

# Unravelling the effect of experimental pain on the corticomotor system using transcranial magnetic stimulation and electroencephalography

Marylie Martel<sup>1,2</sup>, Marie-Philippe Harvey<sup>1,2</sup>, Francis Houde<sup>1,2</sup>, Frédéric Balg<sup>1,3</sup>, Philippe Goffaux<sup>1,3</sup> and Guillaume Léonard<sup>1,2</sup>

<sup>1</sup> Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4; <sup>2</sup> Research Centre on Aging, CIUSSS de l'Estrie-CHUS, Sherbrooke, Quebec, Canada, J1H 4C4; <sup>3</sup> Department of Surgery, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4

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**Corresponding author:** Guillaume Léonard, P.T., Ph.D.  
Research Centre on Aging  
CIUSSS de l'Estrie-CHUS  
1036, rue Belvédère Sud  
Sherbrooke (Québec) J1H 4C4  
Telephone: 819-780-2220, ext. 45246  
Fax: 819-820-6864  
Email: [guillaume.leonard2@usherbrooke.ca](mailto:guillaume.leonard2@usherbrooke.ca)

1 **Abstract**

2 The interaction between pain and the motor system is well-known. For instance, past studies  
3 have shown that pain can alter corticomotor excitability and have deleterious effects on motor  
4 learning. The aim of this study was to better understand the cortical mechanisms underlying the  
5 interaction between pain and the motor system. Experimental pain was induced on 19 young  
6 and healthy participants using capsaicin cream, applied on the middle volar part of the left  
7 forearm. The effect of pain on brain activity and on the corticomotor system was assessed with  
8 electroencephalography (EEG) and transcranial magnetic stimulation (TMS), respectively.  
9 Compared to baseline, resting state brain activity significantly increased after capsaicin  
10 application in the central cuneus (theta frequency), left dorsolateral prefrontal cortex (alpha  
11 frequency), and left cuneus and right insula (beta frequency). A pain-evoked increase in the right  
12 primary motor cortex (M1) activity was also observed (beta frequency), but only among  
13 participants who showed a reduction in corticospinal output (as depicted by TMS recruitment  
14 curves). These participants further showed greater beta M1-cuneus connectivity than the other  
15 participants. These findings indicate that pain-evoked increases in M1 beta power are intimately  
16 tied to changes in the corticospinal system, and provide evidence that beta M1-cuneus  
17 connectivity is related to the corticomotor alterations induced by pain. The differential pattern  
18 of response observed in our participants suggest that the effect of pain on the motor system is  
19 variable from on individual to another; an observation that could have important clinical  
20 implications for rehabilitation professionals working with pain patients.

21 **Introduction**

22 Pain is a rapidly growing area of research, and the last years have shown huge advancement in  
23 our understanding of its neurophysiological process. The development of neuroimaging  
24 techniques have led to the discovery that pain perception is intimately linked to the activation of  
25 a complex cerebral network comprised, among other things, of the primary somatosensory  
26 cortex (S1) and the secondary somatosensory cortex (S2), the anterior cingulate cortex (ACC)  
27 and the insula (IC) (Apkarian et al., 2005, Forster and Handwerker, 2014, Nakata et al., 2014).

28

29 A few neuroimaging studies have also reported an increase in the activity of the primary motor  
30 cortex (M1) in the presence of experimental pain (Apkarian et al., 2000, Tracey et al., 2000,  
31 Burns et al., 2016). A few years ago, Stancák et al. demonstrated, using electroencephalography  
32 (EEG), that the application of a short-lasting painful heat stimuli on the hand decreased the  $\beta$   
33 activity of the sensorimotor cortex (Stancák et al., 2007). Given the inhibitory role that  $\beta$  waves  
34 have on the motor cortex (Pogosyan et al., 2009), the decrease in M1  $\beta$  activity noted by Stancák  
35 and colleagues suggests that the presence of a brief nociceptive stimulus could prime the motor  
36 brain regions (reduction of the inhibition), possibly to facilitate motor withdrawal responses. As  
37 pointed out, the results obtained by Stancák and colleagues were obtained following the  
38 application of brief/escapable, nociceptive stimuli and it remains uncertain whether the same  
39 pattern of results would be obtained with longer/unavoidable nociceptive stimulations.

40

41 The observations made with neuroimaging techniques are consistent with the results of studies  
42 performed with transcranial magnetic stimulation (TMS). TMS studies have shown that  
43 experimental pain stimulation can alter the excitability of the corticomotor system (Farina et al.,  
44 2001, Valeriani et al., 2001). However, contrary to the study by Stancák et al. (that suggest a

45 priming of the motor cortex in the presence of pain), TMS studies generally report reduced  
46 corticospinal excitability following nociceptive stimuli (Boudreau et al., 2007, Mercier and  
47 Leonard, 2011, Schabrun and Hodges, 2012, Schabrun et al., 2013, Rittig-Rasmussen et al.,  
48 2014). Some researchers have suggested that these corticomotor effects could explain the  
49 negative impact that pain can have on motor learning (Boudreau et al., 2007, Rittig-Rasmussen  
50 et al., 2014). Supporting this are the results of Rittig-Rasmussen et al. (Rittig-Rasmussen et al.,  
51 2014) who have observed that the change in corticospinal excitability (increased motor-evoked  
52 potential [MEP] amplitudes) noted following upper trapezius training was completely blocked by  
53 a hypertonic muscle saline injection, with the effect being apparent up to 7 days post-training.

54

55 Interestingly, several neuroimaging and neurostimulation studies have shown that patients  
56 suffering from clinical pain conditions show changes in cortical representation at the M1 level.  
57 For example, in patients suffering from complex regional pain syndrome (CRPS) and from  
58 phantom limb pain, researchers have reported reduced cortical representation of the affected  
59 limb (Karl et al., 2001, Krause et al., 2006). Although compelling, these studies remain  
60 correlational and it is impossible to know if the neuroplastic changes in M1 are *directly* caused  
61 by pain. The use of an experimental pain paradigm, in which the researchers can manipulate the  
62 presence of pain, would make it possible to address this question and determine whether pain is  
63 causally linked to corticomotor changes.

64

65 In this study, TMS and EEG were used concomitantly to better understand the effect of pain on  
66 the motor system. More specifically, the objectives were to evaluate the effect of a  
67 prolonged/inescapable nociceptive stimulation on TMS recruitment curves (a measure believed  
68 to reflect the strength of the corticospinal projections (Devanne et al., 1997, Abbruzzese and

69 Trompetto, 2002)) and on the pattern of EEG activity of the motor brain regions. A second  
70 objective was to determine if these potential changes in the TMS recruitment curve and EEG  
71 activity could be related to changes in functional connectivity between M1 and other brain  
72 regions implicated in the perception of pain.

73

74

## 75 **Materials and Methods**

### 76 *Participants*

77 Nineteen healthy, right handed adults (12 women and 7 men; mean age:  $29 \pm 7$  years old)  
78 participated in the study. To be included in the study, participants had to be aged over 18 years  
79 and be pain-free (absence of painful health condition and no pain upon testing). For security  
80 reasons, individuals with neurological disorders, metal implants in the skull, a pacemaker or  
81 neurostimulator, epilepsy or pregnant were excluded from the study. Participants were asked to  
82 refrain from consuming caffeine for six hours before testing, and tobacco products for two hours  
83 before testing. The research protocol was approved by the ethics committee of the Research  
84 Centre on Aging (Sherbrooke, Quebec, Canada) and each participant provided informed written  
85 consent before participating in the study.

86

### 87 *Transcranial magnetic stimulation (TMS)*

88 Magnetic stimuli were delivered by a 70 mm figure-eight coil connected to a Magstim 200  
89 (Magstim Co., Dyfed, UK). Participants sat in a comfortable chair and two Ag/AgCl surface  
90 recording electrodes (1 cm<sup>2</sup> recording area) were positioned over their left first dorsal  
91 interosseous (FDI) muscle to record motor-evoked potentials (MEP). Electromyographic signals,  
92 elicited by the magnetic stimuli, were amplified and filtered (bandwidth, 200 Hz to 2 kHz) with a

93 CED 1902 amplifier (Cambridge Electronic Design Limited, Cambridge, UK), and digitized at a  
94 sampling rate of 10 kHz using a Power 1401 mk II interface and Spike 2 software (version 7.10;  
95 Cambridge Electronic Design Limited, Cambridge, UK).

96

97 With the coil held  $\sim 45^\circ$  in the mid-sagittal plane, the approximate location of the FDI muscle on  
98 the right hemisphere was explored in 1-cm step until reliable MEP could be evoked in the FDI.  
99 The optimal location for eliciting MEP in the FDI was found (hotspot). This site was then marked  
100 on the scalp of the participants with a marker to ensure consistent coil positioning. Throughout  
101 the experiment, the experimenter frequently reassessed the coil position to ensure that it  
102 remained over the optimal stimulation site. At this point, stimulations of varying intensities were  
103 sent to determine the resting motor threshold (rMT), defined for each participant as the  
104 minimal intensity of stimulation capable of eliciting MEPs of at least 50  $\mu\text{V}$  in 50% of the trials  
105 with the FDI at rest (no muscle contraction). Then, 4 blocks of 10 stimulations were provided  
106 randomly to participants (delay between each stimulation = 5 to 8 sec), with the stimulation in  
107 each block given at the same intensity (i.e., 90, 110, 130, and 150 % of rMT). The peak-to-peak  
108 amplitude of MEP responses were measured off-line and averaged for each participant to derive  
109 mean values. The slope of the recruitment curve (describing the relationship between MEP  
110 amplitude and TMS intensity) was then calculated using hierarchical linear modeling (HLM)  
111 (Roberts et al., 2010).

112

### 113 *Electroencephalography (EEG)*

114 EEG activity was recorded at rest using a 32-channel EEG acquisition system (Brain Products  
115 GmbH, Munich, Germany) with electrodes positioned according to the international 10-20  
116 system. Data were recorded at 500 Hz for 5 minutes in each condition using FCz reference and

117 keeping all electrode impedances below 5 k $\Omega$ . Eye blinks and motion artifacts were removed  
118 from the data using independent component analysis (ICA) denoising (Brain Vision Analyzer,  
119 Brain Products GmbH, Munich, Germany). Data were then re-referenced to the common  
120 average.

121

122 For each participant, 15 non-overlapping, 2-second segments without artifacts were randomly  
123 selected and decomposed in eight frequency bands:  $\delta$  (delta: 1.5–4 Hz),  $\theta$  (theta: 4–8 Hz),  $\alpha$ 1  
124 (alpha 1: 8–10 Hz),  $\alpha$ 2 (alpha 2: 10–13 Hz),  $\beta$ 1 (beta 1: 13–21 Hz),  $\beta$ 2 (beta 2: 21–30 Hz),  $\beta$ 3 (beta  
125 3: 30–60 Hz) and  $\omega$  (omega > 60 Hz). For each segment, intracranial source current densities  
126 were then computed using sLORETA software (Pascual-Marqui, 2002), yielding sources in 6239  
127 5x5x5 mm<sup>3</sup> cortical grey matter voxels in standard MNI space (Fonov et al., 2011). sLORETA  
128 allows the localization of spatially distributed sources of activity without *a priori* on their  
129 number, which is well suited in the context of pain (Apkarian et al., 2005, Tracey and Mantyh,  
130 2007, Schweinhardt and Bushnell, 2010). Current density maps were then averaged across  
131 segments for each subject and condition (i.e., baseline and pain condition).

132

### 133 *Capsaicin application*

134 After the evaluation of baseline TMS and EEG measures, experimental pain was induced by a 1%  
135 capsaicin cream. More specifically, 0.06 ml of capsaicin was applied on the middle volar part of  
136 the left forearm in a perimeter of 4 cm X 4 cm. Capsaicin-induced pain was evaluated by the  
137 participants using a visual analogue scale (VAS; 0 = “no pain”, 10 = “the worst imaginable pain”),  
138 every 5 minutes until the pain sensation stabilized (i.e., when participants rated same intensity  
139 of pain in 2 consecutive VAS pain measures). Once the pain became stable, EEG and TMS  
140 measures were assessed again (see Figure 1).

141

142 *Statistical analysis*

143 Paired-sample t-tests were used to determine if there was a difference between the baseline  
144 and pain condition for the HLM values. Changes in current density power (EEG activity) between  
145 the baseline and pain condition were assessed using paired-sample t-tests across subjects,  
146 independently for each frequency band and each voxel. Statistical significance was assessed  
147 through statistical nonparametric mapping using 5,000 randomizations to account for multiple  
148 comparisons. A threshold on the t-statistic corresponding to  $p < 0.05$  was used to uncover pain-  
149 evoked activation maps and identify regions of the brain displaying changes in activity between  
150 the rest and pain conditions.

151

152 Because the analyses revealed no consistent changes in TMS measures and EEG activity  
153 between the baseline and pain condition (see results section), separate functional connectivity  
154 analyses were conducted in participants who showed a reduction in corticospinal output and an  
155 increase in M1  $\beta$  activity (group 1), and in participants who did not (group 2). For each group,  
156 linear lagged connectivity was assessed in the  $\beta$  band frequency using sLORETA software  
157 between M1 (region of interest) and other brain regions in which an increase in activity was  
158 observed during the pain condition. These functional connectivity analyses allowed us to  
159 evaluate if the activation of M1 was related to an interaction with other brain structures also  
160 activated in the presence of pain (Apkarian et al., 2000, Tracey et al., 2000).

161

162

163

164



165 **Results**

166 *Pain assessment*

167 Every participant experienced pain following capsaicin application (mean pain intensity =  $4 \pm 2$ ).

168 On average, 42 minutes were required after capsaicin application before the pain stabilized.

169

170 *Effect of experimental pain on TMS recruitment curves*

171 TMS recruitment curves obtained before and after capsaicin application are presented in Figure

172 2. As can be seen from this figure, pain did not affect corticospinal output, as evidenced by the

173 comparable TMS recruitment curves obtained for the baseline and pain conditions. The absence

174 of difference between the two conditions was confirmed by the statistical analysis, with the

175 paired-sample t-test showing no difference in HLM slope values between the baseline and pain

176 condition ( $p = 0.26$ ). Pearson correlational analyses showed that there were no relationships

177 between the change in the slope of the recruitment curve and the time needed for pain to reach

178 a plateau ( $r = -0.02$ ;  $p = 0.92$ ) and between the change in the slope of the recruitment curve and

179 the intensity of pain reported by the participants ( $r = -0.21$ ;  $p = 0.36$ ).

180

181 *Effect of experimental pain on brain activity*

182 Source localization analyses conducted to compare brain responses between the baseline and

183 pain condition revealed a significant increase in brain activity across the central cuneus ( $x = 0$ ,  $y =$

184  $-85$ ,  $z = 10$  at theta frequency), the left dorsolateral prefrontal cortex (DFPLC) ( $x = -45$ ,  $y = 30$ ,  $z =$

185  $35$  at alpha frequency), and the left cuneus ( $x = -20$ ,  $y = -90$ ,  $z = 35$ ) and right insula ( $x = 35$ ,  $y = -5$ ,

186  $z = 20$  both at the beta frequency) while participants were in the pain condition (all  $t_s > 4.40$ ,

187 corresponding to  $p < 0.05$ ). No changes were noted in other brain regions, including M1 (all  $p$ -

188 values  $> 0.05$ ).

189 *Between-group analyses*

190 Careful examination of the data revealed that about two thirds of the participants (n = 12)  
191 showed a decrease in corticospinal output (reduced TMS recruitment curve slope) during the  
192 pain condition while the other third (n = 7) showed an increase in corticospinal output  
193 (increased TMS recruitment slope; see Figure 3 A, B and Figure 4). These observations brought  
194 us to evaluate and to compare the changes in EEG brain activity and functional connectivity  
195 between these two groups of participants.

196

197 The between-group analysis first revealed that, compared to participants who showed an  
198 increase in corticospinal output, participants who showed a decrease in corticospinal output  
199 also showed greater right M1 beta frequency activity (x= 35, y= -15, z=50; t = 4.69, p = 0.049) in  
200 the “pain condition” (see Figure 5). Importantly, this group difference was absent at baseline (all  
201 ts < 4.80, p > 0 .48). Between-group comparisons, looking at changes in EEG functional  
202 connectivity, showed that, compared to participants who showed an increase in corticospinal  
203 output, those who showed a decrease demonstrated greater pain-related beta M1-cuneus  
204 connectivity (t = 3.58, p = 0.03). Again, these between group differences in beta M1-cuneus  
205 connectivity were not found at baseline (t = 3.73, p = 0.73). No other connectivity change was  
206 observed (all p-values > 0.05).

207

208

## 209 **Discussion**

210 The current study’s objective was to better understand the corticomotor changes induced by  
211 pain. More specifically, we wanted to determine if a prolonged/inescapable nociceptive  
212 stimulation pain, induced with a capsaicin cream, could modify TMS recruitment curves as well

213 as EEG activity of the motor cortex, and if these eventual alterations could be associated to  
214 functional connectivity changes. Our analyses revealed that capsaicin pain produced variable  
215 effects, with approximately two thirds of participants showing a reduced TMS recruitment curve  
216 slope. Participants who showed this type of decrease also showed an increase in M1  $\beta$  activity.

217

#### 218 *Effect of pain on cortical representation and corticospinal output*

219 In the past years, many studies have revealed the presence of functional reorganizations in the  
220 somatosensory and motor system of pain patients. For example, Krause et al. observed that  
221 patients with complex regional pain syndrome (CRPS) had a smaller corticomotor representation  
222 of the affected limb, compared to pain-free participants (Krause et al., 2006). Flor et al. reported  
223 similar changes in the primary somatosensory cortex (S1) in people suffering from phantom pain  
224 (Flor, 2003). Interestingly, researchers observed the presence of a positive correlation between  
225 pain intensity and the amplitude of cortical reorganization in amputee patients, suggesting that  
226 these neuroplastic changes could play an important role in the physiopathology of persistent  
227 pain (Flor et al., 1995).

228

229 The idea that cortical reorganization could play an important role in the physiopathology of  
230 chronic pain was reinforced by Maihofner et al. and Pleger et al., who observed a normalization  
231 of the cortical changes in CRPS patients after treatment, once pain subsided (Maihofner et al.,  
232 2004, Pleger et al., 2005). The results of Maihofner et al. and Pleger et al. support the idea that  
233 pain could drive cortical reorganization; however, the ultimate way to confirm the presence of a  
234 causal relationship between pain and cortical changes is to experimentally manipulate the  
235 presence of pain, as it is the case in this study. Our results show that pain can, indeed, drive  
236 changes in the corticomotor system, but that its effect is not uniform across all individuals.

237 Nevertheless, we must remember that the results obtained from experimental pain paradigm  
238 cannot be directly generalized to clinical pain populations. It should also be noted that the effect  
239 of pain on the motor system can vary depending on the duration of the painful stimulus (phasic  
240 vs tonic pain), the submodality (deep vs superficial pain), and the location (proximal vs distal  
241 pain) (Valeriani et al., 1999, Farina et al., 2001, Le Pera et al., 2001, Valeriani et al., 2001, Cheong  
242 et al., 2003, Svensson et al., 2003, Mercier and Leonard, 2011). Replicating the present results  
243 with different experimental pain paradigms and pursuing research in pain populations is  
244 essential before any final conclusions can be made.

245

#### 246 *Effect of pain on EEG activity of the motor cortex*

247 Several neuroimaging studies have shown that experimental pain can affect the activity of the  
248 motor cortex (Apkarian et al., 2000, Tracey et al., 2000, Burns et al., 2016). For the most part,  
249 these studies were done using functional magnetic resonance imaging (fMRI). Although useful –  
250 in particular because of its ability to measure changes in deep areas of the brain – it is important  
251 to remember that fMRI BOLD responses reflect changes in cerebral blood flow, cerebral blood  
252 volume and cerebral metabolic rate of oxygen following neural activation (Fox and Raichle,  
253 1986, Uludag et al., 2009, Attwell et al., 2010). As such, changes in BOLD can, at best, be related  
254 to changes in neural activity and cannot be interpreted specifically in terms of excitatory  
255 (increase in the activity of excitatory neurons) or inhibitory (increase in the activity of inhibitory  
256 neurons) activity. Contrary to fMRI, EEG directly measures the neuroelectric activity of brain  
257 cells, allowing a better characterization of neuronal changes (Aine, 1995). In this study, the EEG  
258 analyses have revealed that the majority of participants showed increased contralateral M1  $\beta$   
259 frequency activity during pain, suggesting that pain increases the inhibitory activity in this area  
260 (Pogosyan et al., 2009). The biological reasons for these cortical changes remain hypothetical. A

261 possible explanation is that increased  $\beta$  activity could force the injured individual to limit his  
262 movements, in order to promote healing. However, in certain cases, this inhibitory effect could  
263 be detrimental, for example by interfering with motor learning and rehabilitation (Boudreau et  
264 al., 2007, Bouffard et al., 2014).

265

266 In the past years, accumulating evidence stemming from paired-pulse TMS studies has  
267 suggested that chronic pain populations display changes in GABA-mediated intracortical  
268 inhibition (see for instance Parker et al. (2016) for a review). Perhaps the most compelling  
269 observations are the ones made by Lefaucheur and colleagues (Lefaucheur et al., 2006). In this  
270 study, Lefaucheur and colleagues observed that (1) neuropathic pain patients had reduced  
271 intracortical inhibition, when compared to age-matched healthy controls, (2) application of high-  
272 frequency (10 Hz) repetitive TMS (rTMS) in these pain patients increased intracortical inhibition,  
273 and (3) there was a significant association between the extent of pain relief and the increase in  
274 intracortical inhibition observed following the application of rTMS. Changes in GABA-mediated  
275 intracortical inhibition ([SICI](#)) have also been documented with experimental pain paradigms  
276 (Fierro et al., 2010, Schabrun and Hodges, 2012). Results from these studies indicate that the  
277 effect of experimental pain on SICI may depend on the nature/location of the nociceptive  
278 stimulus; while Fierro et al. (Fierro et al., 2010) observed reduced SICI following a topical  
279 capsaicin application (superficial cutaneous pain), Schabrun & Hodges (Schabrun and Hodges,  
280 2012) reported increased SICI following injection of a hypertonic saline solution (deep muscle  
281 pain). Changes in intracortical facilitation (ICF) were also noted by Schabrun & Hodges (Schabrun  
282 and Hodges, 2012), but not by Fierro et al. (Fierro et al., 2010). These findings help to better  
283 understand the role played by intracortical circuits and remind researchers that the effect of

284 pain on the corticomotor system likely varies depending on the type of pain (clinical vs  
285 experimental pain; deep vs superficial pain).

286

287 The increase in  $\beta$  power observed in the majority of our participants contrast with the results of  
288 Stancák and colleagues, who showed that thermode induced pain *decreased* M1  $\beta$  activity  
289 (Stancák et al., 2007). This discrepancy could be explained by the fact that prolonged pain (e.g.  
290 capsaicin) and brief pain (e.g. thermode) stimulation may foster the emergence of different  
291 motor strategies. Whereas immobilization can be a successful strategy in the former case, this  
292 same response could be detrimental in the second case, when it is possible for the individual to  
293 remove the body part away from the painful stimuli. Decreasing  $\beta$  activity during  
294 brief/escapable nociceptive stimulation could promote movement and help the individual avoid  
295 potential threats.

296

297 Interestingly, associations between M1  $\beta$  power and GABA concentration have been observed  
298 by Baumgarten and colleagues (2016). Similarly, Farzan and colleagues (2013) noted that the  
299 duration of the silent period (a TMS measure mediated by GABA receptors (Abbruzzese and  
300 Trompetto, 2002, Jono et al., 2016)) is related to  $\beta$  oscillations. Taken together, these  
301 observations suggest that the changes observed in corticospinal output in some of our  
302 participants could be linked to changes in GABA activity.

303

#### 304 *Effect of pain on other brain areas*

305 The EEG analysis revealed an increase of the activity of the insula, DFPLC and cuneus in the pain  
306 condition in all participants, when compared to baseline. The role of the insula and DFPLC in  
307 pain perception and modulation has been well documented in previous pain studies (Rainville et

308 al., 2000, Borckardt et al., 2007); however, the activation of the cuneus in the pain condition is  
309 more unexpected. A previous study, from our research group, did suggest that a brain area  
310 adjacent to the cuneus could play a significant role in the perception of pain (Goffaux et al.,  
311 2014). In this past study, we observed that individuals who showed increased activity in the  
312 precuneus in the presence of experimental pain also showed the promptest response to pain.  
313 Traditionally linked to the treatment of visual information (Corbetta et al., 1995, Nobre et al.,  
314 2003), the cuneus also plays an important role in the integration of sensory information, as well  
315 as cognitive processes such as attention, learning and memory (Cabeza et al., 2002, Makino et  
316 al., 2004).

317

318 The functional connectivity analyzes, done on the subgroup of participants for whom pain  
319 reduced corticospinal output, further highlighted the potential role that the cuneus could play in  
320 pain processes. These analyses have shown that the application of a capsaicin cream increases  
321 the functional connectivity between the motor cortex and the cuneus in individuals who show a  
322 reduced TMS recruitment curve slope. These results reinforce the role that the cuneus could  
323 play as a significant brain area for the integration of sensory and attentional information. This  
324 integrative function of the cuneus makes it an ideal cerebral structure, capable of modulating  
325 the activity and organization of the motor cortex based on the ascending sensory information  
326 and on the context in which the individual is placed and asked to interact.

327

328

### 329 **Limits**

330 The most important limit of this study probably relates to the inconsistent effect produced by  
331 pain on the corticomotor system. Indeed, it should be reminded that the most compelling

332 findings (i.e., increased M1  $\beta$  activity and reduced corticospinal output) were found in a  
333 subsample of participants. Future studies need to be conducted to determine if these results  
334 can be consistently reproduced and validate that the observed TMS and EEG changes are not  
335 spurious effects only. An additional limitation concerns the absence of control group. Although  
336 the TMS and EEG measures have been proven to be reliable (Cacchio et al., 2009, Cannon et al.,  
337 2012, Ngomo et al., 2012), the addition of a control group would have been an important asset  
338 for the study to document the stability of the TMS and EEG measures over time. Finally, it  
339 should be noted that the effect of pain on TMS and EEG measures was investigated only once  
340 (i.e., when pain stabilized). Again, futures studies, looking into the long-term effects are  
341 warranted.

342

### 343 **Conclusion**

344 In conclusion, our results show that tonic experimental pain increases M1  $\beta$  activity in certain  
345 individuals, and that this increase in  $\beta$  activity is intimately tied to corticomotor and functional  
346 connectivity changes. These observations remind us that the cerebrum works as an integrated  
347 system of circuits and that certain brain areas, other than those classically involved in pain  
348 perception and modulation can be affected by nociceptive stimulations. The differential pattern  
349 of response observed in our participants suggest that the effect of pain on the motor system is  
350 variable from on individual to another; an observation that could have important clinical  
351 implications for rehabilitation professionals working with pain patients.



352 **Conflict of interest**

353 The authors have no conflict of interest to report.

354

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357 data collection.

358

359 **Ethical approval**

360 All procedures performed in studies involving human participants were in accordance with the  
361 ethical standards of the institutional and/or national research committee and with the 1964  
362 Helsinki declaration and its later amendments or comparable ethical standards.

363

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