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Cortical motor output decreases after neuromuscular fatigue induced by electrical stimulation of the plantar flexor muscles

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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 **1.Introduction**

2 Neuromuscular electrical stimulation (NMES) is a peripheral type of solicitation that consists
3 of setting off a muscular contraction electrically by activating intramuscular nerve branches
4 (Hultman et al., 1983). Despite widespread clinical use, NMES presents a strong limitation: it
5 provokes rapid and excessive neuromuscular fatigue (Doucet et al., 2012, Downey et al.,
6 2011). In rehabilitation, low stimulation frequencies are often preferred as they delay the
7 development of neuromuscular fatigue compared with high stimulation frequencies (Gorgey
8 et al., 2009). Therefore, previous investigations of NMES-induced neuromuscular fatigue
9 were conducted at low frequencies, notably over the plantar flexors (Papaiordanidou et al.,
10 2010b) and the thumb abductor muscle (Papaiordanidou et al., 2010a). Opposite effects were
11 reported, depending on the nature of the stimulated muscle: whereas NMES applied over the
12 high fatigue-resistant plantar flexor muscles mainly induced supra-spinal alterations
13 (Papaiordanidou et al., 2010b), the authors failed to report the same effects over the low
14 fatigue-resistant thumb abductor muscle (Papaiordanidou et al., 2010a). In both studies, given
15 the preservation of spinal excitability, the presence or absence of supra-spinal alterations were
16 deduced at the peripheral level through (i) the level of voluntary activation (LOA) and (ii) the
17 root mean square (RMS) of the electromyographic (EMG) activity normalized by the
18 maximal M-wave ($RMS_{EMG/M}$), two markers that may lead to misinterpretation. Indeed, LOA
19 sometimes overestimates the neural drive (Kooistra et al., 2007), while RMS_{EMG} may
20 underestimate it because of the amplitude cancellation phenomenon (Keenan et al., 2005).
21 Therefore, if based only on the use of peripheral markers, the existence of supra-spinal
22 alterations in electrically induced fatigue remains mainly speculative.

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

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

29 One might therefore wonder which brain areas would be of interest in a neuroimaging-based
30 investigation of electrically induced neuromuscular fatigue. The primary motor area (M1) is
31 incontestably of relevance in this context as it represents the main motor output from the
32 cortex to the spinal cord. Second, the primary somatosensory cortex (S1) is a candidate as it
33 houses projections from the group III and IV afferents (Almeida et al. 2004; Chen et al. 2009;
34 Kalliomaki et al. 1998; Schouenborg et al. 1986), which are strongly suspected of
35 involvement in the neuromuscular fatigue under NMES (Boerio et al. 2005; Papaiordanidou et
36 al. 2010b). The potential involvement of the S1 area in central motor drive inhibition is
37 supported by several studies reporting increased activity in this area during electrical nerve
38 stimulation (Forss et al., 1994, Hlushchuk & Hari, 2006). Also, the increase in S1 activity was
39 found to occur in parallel to a decrease in primary motor cortex (M1) activity (Chen et al.,
40 1999, Hlushchuk & Hari, 2006) and excitability (Chen et al., 1999, Tokimura et al., 2000).
41 Accordingly, an inhibitory influence exerted by the S1 area toward the M1 area has been
42 hypothesized (Sutherland, 2006).

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

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

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

54

55 **2. Materials and methods**

56

57 2.1. Participants

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

66

67 2.2. Pre-experiment

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

72

73 2.3. Experimental design

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

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

85

86 2.4. Fatigue protocol

87 The plantar flexor muscles of the dominant leg, formed by the gastrocnemii medialis and
88 lateralis and the soleus muscles, were electrically stimulated by a constant-current stimulator
89 (Cefar Physio 4, Cefar Medical, Lund, Sweden) delivering rectangular symmetric, biphasic
90 pulses at maximal tolerated intensity. The two rectangular 50-cm² surface electrodes
91 (Medicomplex, Ecublens, Switzerland) were respectively placed 5 cm under the popliteal fossa
92 and at the level of the insertion point of the two gastrocnemii muscles on the Achilles tendon.
93 Train characteristics were the following: frequency 30 Hz, pulse duration 450 μ s, duty cycle
94 40% (4 s on, 6 s off), and intensity: maximal tolerated, readjusted at the beginning of each
95 sequence to reach the maximal tolerated threshold continuously (Maffiuletti, 2010). The mean
96 intensity was 33.83 ± 7.7 mA. The mean percentage of maximal voluntary torque evoked by
97 the stimulation trains was $44.06 \pm 13.82\%$.

98

99

100

101 2.5. Measurements

102

103 2.5.1. Mechanical recording

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

114

115 2.5.2. EMG recording

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

125

126 2.5.3. Electrically evoked potentials

127 Muscle excitability was evaluated by the superimposed M-wave (M_{sup}), spinal excitability by
128 the superimposed H-reflex (H_{sup}), 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, Hertfordshire, UK). Rectangular monophasic pulses of
132 200 μ s 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 M_{sup} 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 H_{sup} .

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

151 activity increases, the increase in regional cerebral blood flow is ten times higher than the
152 increase in regional oxygen consumption, leading to local hyperoxygenation (Fox et al.,
153 1988), and a subsequent increase in fNIRS-measured oxy-hemoglobin (HbO) with a decrease
154 in fNIRS-measured deoxy-hemoglobin (HbR) (Colier et al., 1999). The fNIRS optodes were
155 positioned over the scalp in accordance with the extended modified international EEG 10-10
156 systems (Fig. 2). One receptor and four emitters were placed around C₃ and CP₃ (left
157 hemisphere) for right leg stimulation or C₄ and CP₄ (right hemisphere) for left leg stimulation
158 at a 3.5-cm interoptode distance. This probe arrangement allowed us to target the contralateral
159 (to the stimulated dominant leg) primary motor cortex (M1) and the contralateral
160 somatosensory cortex (S1), through the rostral and caudal parts, respectively. Four recording
161 paths were obtained: two over M1 and two over S1. To target the contralateral PFC area, one
162 receptor and one emitter were also placed around Fp1 (left hemisphere) for stimulation of the
163 right leg or Fp2 (right hemisphere) for stimulation of the left leg. fNIRS data were corrected
164 by implementing a specific differential pathlength factor ($4.99 + 0.067 \times \text{age}^{0.814}$) (Duncan et
165 al., 1996), which yielded more accurate measurement of the concentration changes,
166 converting the concentration changes in [HbO] and [HbR] to μM units (Delpy et al., 1988).
167 The sampling frequency was set at 10 Hz. The NIRS signals were low-pass filtered (finite
168 impulse response) with a cut-off frequency of 0.7 Hz to remove heart rate signal (Huppert et
169 al., 2009). Specific events were set in the Oxysoft software (V6.0, Artinis, the Netherlands) to
170 distinguish the beginning and the end of each MVC.

171

172 2.6. Neuromuscular tests

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

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

183

184 2.7. Data analysis

185 The peak of the mechanical response (Pt) during the three resting evoked potentials of the
186 neuromuscular tests was calculated and averaged. The torque evoked by the stimulation trains
187 was recorded and averaged for the first three trains of the first NMES sequence (trains 1-3)
188 and for the last three trains of each NMES sequence (trains 15-17, 32-34, 49-51, 66-68 and
189 83-85) in order to obtain muscle response to the stimulation trains. For each block of
190 neuromuscular tests, the highest torque plateau of the two MVCs that lasted 500 ms was
191 considered as the maximal voluntary torque. During the first of the two MVCs, the LOA was
192 calculated as follows (Allen et al., 1995):

$$193 \quad LOA (\%) = [1 - (\textit{superimposed doublet}/\textit{control doublet}) \times 100]$$

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

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

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

206

207 2.8. Statistical analysis

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

217

218 **3. Results**

219

220 3.1. Pre-experiment check-up

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

225

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}/M_{sup} declined significantly during the
241 experimental session ($F=3.4$, $p<0.01$) from post34 ($p<0.01$). V/M_{sup} exhibited a significant
242 decrease ($F=2.78$, $p<0.05$) immediately at post17 ($p<0.05$). H_{sup}/M_{sup} 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).

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

253 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
254 0.76 ± 0.33 μM at pre, post17, post 34, post51, post68 and post85, respectively, and was not
255 significantly modified ($F=1.43$, $p=0.24$). [HbR] was -0.13 ± 0.13 , -0.06 ± 0.07 , -0.04 ± 0.8 , -
256 0.03 ± 0.07 , -0.16 ± 0.12 and -0.07 ± 0.14 μM at pre, post17, post34, post51, post68 and post85,
257 respectively, and was not significantly modified throughout the experimental session ($F=1.14$,
258 $p=0.35$).

259

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

262

263 **4. Discussion**

264

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

271

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

275 The neuromuscular fatigue induced by the NMES protocol was evidenced by the decrease in
276 maximal voluntary torque. This torque alteration may have originated at peripheral, spinal or
277 supra-spinal levels. Regarding the peripheral level, a decrease in M_{sup} and peak twitch
278 amplitude was observed, consistent with neuromuscular excitability and muscle contractility
279 failure, respectively. These results support other studies investigating higher stimulation
280 frequencies (75 Hz) and/or other muscular groups (Boerio et al. 2005; Papaiordanidou et al.
281 2010a; Zory et al. 2005).

282 Confirming previous studies (Boerio et al., 2005, Papaiordanidou et al., 2010b),
283 neuromuscular fatigue could not be ascribed to spinal excitability alterations since the
284 H_{sup}/M_{sup} ratio did not change. Conversely, LOA and EMG_{RMS}/M_{sup} decreased throughout
285 the protocol. The decrease in these peripheral indices of the descending neural drive is
286 consistent with a drop in the central motor drive. In accordance with earlier conclusions on
287 the nature of the CNS alterations under NMES (Boerio et al., 2005, Papaiordanidou et al.,
288 2010b), and because in the present study no modification in spinal excitability was found, the
289 decrease in the central motor drive most probably was derived from the supra-spinal level.

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

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

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

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

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

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

329 The decrease in cortical activation over the M1 and S1 areas was evident from the very first
330 NMES sequence. Previous studies dealing with voluntary fatigue contractions reported a
331 different activation pattern, showing an initial increase in cortical activity followed by a final
332 decrease in cortical activity near the exhaustion threshold (Liu et al., 2002, Shibuya & Tachi,
333 2006, Shibuya & Kuboyama, 2010). The early decrease in cortical activity after NMES is in
334 accordance with the characteristics of electrically induced contractions as opposed to
335 voluntary contractions. Here, we suggest that the higher metabolic stress induced by NMES
336 (Jubeau et al., 2012, Theurel et al., 2007) led to an early elevated firing rate of the group III
337 and IV afferents, which in turn caused the recruitment of inhibitory interneurons within the
338 brain to decrease the central motor drive.

339

340 Methodological consideration:

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

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

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

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

364

365 **5. Conclusion**

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

372

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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.

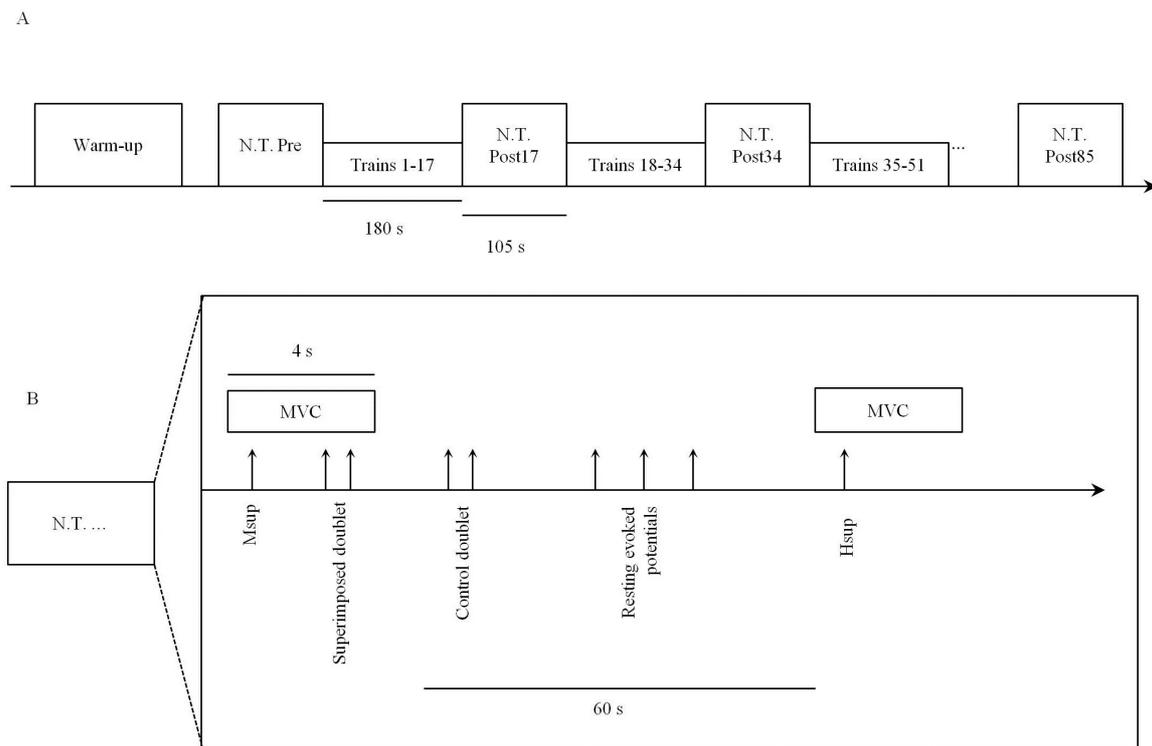


Fig. 2. Schematic representations of the placement of the fNIRS optodes. The emitters (E) were placed at 3.5 cm from the receptor (R). The optodes were positioned according to the extended EEG 10-20 system: around C₃ and CP₃ (or C₄ and CP₄) to target the primary motor and somatosensory cortex, around CP₁ (or CP₂) to target the prefrontal cortex. C_Z is situated at the middle of the distance between the nasion and theinion. CP_Z is situated at 10% of the distance between the nasion and theinion. The optodes were placed on the contralateral side.

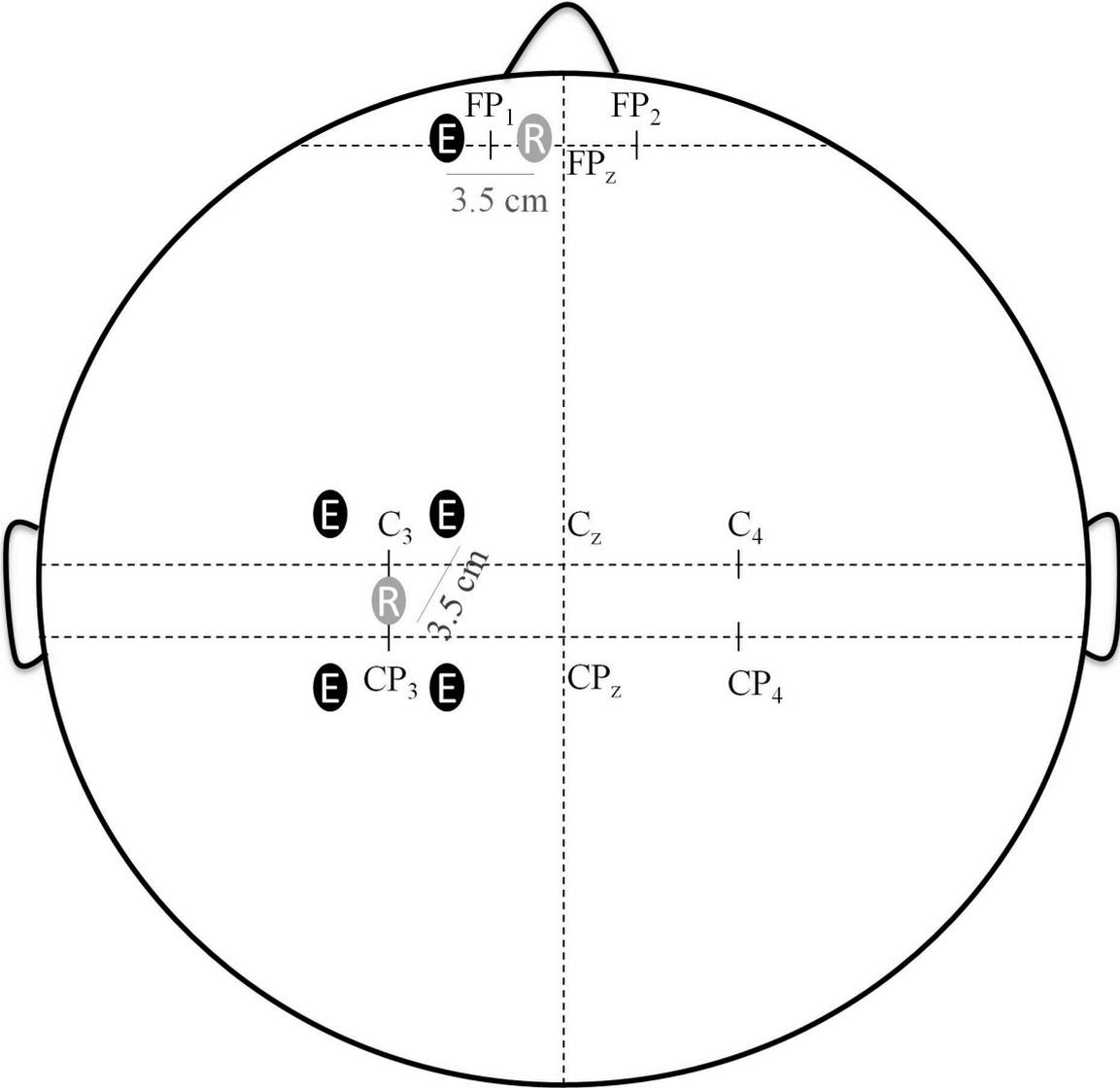


Fig. 3. (A) Torque evoked by the NMES trains. (B) Torque produced during maximal voluntary contractions. ** $p < 0.01$ and *** $p < 0.001$ significantly different from pre values.

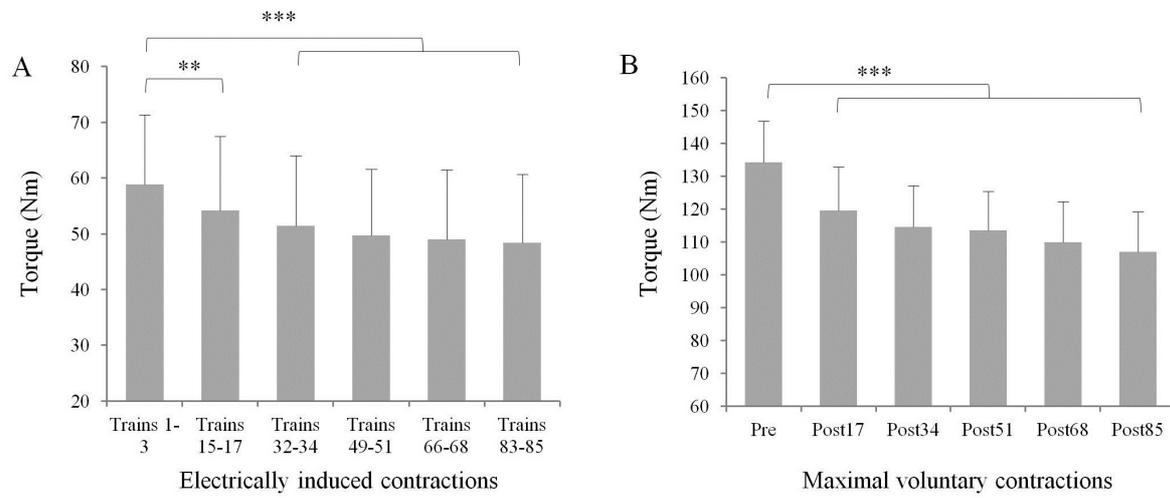


Fig. 4. (A) Primary motor cortex oxygenation. (B) Primary somatosensory cortex oxygenation. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ significantly different from pre values.

Vertical bars represent SE.

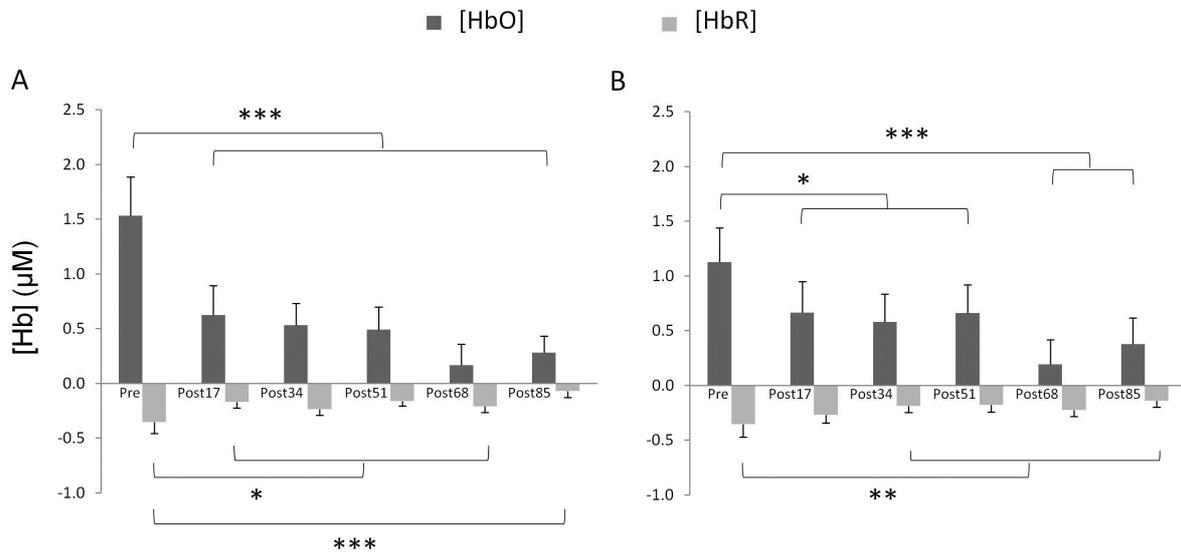


Fig. 5. Typical response of one subject for superimposed evoked potentials during maximal voluntary contractions (Msup, V-wave and Hsup), level of voluntary activation (twitch-like increment in torque) and cortical activity changes over the M1 area (HbO and HbR), before (pre) and after NMES (post85).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

