Mark III, Artinis, the Netherlands) as described
150 elsewhere (Derosiere et al., 2014). Briefly, it is based on neurovascular coupling: when neural

activity increases, the increase in regional cerebral blood flow is ten times higher than the increase in regional oxygen consumption, leading to local hyperoxygenation (Fox et al., 1988), and a subsequent increase in fNIRS-measured oxy-hemoglobin (HbO) with a decrease in fNIRS-measured deoxy-hemoglobin (HbR) (Colier et al., 1999). The fNIRS optodes were positioned over the scalp in accordance with the extended modified international EEG 10-10 systems (Fig. 2). One receptor and four emitters were placed around C_3 and CP_3 (left hemisphere) for right leg stimulation or C_4 and CP_4 (right hemisphere) for left leg stimulation at a 3.5-cm interoptode distance. This probe arrangement allowed us to target the contralateral (to the stimulated dominant leg) primary motor cortex (M1) and the contralateral somatosensory cortex (S1), through the rostral and caudal parts, respectively. Four recording paths were obtained: two over M1 and two over S1. To target the contralateral PFC area, one receptor and one emitter were also placed around Fp1 (left hemisphere) for stimulation of the right leg or Fp2 (right hemisphere) for stimulation of the left leg. fNIRS data were corrected by implementing a specific differential pathlength factor ($4.99 + 0.067 \times age^{0.814}$) (Duncan et al., 1996), which yielded more accurate measurement of the concentration changes, converting the concentration changes in [HbO] and [HbR] to μM units (Delpy et al., 1988). The sampling frequency was set at 10 Hz. The NIRS signals were low-pass filtered (finite impulse response) with a cut-off frequency of 0.7 Hz to remove heart rate signal (Huppert et al., 2009). Specific events were set in the Oxysoft software (V6.0, Artinis, the Netherlands) to distinguish the beginning and the end of each MVC.

2.6. Neuromuscular tests

The neuromuscular tests were conducted during two MVCs with electrically evoked potentials, voluntary activation assessment, and measurement of cerebral oxygenation. Each subject was verbally encouraged during each MVC, which lasted for 4 s. During the plateau

of the first MVC, a single stimulation at I_{Mmax} was first delivered. Immediately after, a superimposed doublet (100Hz) at I_{Mmax} followed, as well as a control doublet 2 s after the relaxation, according to the twitch interpolation technique (Allen et al., 1995). During the plateau of the second MVC, a single stimulation at I_{Hmax} was delivered. The MVCs were separated by a 1-min resting period, during which three resting stimulations at I_{Mmax} were delivered to calculate the mechanical response (Pt). The design of the neuromuscular tests is presented in Fig. 1B.

2.7. Data analysis

The peak of the mechanical response (Pt) during the three resting evoked potentials of the neuromuscular tests was calculated and averaged. The torque evoked by the stimulation trains was recorded and averaged for the first three trains of the first NMES sequence (trains 1-3) and for the last three trains of each NMES sequence (trains 15-17, 32-34, 49-51, 66-68 and 83-85) in order to obtain muscle response to the stimulation trains. For each block of neuromuscular tests, the highest torque plateau of the two MVCs that lasted 500 ms was considered as the maximal voluntary torque. During the first of the two MVCs, the LOA was calculated as follows (Allen et al., 1995):

$$LOA\ (\%) = [1 - (superimposed\ doublet / control\ doublet) \times 100]$$

The RMS of the soleus EMG activity (RMS_{EMG}) was calculated during the maximal torque plateau (as defined above) during each sequence of neuromuscular testing. The peak-to-peak amplitudes of Msup, V-wave and Hsup were calculated. EMG_{RMS}, V-wave and Hsup were normalized with respect to Msup to avoid any influence of peripheral changes on the central parameters: EMG_{RMS}/Msup, Hsup/Msup, V/Msup.

The area under the curve (AUC) of [HbO] and [HbR] was calculated and used as an index of cortical activity (Shimodera et al., 2012). AUC of [HbO] and [HbR] from each channel over

the M1, S1 and PFC areas was calculated and normalized over time from the beginning to the end (labeled with the specific events set in the fNIRS software during recording) of the best of the two MVCs performed during the neuromuscular tests. Data from the two channels over M1 and from the two channels over S1 were then averaged, resulting respectively in an overall response of the M1 and S1 areas.

2.8. Statistical analysis

All statistical analyses were performed using Statistica software (StatSoft, Inc., version 6.0, Tulsa, OK, USA). All variables recorded before, during and after the NMES protocol (or resting periods for pre-experiment check-up) were tested using a one-way repeated measure ANOVA (time effect): before NMES (pre), after 17 trains (post17), after 34 trains (post34), after 51 trains (post51), after 68 trains (post68) and after 85 trains (post85). The underlying assumptions of ANOVA were checked using Skewness-Kurtosis coefficients (normality of distribution) and the Mauchly test (sphericity of variance). When the ANOVA F ratio was significant ($p<0.05$), means were compared by a LSD post-hoc test. Data are reported as means and standard deviation (SD) unless specified.

3. Results

3.1. Pre-experiment check-up

The torque recorded during MVCs did not change significantly throughout the control condition ($F=0.62$, $p=0.68$). Likewise, there was no significant changes nor in LOA neither in evoked potential properties (all $p>0.15$). Furthermore, the cortical activity remained unchanged during the control session over the M1 and S1 areas (all $p>0.31$).

226 3.2. Stimulation trains and maximal voluntary contraction torques

227 The torque evoked during the stimulation trains decreased during the NMES protocol
228 (F=16.2, p<0.001). The decrease was significant from post17 (p<0.01) and remained
229 significant until the end of the fatigue protocol (-8.2 to -14.8% between trains 15-17 to trains
230 83-85; Fig. 3A).

231 The torque produced during MVCs decreased throughout the NMES protocol (F=21.5,
232 p<0.001). The decrease was significant from post17 (p<0.001) and remained significant until
233 the end of the protocol (-10.8 to -19.9% between post34 and post85; Fig. 3B).

234

235 3.3. LOA, mechanical response and evoked potentials

236 Detailed results of the LOA, mechanical response and evoked potentials are given in Table 1.

237 A significant decrease in LOA (F=9.06, p<0.001) was observed immediately after the first 17
238 NMES trains (p<0.01). The Pt values decreased significantly (F=16.28, p<0.001) from post34
239 (p<0.001). Msup amplitude decreased significantly during the NMES protocol (F=12.27,
240 p<0.001) from post17 (p<0.001). EMG_{RMS}/Msup declined significantly during the
241 experimental session (F=3.4, p<0.01) from post34 (p<0.01). V/Msup exhibited a significant
242 decrease (F=2.78, p<0.05) immediately at post17 (p<0.05). Hsup/Msup was not significantly
243 modified (F=1.135, p=0.35).

244

245 3.4. Cortical activity

246 A significant decrease in [HbO] and increase in [HbR] were found over the M1 area during
247 the experimental session (F=8.49, p<0.001 and F=4.03, p<0.01, respectively). These
248 significant modifications of [HbO] and [HbR] occurred at post17 (p<0.001 and p<0.05) and
249 persisted until the end of the protocol (Fig. 4A).

A comparable result ([HbO] decrease and [HbR] increase) was found over the S1 area (F=3.85, $p<0.01$ and F=2.63, $p<0.05$, respectively). The decrease in [HbO] was significant at post 17 ($p<0.05$), while [HbR] increased significantly from post34 ($p<0.01$) (Fig. 4B).

Over the PFC area, [HbO] was 1.17±0.31, 1.3±0.32, 1.27±0.31, 0.8±0.22, 0.97±0.19 and 0.76±0.33 µM at pre, post17, post 34, post51, post68 and post85, respectively, and was not significantly modified (F=1.43, $p=0.24$). [HbR] was -0.13±0.13, -0.06±0.07, -0.04±0.8, -0.03±0.07, -0.16±0.12 and -0.07±0.14 µM at pre, post17, post34, post51, post68 and post85, respectively, and was not significantly modified throughout the experimental session (F=1.14, $p=0.35$).

Figure 5 represents raw data of LOA, Msup, V-wave, Hsup and fNIRS signal from one individual participant at pre and post85.

4. Discussion

The present study aimed to identify the supra-spinal factors involved in neuromuscular fatigue induced by low frequency stimulation of the plantar flexors. The results showed the development of neuromuscular fatigue, as evidenced by the significant MVC torque decrease, which was accompanied by changes occurring at the supra-spinal level. The major finding of the study was an early and progressive decline in cortical activity over the M1 and S1 areas during MVCs following NMES trains.

Since the pre-experiment check-up did not reveal any parameters modifications (maximal voluntary torque, superimposed evoked potential and cortical activity), it can be assert that the NMES protocol caused all the identifying results in the experiment.

The neuromuscular fatigue induced by the NMES protocol was evidenced by the decrease in maximal voluntary torque. This torque alteration may have originated at peripheral, spinal or supra-spinal levels. Regarding the peripheral level, a decrease in Msup and peak twitch amplitude was observed, consistent with neuromuscular excitability and muscle contractility failure, respectively. These results support other studies investigating higher stimulation frequencies (75 Hz) and/or other muscular groups (Boerio et al. 2005; Papaiordanidou et al. 2010a; Zory et al. 2005).

Confirming previous studies (Boerio et al., 2005, Papaiordanidou et al., 2010b), neuromuscular fatigue could not be ascribed to spinal excitability alterations since the Hsup/Msup ratio did not change. Conversely, LOA and EMG_{RMS}/Msup decreased throughout the protocol. The decrease in these peripheral indices of the descending neural drive is consistent with a drop in the central motor drive. In accordance with earlier conclusions on the nature of the CNS alterations under NMES (Boerio et al., 2005, Papaiordanidou et al., 2010b), and because in the present study no modification in spinal excitability was found, the decrease in the central motor drive most probably was derived from the supra-spinal level.

As in a previous study conducted at higher frequencies (Laurin et al., 2012), we observed a V/Msup decrease, a change in a variable that likely reflects both spinal excitability (Crone & Nielsen, 1994) and descending neural drive (Aagaard et al., 2002). Because spinal excitability was not modified, it is assumed that the reported V/Msup decline was instead caused by a decrease in the descending neural drive upstream the spinal level. Thus, the V/Msup kinetic is in line with the involvement of supra-spinal impairments in decreasing the central motor drive under NMES.

Taken individually, electrophysiological parameters like V/Msup, LOA and EMG_{RMS}/Msup cannot provide information on the supra-spinal mechanisms responsible for torque decline. Furthermore, because they are recorded at the peripheral level, the decrease in central motor

drive reflected by these markers, even when normalized over spinal and muscle properties, is based only on an extrapolation. In the current study, we used a new complementary approach to assess supra-spinal alterations by combining the results derived from classical electrophysiological data with neuroimaging. The fNIRS data exhibited a decrease in [HbO] and an increase in [HbR] over the M1 and S1 areas as neuromuscular fatigue developed, a typical sign of decreased neural activity (Wenzel et al., 2000). These results, which corroborate the electrophysiological data, are consistent with an immediate decrease in neural activity from the first NMES sequence. Furthermore, taken together, they provide the first evidence that a cortical motor output decline is implicated in the loss in voluntary force production after NMES.

The PFC area did not exhibit any changes in [HbO] or [HbR] throughout the fatiguing protocol. This area is notably involved in motivation (Kouneiher et al., 2009) and is considered as the main facilitation structure of the brain (Tanaka & Watanabe, 2012). Other studies have reported a decrease in [HbO] within the PFC area during voluntary whole-body exercise just before task failure, suggesting its possible involvement in decreasing neural drive (Rupp & Perrey, 2008, Shibuya et al., 2004). The same pattern could not be found with electrically induced fatigue, thus the decrease in cortical motor output cannot be ascribed to alterations in motivation or facilitation processes from the PFC area during this specific task.

One of the objectives of the study was to better understand the supra-spinal mechanisms responsible for voluntary muscle torque decline after NMES. Our initial hypothesis was that an increase in inhibitory input from the S1 to the M1 area would explain the decrease in cortical motor output. Because the activation of inhibitory interneurons increases local energy consumption (Jueptner & Weiller, 1995), areas involved in the onset of motor output inhibition should appear overactivated. In the current study, both the M1 and S1 areas were deactivated, so it can be assumed that the onset of the inhibition process took place upstream

of these two primary cortical areas. It is thus possible to suggest the involvement of deeper structures. The thalamus, the insular and the cingulate cortex should be considered, as their implication in the inhibition of central motor drive has been clearly suggested during volitional fatigue (Tanaka et al., 2013, Hilty et al., 2011a, Jouanin et al., 2009).

The decrease in cortical activation over the M1 and S1 areas was evident from the very first NMES sequence. Previous studies dealing with voluntary fatigue contractions reported a different activation pattern, showing an initial increase in cortical activity followed by a final decrease in cortical activity near the exhaustion threshold (Liu et al., 2002, Shibuya & Tachi, 2006, Shibuya & Kuboyama, 2010). The early decrease in cortical activity after NMES is in accordance with the characteristics of electrically induced contractions as opposed to voluntary contractions. Here, we suggest that the higher metabolic stress induced by NMES (Jubeau et al., 2012, Theurel et al., 2007) led to an early elevated firing rate of the group III and IV afferents, which in turn caused the recruitment of inhibitory interneurons within the brain to decrease the central motor drive.

Methodological consideration:

We did not assess the gastrocnemius EMG activity. At 110°-knee joint angle, the contribution of the gastrocnemius muscles to plantar flexor torque should be between 30% and 40% (Cresswell et al., 1995; Fukunaga et al., 1992). After the electrically-induced fatigue, we observed a decline in the plantar flexor torque without the possibility to identify the contribution of each muscle in the declining torque. Although we could only report a decline of the soleus EMG_{RMS} activity, a similar decline in the gastrocnemius musles is strongly expected (Papaiordanidou et al. 2010b).

The superimposed evoked potentials during MVCs were elicited on the tibial nerve using stimulation intensities determined at rest. By doing so, activity-dependent changes in motor

axonal excitability during MVCs may have led to unsuitable stimulation intensities. To overcome this issue, the maximal intensity to elicit Msup was increased by 20%. Indeed, it has been shown that the plateau of the maximal superimposed M-wave during MVC is obtained by stimulation of at least 120% of the maximal M-wave determined at rest (Racinais et al., 2013). Conversely, we did not increase the submaximal intensity to elicit superimposed H-waves during MVCs. By using intensity determined at rest, the superimposed H-waves obtained in the study were presumably not maximal but were rather on the ascending part of the recruitment curve (Racinais et al., 2013). Yet, it has been shown that the reliability of the superimposed H-reflex is higher when assessed in the ascending part of the recruitment curve (Grospretre & Martin, 2012).

LOA and superimposed evoked potentials are subjects to variability and were recorded only once during each neuromuscular tests session. However, no changes were found in the control condition for any recorded parameter, supporting that NMES rather than variability was likely responsible for LOA and superimposed evoked potentials changes.

5. Conclusion

In conclusion, the present study provides evidence that a cortical motor output decrease is involved in the loss of force after NMES at low frequencies. The decrease in central motor drive was demonstrated from the very first NMES sequence by the consistency between the kinetics of the electrophysiological and the fNIRS-measured hemodynamics data over the M1 area. The fact that the S1 activity also decreased after NMES indicates that S1 is not involved in the inhibition of the cortical motor output during this specific task.

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376

377 **Conflict of interest**

378 No conflict of interest, financial or otherwise, is declared by the authors.

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Tables:

Table 1. EMG activity, voluntary activation level, superimposed evoked potentials and mechanical responses.

	Pre	Post 17	Post 34	Post 51	Post 68	Post 85
LOA (%)	93.33 (1.31)	87.96 (1.82)**	87.69 (1.97)***	86.27 (2.03)***	84.3 (2.81)***	83.5 (2.59)***
Pt (Nm)	21.53 (1.1)	21.38 (1.08)	20.23 (0.96)***	19.92 (0.95)***	19.44 (0.88)***	19.28 (0.81)***
Msup (mV)	10.9 (1)	9.13 (0.91)***	9.13 (0.91)***	8.63 (0.88)***	8.82 (0.9)***	8.27 (0.73)***
EMG_{RMS}/Msup	0.0224 (0.002)	0.0223 (0.002)	0.0198 (0.001)*	0.0204 (0.001)**	0.0198 (0.001)*	0.0203 (0.001)*
Hsup/Msup	0.398 (0.03)	0.455 (0.046)	0.414 (0.036)	0.396 (0.034)	0.355 (0.036)	0.413 (0.038)
V/Msup	0.321 (0.025)	0.237 (0.031)*	0.252 (0.027)*	0.218 (0.038)**	0.227 (0.031)**	0.220 (0.035)**

Values are mean (SE). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ significantly different from pre values.

Figure:

Fig. 1. Schematic representation of the experimental session. (A) Experimental design. (B) Decomposition of a sequence of neuromuscular tests performed after each sequence of trains. N.T: Neuromuscular Tests. Details on resting evoked potentials are given in *electrically evoked potentials* section.

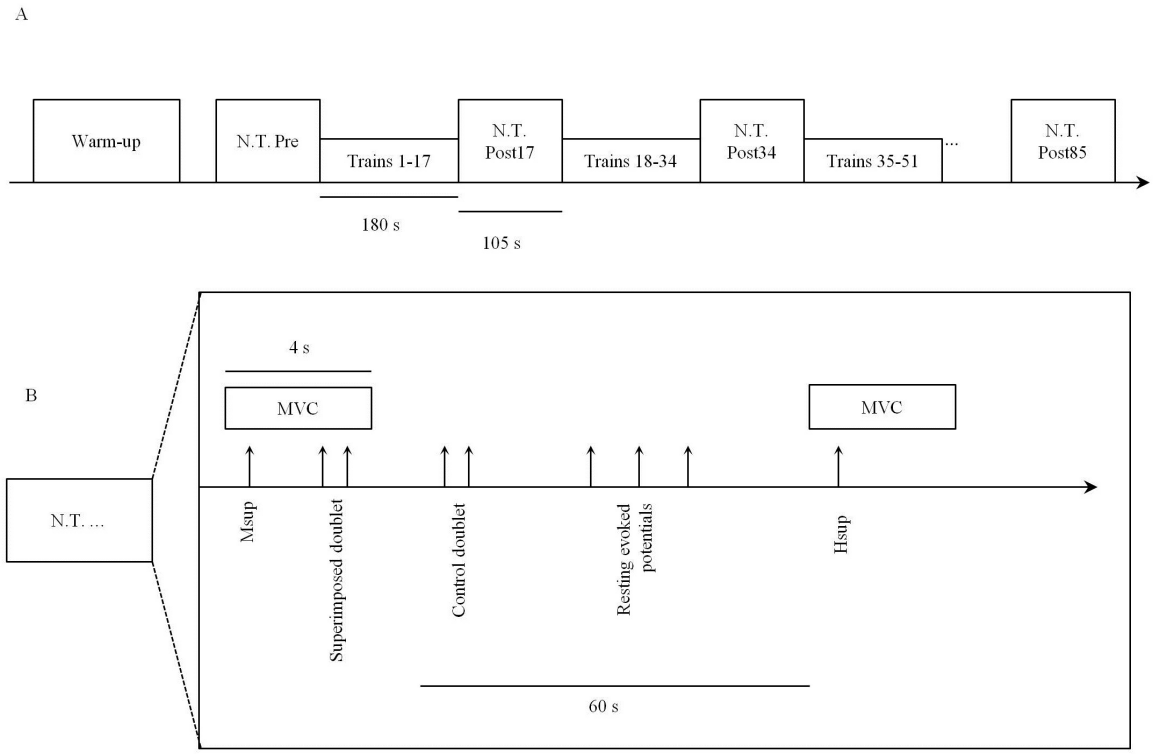

Fig. 2. Schematic representations of the placement of the fNIRS optodes. The emitors (E) were placed at 3.5 cm from the receptor (R). The optodes were positioned according to the extended EEG 10-20 system: around C_3 and CP_3 (or C_4 and CP_4) to target the primary motor and somatosenry cortex, around CP_1 (or CP_2) to target the prefrontal cortex. C_z is situated at the middle of the distance between the nasion and the inion. CP_z is situated at 10% of the distance between the nasion and the inion. The optodes were placed on the contralateral side.

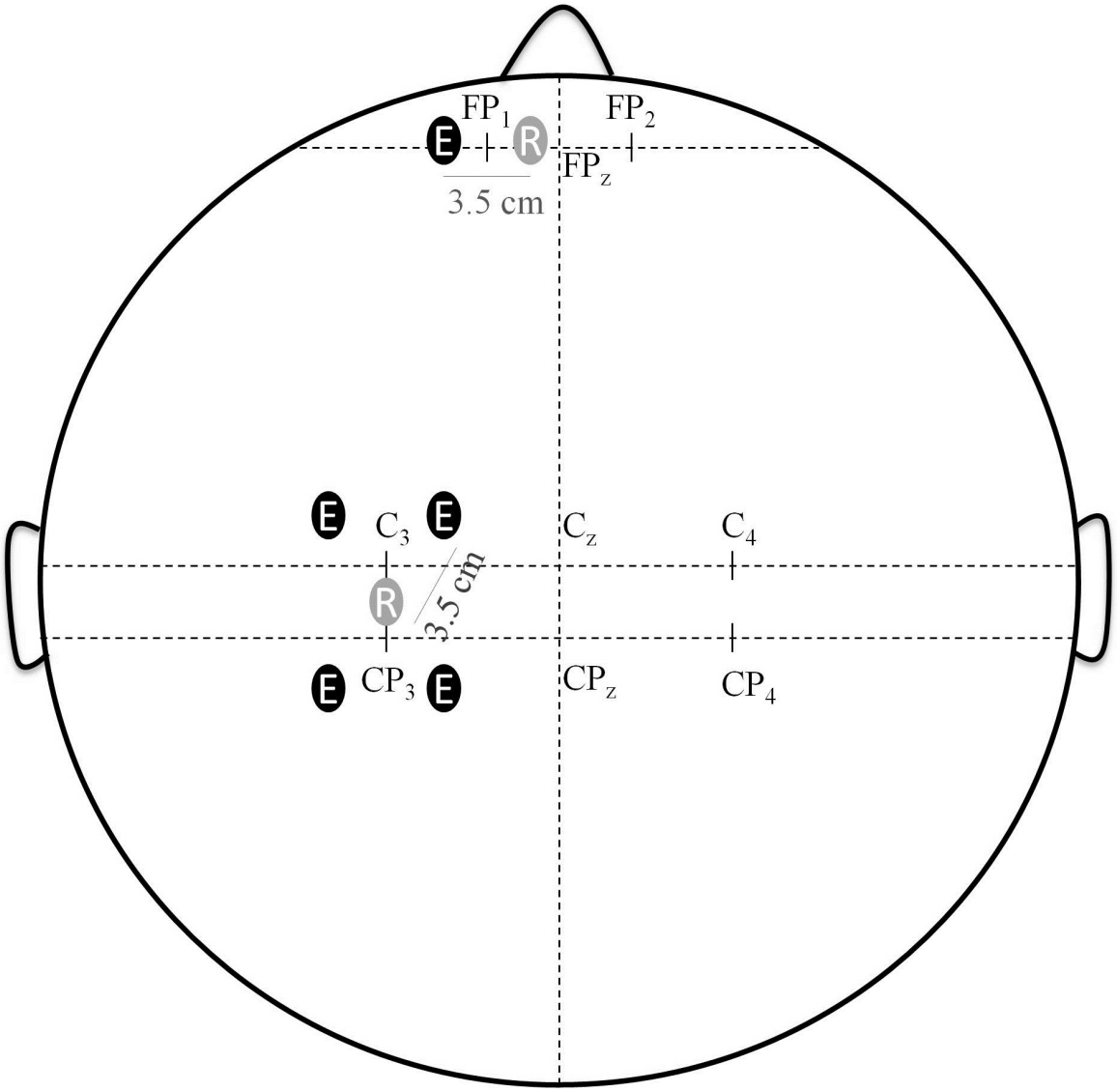

Fig. 3. (A) Torque evoked by the NMES trains. (B) Torque produced during maximal voluntary contractions. ** $p < 0.01$ and *** $p < 0.001$ significantly different from pre values.

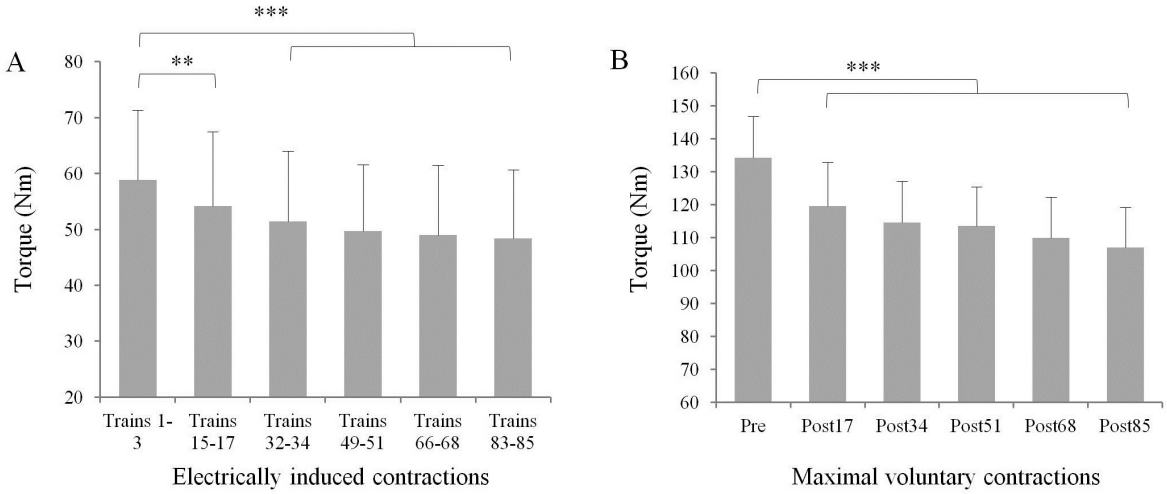

Fig. 4. (A) Primary motor cortex oxygenation. (B) Primary somatosensory cortex oxygenation. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ significantly different from pre values. Vertical bars represent SE.

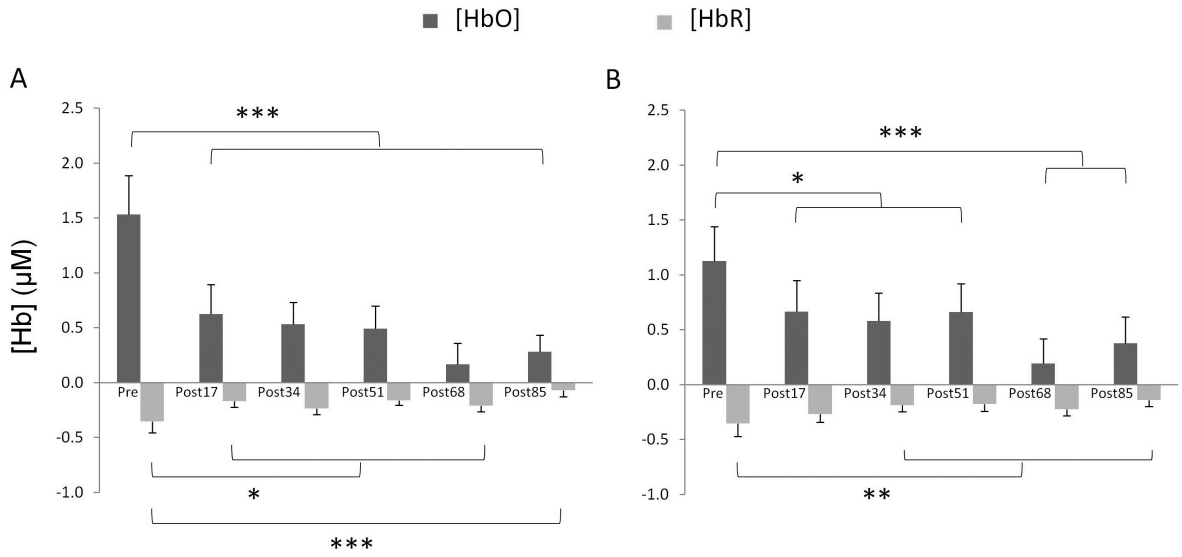

Fig. 5. Typical response of one subject for superimposed evoked potentials during maximal voluntary contractions (Msup, V-wave and Hsup), level of voluntary activation (twitch-like increment in torque) and cortical activity changes over the M1 area (HbO and HbR), before (pre) and after NMES (post85). Arrows indicate tibial nerve stimulation.