

# Is the firing rate of motor units in different vastus medialis regions modulated similarly during isometric contractions?

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1 Title:

2 Is the firing rate of motor units in different vastus medialis regions modulated similarly during  
3 isometric contractions?

4  
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33 Title:

34 Is the firing rate of motor units in different vastus medialis regions modulated similarly during  
35 isometric contractions?

36

37 Abstract:

38 **Introduction:** Previous evidence suggests the fibres of different motor units reside within  
39 distinct vastus medialis (VM) regions. Whether the activity of these motor units may be  
40 modulated differently remains unknown. Here we assess the discharge rate of motor units  
41 detected proximo-distally from VM to address this issue.

42 **Methods:** Surface electromyograms (EMGs) were recorded proximally and distally from VM  
43 while ten healthy subjects performed isometric contractions. Single motor units were  
44 decomposed from surface EMGs. The smoothed discharge rates of motor units identified from  
45 the same and from different VM regions were then cross-correlated.

46 **Results:** During low-level contractions, the discharge rate varied more similarly for distal  
47 (cross-correlation peak; interquartile interval: 0.27-0.40) and proximal (0.28-0.52) than for  
48 proximo-distal pairs of VM motor units (0.20-0.33;  $P=0.006$ ).

49 **Discussion:** The discharge rates of motor units from different proximo-distal VM regions show  
50 less similarity in their variations than those of pairs of units either distally or proximally.

51

52

53 Keywords: motor unit; vastus medialis; surface electromyography; compartmentalization;  
54 knee; quadriceps.

**55 Introduction**

56 Architectural differences seem to account for functional differences within the vastus medialis  
57 (VM) muscle. Previous studies reported that distal VM fibres are oriented more obliquely to  
58 the quadriceps tendon than the proximal fibres<sup>1,2</sup>. In their review, Smith et al.<sup>3</sup> specifically  
59 observed the orientation of VM distal and proximal fibres in relation to the quadriceps tendon  
60 respectively ranges from 40° to 77° and from 11° to 35°. Furthermore, *in-vivo*<sup>4,5</sup> and cadaveric<sup>1</sup>  
61 studies reported that distal VM fibres attached directly to the medial edge of the patella.  
62 Collectively, these findings indicate the activation of fibres in different, proximo-distal VM  
63 regions results in force vectors oriented in different directions<sup>6</sup>. Such differential, architectural  
64 organisation has led to the consideration that fibres in distinct VM regions may contribute to  
65 distinct functions<sup>2,6</sup>. Whether the nervous system may selectively activate fibres in distinct VM  
66 regions is an open and potentially relevant issue for the rehabilitation of patients with knee  
67 musculoskeletal disorders such as patellofemoral pain syndrome<sup>7,8</sup>.

68

69 The possibility of controlling distinct VM regions presumes a specific, neuromuscular  
70 organisation. First, VM motor units (MUs) must have small territories in relation to the muscle  
71 proximo-distal axis. Otherwise, activation of individual motor neurones would result in  
72 contraction of fibres spanning a large muscle region, resulting in force vectors with similar  
73 directions for different MUs. Investigations using scanning electromyography indicated that  
74 in-depth territory of VM motor units is remarkably small, ranging between 2 to 8 mm in healthy  
75 subjects<sup>9</sup>. More recently, Gallina and Vieira<sup>2</sup> observed the action potentials of single MUs are  
76 represented locally proximo-distally in the VM, suggesting that the territory of some VM motor  
77 units is relatively small. In agreement with this view, Lin et al.<sup>6</sup> observed that the patella shifted  
78 medially when stimulation pulses were delivered to the distal VM region and proximally when  
79 the proximal VM region was stimulated. In addition to MUs with small territories, the

80 activation of distinct VM regions demands preferential access to these MUs within the nervous  
81 system; if all VM motor neurones receive a common input, the activity of different proximo-  
82 distal VM fibres would be modulated similarly.

83

84 In this study, we therefore investigated whether the activity of MUs represented in surface  
85 electromyograms (EMGs) detected from different VM regions are modulated differently. We  
86 specifically asked: is the firing rate of MUs identified proximally and distally in the VM  
87 modulated equally strongly during isometric, torque-varying contractions? If MUs with  
88 territories in different VM regions receive different inputs, then, we expect the firing rate of  
89 pairs of MUs identified from the same VM region, either distal or proximal, to be modulated  
90 more similarly than that of pairs of units taken from different VM regions. Addressing this  
91 question would contribute to advancing our knowledge of the functional organisation of MUs  
92 within the VM.

93 **Methods**94 *Participants*

95 Ten healthy, male subjects (range: 24-32 years; 168-182 cm; 70-85 kg) were recruited to  
96 participate in the study. Participants did not report any knee injuries at the time of the  
97 experiments and all provided written informed consent. The study was conducted in accordance  
98 with the latest revision of the Declaration of Helsinki and approved by our University Hospital  
99 Ethics Committee (HUCFF/UFRJ – 127/2013).

100

101 *Experimental protocol*

102 Knee extension torque was measured with participants seated comfortably on a dynamometer  
103 chair (Biodex System 4, New York, USA), with their right knee flexed at 80° and aligned as  
104 coaxially as possible to the dynamometer axis of rotation. Two isometric, maximal voluntary  
105 contractions (MVCs) lasting 5 s each were performed, with a rest period of at least 2 min in-  
106 between. The peak torque, averaged across the two MVCs, was considered as the maximal  
107 knee extension torque. After that, torque-varying, isometric contractions at two force levels  
108 were applied. Participants were asked to increase knee torque from rest to a submaximal target  
109 level in 5 s, to hold it at that level for 10 s and then to return to rest in 5 s. This trapezoidal  
110 profile was repeated four times for each of two contraction levels, 20% and 40% MVC. The  
111 contraction level was randomised and a rest period of at least 5 min was provided in-between.  
112 Visual feedback of knee extension torque was presented on a computer monitor. Data  
113 collection started after participants had trained with visual feedback and could successfully  
114 follow the trapezoidal profiles; the familiarisation session started at least 3 min after MVCs.

115

116 *Electrode placement and EMGs recordings*

117 Two adhesive arrays of eight, silver-bar electrodes each (10 mm inter-electrode distance; Spes  
118 Medica, Battipaglia, Italy) were used to sample surface EMGs. The reference electrode was  
119 placed over the patella and conductive paste (TEN 20 Conductive Paste, Weaver, Aurora, USA)  
120 was used to assure electrical contact between electrodes and skin. Prior to positioning the  
121 arrays, the skin was shaved and cleaned with abrasive paste. With a dry array of eight silver-  
122 bar electrodes (LISiN-Politecnico di Torino, Turin, Italy), EMGs were visually inspected while  
123 participants gently, isometrically loaded their knee extensors. The array orientation was then  
124 changed until the propagation of action potentials of individual MUs could be clearly observed  
125 across electrodes; this orientation was deemed parallel to the average orientation of fibres  
126 underneath electrodes<sup>2,10</sup>. This procedure was repeated with the dry array centred roughly at  
127 the VM distal and proximal regions, defined through palpation. Adhesive arrays were then  
128 centred at these locations. An example of the position of adhesive arrays and the propagation  
129 of motor unit action potentials is provided in Figure 1.

130

131 Surface EMGs were recorded in monopolar derivation and amplified by a variable factor,  
132 ranging from 2,000 to 10,000 (10-900 Hz bandwidth amplifier; CMRR > 100 dB; EMG-USB2,  
133 OTBioelettronica, Turin, Italy). EMGs and the torque signal were digitised synchronously at  
134 2048 samples/s using a 12-bit A/D converter, with 5 V dynamic range.

135

### 136 *Assessing the variation in motor unit firing rates*

137 Raw surface EMGs were first visually inspected for power line interference and contact  
138 problems. Low-quality EMGs were not observed among the 320 (10 subjects x 8 electrodes x  
139 2 VM portions x 2 contraction levels) monopolar signals collected.

140

141 Similarities in modulation of MUs detected from different VM regions were assessed through  
142 cross-correlation. First, monopolar EMGs were band-pass filtered with a 4<sup>th</sup> order Butterworth  
143 filter (15-350 Hz cut-off frequencies). Filtered EMGs were decomposed into their constituent  
144 trains of motor unit action potentials<sup>11,12</sup>. Decomposition was applied separately for each  
145 contraction level and array of electrodes, providing the firing instants of MUs recruited for  
146 different effort levels and represented in different VM regions. The coefficient of variation of  
147 the inter-spike interval was computed for each MU considering periods of constant torque.  
148 Additionally, the instantaneous firing rate of individual MUs was computed as the  
149 multiplicative inverse of the inter-spike interval, linearly interpolated at 50 Hz, smoothed with  
150 a low-pass Butterworth filter (4<sup>th</sup> order, 3 Hz cut-off frequency<sup>12</sup>) and demeaned. For each  
151 participant, the resulting, smoothed firing rate profiles were cross-correlated for pairs of MUs:  
152 i) obtained from the same array, either proximal or distal; ii) identified from different arrays.  
153 The normalised, cross-correlation functions were calculated considering the longest period  
154 within which all MUs were active, separately for each of the four, consecutive trapezoidal  
155 contractions. The peak of the cross-correlation function, taken for lags from -100 to 100 ms<sup>13</sup>  
156 and averaged across the four consecutive contractions, was considered a measure of how  
157 similarly the firing rate of pairs of MUs varied for each individual and contraction level.

158

159 Only MUs recruited at torque levels lower than 90% of the target level were considered for  
160 analysis. The recruitment threshold of individual units was calculated as the torque value  
161 observed at the time of the first discharge, averaged across the four, trapezoidal contractions<sup>14</sup>.  
162 Moreover, MUs whose action potentials were clearly represented in both arrays of electrodes  
163 or that did not discharge continuously (< 200 discharges; 4 repetitions x 10 s plateau x 5  
164 discharges per second) were also excluded from analysis. Figure 2 shows examples of surface  
165 EMGs triggered and averaged at the time of the firing of individual MUs identified proximally



166 and distally from VM. While each of the two MUs shown in Figure 2A is represented mainly  
167 either in the distal or proximal array, potentials of the MUs shown in Figure 2B appear clearly  
168 in both arrays.

169

#### 170 *Statistics*

171 Given the data distribution was not Gaussian (Kolmogorov-Smirnov test;  $P < 0.029$ ) non-  
172 parametric analysis was considered for inferential statistics. Wilcoxon rank sum test was  
173 applied to compare the recruitment threshold of MUs identified for 20% and 40% MVC. The  
174 strength of the relationship between recruitment threshold and the interquartile interval of the  
175 MU firing rates was assessed with Pearson correlation analysis. The Kruskal-Wallis test was  
176 applied separately for 20% and 40% MVC to test for differences in the peak of the cross-  
177 correlation function computed for pairs of MUs in the same and in different VM regions; the  
178 Dunn-Sidak test was used for post-hoc comparisons. Statistical analysis was carried out with  
179 Matlab (Version 8.5, The MathWorks Inc., Natick, Massachusetts, USA). The level of  
180 significance was set at 5%.

**181 Results**

182 A total of 80 MUs were identified for the ten participants. Six MUs were excluded because  
183 their action potentials were observed in both arrays; all were identified proximally during 40%  
184 MVC. The firing characteristics of all MUs analysed are shown in Table 1 (20% MVC) and  
185 Table 2 (40% MVC), separately for each subject and VM region. A median of 4 MUs were  
186 analysed per subject and contraction level.

187

*188 Motor units recruited for the different contraction levels*

189 During the 20% MVC trapezoidal contraction, MUs first discharged when knee torque reached  
190 8.0% (6.2%-11.5%) MVC (median and interquartile interval). Conversely, the first discharge  
191 of MUs decomposed during the 40% MVC were observed at a significantly greater torque value  
192 (25.6%, 19.0%-29.0% MVC; Figure 3; Wilcoxon test;  $P < 0.0005$ ;  $N = 74$  MUs, 40 distal and  
193 34 proximal units). As shown in Figure 3, variations in the MU discharge rates were  
194 significantly negatively correlated with recruitment threshold, both for 20% and 40% MVC  
195 contractions (Pearson  $R < -0.47$  and  $P < 0.002$  for both cases). MUs recruited at higher torque  
196 levels showed less variation in firing rate.

197

*198 Cross-correlation function*

199 The firing rate of MUs identified from EMGs detected proximally and distally was modulated  
200 differently. Figure 4 shows the smoothed, instantaneous firing rate of four MUs decomposed  
201 for a representative participant and the resulting, cross-correlation functions. All four MUs  
202 were recruited at torque values well below the target, 20% MVC torque level (Figure 4A). Two  
203 of these units were decomposed from EMGs collected distally and their action potentials were  
204 clearly represented in the distal array of electrodes, whereas the two MUs decomposed  
205 proximally were represented predominantly in EMGs detected proximally (cf. bottom and top

206 traces in Figure 4B). The cross-correlation functions calculated for pairs of MUs decomposed  
207 from the same array of electrodes showed a clear, single peak at zero lag (Figure 4C). In  
208 contrast, pairs of units decomposed from different arrays either showed a markedly small cross-  
209 correlation value around 0 s or did not show a distinct peak.

210

211 Group data revealed the firing patterns of MUs decomposed for either the proximal or distal  
212 VM region were more similarly modulated than those of units decomposed proximo-distally.  
213 Cross-correlation functions were computed for 116 pairs of MUs; 28 pairs of distal units, 16  
214 pairs of proximal units and 73 proximo-distal pairs. Of the distal, proximal and crossed pairs,  
215 17, 9 and 39 were respectively obtained for the 20% MVC contraction and, then, there were  
216 instances with no or one motor unit being identified from a given VM region. The distribution  
217 of the peak of the cross-correlation function is shown in Figure 5 for all MU combinations.  
218 Kruskal-Wallis test revealed a significant difference in cross-correlation values between VM  
219 regions at 20% MVC. The firing rate variation was significantly more similar for pairs of MUs  
220 identified from the same VM region, both distal (interquartile interval: 0.27-0.40) and proximal  
221 (0.28-0.52), than for pairs of units in different regions (0.20-0.33;  $P = 0.006$ ). For 40% MVC,  
222 no significant differences in cross-correlation peaks were observed for any pairwise  
223 comparisons between distal (0.23-0.40), proximal (0.27-0.36) and crossed MUs pairs (0.22-  
224 0.33;  $P = 0.668$ ).

**225 Discussion**

226 Our main finding showed that MUs decomposed during 20% MVC from the same array, either  
227 distal or proximal, discharged with variations that were significantly more similar than those of  
228 units decomposed from different arrays. As discussed below, at least for low-level contractions,  
229 these results suggest motor neurones serving predominantly either the distal or proximal VM  
230 fibres may receive different synaptic input. The differential activation of distal and proximal  
231 VM motor units may therefore contribute to regulating the direction of the knee extension, force  
232 vector.

233

*234 Assessing different populations of vastus medialis motor units*

235 Notwithstanding the validity of the decomposition algorithm<sup>12,15</sup>, decomposition results are  
236 typically limited to the identification of a relatively small number of MUs<sup>16</sup>. Among the MUs  
237 recruited, those with greater amplitude are more likely to be identified for a given, fixed  
238 contraction level<sup>17</sup>. Results shown in Figure 3 suggest, indeed, different populations of MUs  
239 were identified for 20% and 40% MVC. Moreover, corroborating previous findings<sup>14,18</sup>, MUs  
240 recruited at progressively greater torque levels discharged at lower rates (Figure 3). Even  
241 though the results presented here may not be generalised to all VM MUs, they apply to MUs  
242 recruited at torque levels often elicited during daily activities<sup>19</sup>.

243

*244 Is the activity of proximo-distal motor units modulated differently in vastus medialis?*

245 Different methods have been proposed to assess how similarly the activity of different MUs is  
246 modulated<sup>20</sup>. In agreement with previous studies<sup>13,21-23</sup>, here we assessed similarities in the  
247 activity of VM motor units using the cross-correlation function. This association between  
248 motor neurone activity and cross-correlation function is a corollary following the well-  
249 established notion that variations in the synaptic drive impinging upon a given population of

250 active motor neurones lead to a concurrent and similar variation in their firing rate<sup>18,24</sup>.  
251 Interestingly, our key results show significant differences for 20% MVC in the cross-correlation  
252 function evaluated for pairs of units detected from the same and from different VM regions.  
253 When considering pairs of units detected from the same VM region, cross-correlation values  
254 0.27-0.52 (interquartile interval) were somewhat similar to those reported in the literature for  
255 other muscles (typically within the 0.3-0.6 range<sup>13,21,22,25-27</sup>). When considering crossed-pairs  
256 of units (proximal-distal), cross-correlation values were ~30% smaller (0.20-0.33; Figure 5).  
257 In view of these arguments, our results are consistent with the possibility that motor neurones  
258 serving fibres located in different VM regions do not share the same, synaptic input during  
259 isometric, knee extension contractions.

260

261 According to the common drive principle, the firing rate of active MUs is modulated in  
262 unison<sup>23,28</sup> with the active MUs sharing the same synaptic input. However, it is known that  
263 activation of different volumes of individual muscles, in particular of those with broad  
264 attachment, contributes to different joint motions (e.g., deltoid<sup>29</sup> and trapezius<sup>30</sup>). Such  
265 selective activation of muscles sub-volumes implies that motor neurones serving different sub-  
266 volumes receive distinct, net inputs. Following this reasoning, it is possible that different pools  
267 of MUs, each elicited for a specific purpose (e.g. to regulate force direction or to endure a  
268 fatiguing contraction<sup>13,31,32</sup>), receive different inputs. Results presented here suggest this  
269 concept may be extended, at least in VM, to motor neurones serving different, proximo-distal  
270 muscle regions.

271

272 A final consideration on the regional modulation of MU firing rates concerns the different  
273 contraction levels. During 40% MVC, the cross-correlation peak did not differ between pairs  
274 of crossed units and of units detected from the same region (Figure 5). Different factors may

275 have contributed to the lack of statistical significance. First, it is possible that the proximo-  
276 distal differentiation of MUs' activity becomes less relevant for torque demands higher than  
277 20% MVC, as a relatively greater proportion of VM motor units is recruited. Second, as  
278 discussed below, we cannot exclude the possibility that fibres of some MUs spanned an  
279 extensive, proximo-distal VM region. Third, in the EMGs, the interferential activity of different  
280 MUs increases markedly with the contraction level<sup>33</sup>, hampering the distinction of action  
281 potentials of individual MUs. As a consequence, the decomposition of EMGs collected at  
282 greater force levels may not work as effectively as it does at lower contraction levels<sup>33</sup>.

283

284 *What are the potential causes and consequences of regional differences in motor unit*  
285 *modulation?*

286 In this study, MU firings observed in different arrays are expected to reflect the discharges of  
287 motor neurones innervating fibres localised proximally or distally. Due to the close association  
288 between the location of active muscle fibres and their EMGs amplitude distribution, surface  
289 potentials are greater when collected above than far from the MU territory<sup>34</sup>. Indeed, when  
290 regional activation is elicited through selective, intra-muscular VM stimulation, the peak of the  
291 EMGs distribution can be observed near the stimulating electrode and signals recorded from  
292 electrodes 60 mm far from the distribution peak are dramatically small<sup>35</sup>. For these reasons,  
293 motor unit action potentials identified in either array in this study likely belong to units located  
294 predominantly proximally or distally within the VM.

295

296 During low-level contractions, MUs in different VM regions may be recruited based on their  
297 function. Due to distributed insertion along the patella medial edge<sup>4</sup> and to variations in fibre  
298 orientation within the VM<sup>3</sup>, distinct VM regions may contribute differentially to the patellar,  
299 proximo-medial translation<sup>6</sup>. The nervous system may take advantage of this anatomical

300 arrangement to preferentially recruit populations of VM fibres producing force in specific  
301 directions. In such case, fluctuation in the neural drive would affect MUs serving fibres within  
302 relatively small VM regions<sup>2</sup>. Our current findings seem to support this hypothesis (Figure 4  
303 and 5). Given the spectrum of orientations of VM fibres<sup>2</sup>, the potentially weak, mechanical  
304 linkage between VM fibres<sup>6</sup> and the differential modulation of activity of proximal and distal  
305 MUs (Figure 4 and 5), it is therefore possible the nervous system relies on the activation of  
306 different MUs to specifically control force direction in the VM. If this is the case, subjects  
307 could learn or be trained to selectively activate distinct VM regions (for example, through EMG  
308 biofeedback), opening new fronts for the rehabilitation of patients with knee musculoskeletal  
309 disorders such as patellofemoral pain syndrome.

310 **Abbreviations**

311 EMGs – surface electromyograms

312 IZ – innervation zone

313 MUs – motor units

314 MVC – maximal voluntary contraction

315 VM – vastus medialis



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401

402 **Tables**

403 **Table 1:** Firing characteristics of motor units analysed for 20% MVC contractions, separately  
 404 for each subject and vastus medialis region.

Subjects	Number of MUs		Recruitment threshold (%MVC)		Mean inter-spike interval (s)		Coefficient of variation of inter-spike interval	
	Prox	Dist	Prox	Dist	Prox	Dist	Prox	Dist
#1	3	2	4.22-6.53	7.88-8.74	0.07-0.11	0.08-0.09	0.39-0.50	0.32-0.38
#2	2	2	2.63-6.19	5.87-11.55	0.09-0.10	0.11-0.14	0.38-0.45	0.39-0.43
#3	2	3	7.00-13.18	11.03-16.89	0.11-0.12	0.12-0.16	0.37-0.44	0.31-0.42
#4	1	0	16.11		0.13		0.36	
#5	2	4	6.99-8.89	5.01-8.41	0.08-0.10	0.08-0.09	0.29-0.38	0.19-0.39
#6	2	2	4.72-8.25	6.13-10.13	0.08-0.13	0.08-0.09	0.37-0.48	0.37-0.41
#7	2	2	13.99-14.74	2.67-6.55	0.11-0.13	0.09-0.10	0.44-0.48	0.45-0.47
#8	1	3	14.44	7.71-16.00	0.09	0.11-0.14	0.35	0.41-0.50
#9	0	1		10.95		0.08		0.43
#10	2	2	6.23-8.87	4.84-11.98	0.07-0.08	0.09-0.12	0.32-0.43	0.43-0.47

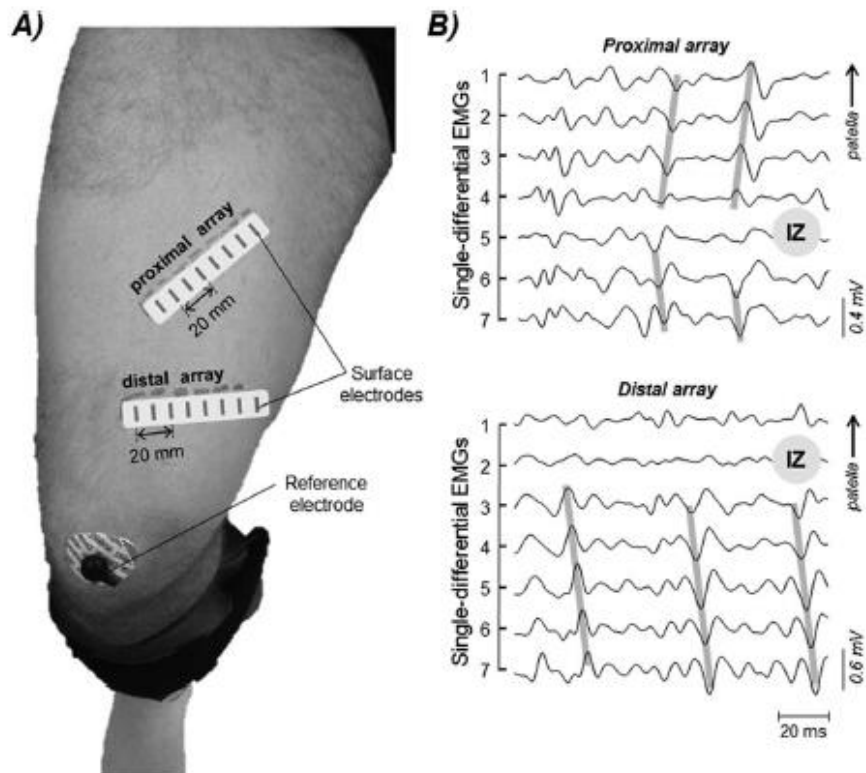
405 MUs: motor units; MVC: maximal voluntary contraction; Prox: proximal array; Dist: distal  
 406 array.

407 **Table 2:** Firing characteristics of motor units analysed for 40% MVC contractions, separately  
 408 for each subject and vastus medialis region.

Subjects	Number of MUs		Recruitment threshold (%MVC)		Mean inter-spike interval (s)		Coefficient of variation of inter-spike interval	
	Prox	Dist	Prox	Dist	Prox	Dist	Prox	Dist
#1	2	2	21.61-27.13	29.79-32.24	0.08-0.09	0.09-0.14	0.36-0.38	0.41-0.49
#2	2	2	20.38-30.14	16.42-18.31	0.10-0.11	0.07-0.09	0.37-0.49	0.32-0.43
#3	1	1	13.02	14.12	0.08	0.08	0.36	0.34
#4	2	3	26.88-33.53	10.90-26.25	0.11-0.11	0.10-0.10	0.40-0.41	0.44-0.48
#5	2	2	20.01-23.84	9.47-25.46	0.10-0.11	0.08-0.10	0.32-0.45	0.37-0.40
#6	2	3	20.06-27.56	29.12-29.92	0.08-0.09	0.09-0.12	0.35-0.38	0.39-0.44
#7	2	1	16.56-29.01	28.59	0.11-0.12	0.09	0.49-0.50	0.44
#8	1	2	18.98	13.13-19.05	0.11	0.08-0.11	0.50	0.36-0.41
#9	1	1	25.75	27.83	0.08	0.11	0.46	0.50
#10	2	2	22.22-31.65	27.82-29.78	0.12-0.13	0.09-0.10	0.46-0.47	0.35-0.39

409 MUs: motor units; MVC: maximal voluntary contraction; Prox: vastus medialis proximal  
 410 region; Dist: vastus medialis distal region.

## Figure captions



**Figure 1: Electrode positioning and raw, differential electromyograms.**

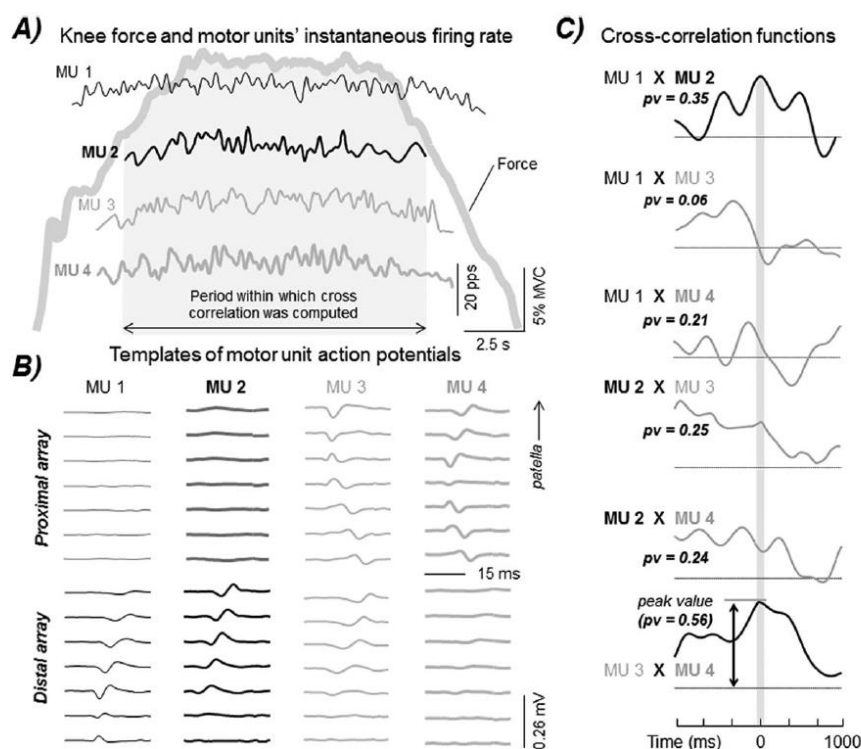
A schematic representation of the position of surface electrodes over the vastus medialis (VM) muscle is shown in panel A. A short epoch (100 ms) of raw, single-differential EMGs detected by both arrays is shown in panel B. Innervation zone (IZ; shaded circles) and propagation (thick, grey lines) of motor unit action potentials are clearly seen in both VM regions, indicating both arrays were aligned roughly parallel to VM proximal and distal fibres.





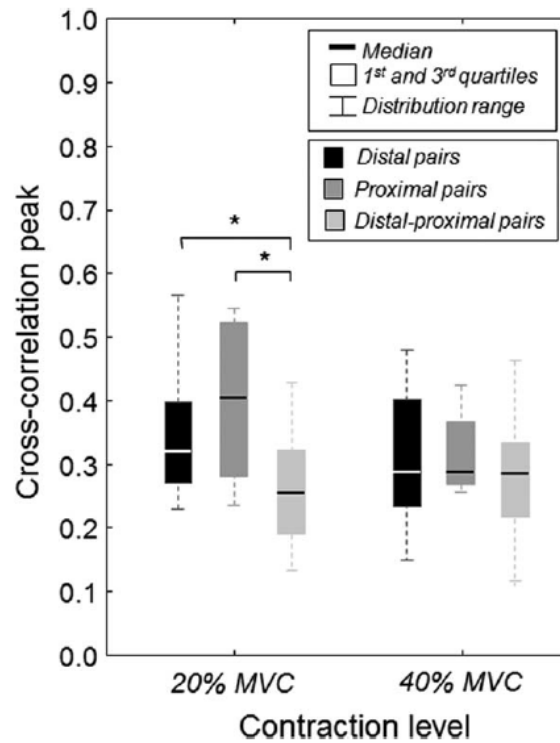
**Figure 3: Motor unit firing rates and recruitment threshold.**

The scatter plot shows how changes (ordinate; interquartile interval) in the firing rate of motor units varied with the torque value matching the units' first discharge (abscissa; recruitment threshold). Motor units decomposed from surface EMGs detected during 20% MVC ( $N = 38$ ) are represented with circles whereas crosses denote units decomposed for 40% MVC contractions ( $N = 36$ ).

**Figure 4: Example of regional modulations in the activity of vastus medialis motor units.**

A, shows the knee extension torque and the smoothed, instantaneous firing rate of four decomposed motor units for a single, representative participant during one trapezoidal, torque-varying contraction. The firing rate of units decomposed proximally is represented with black traces while that of distal units is shown with grey traces. The recruitment threshold of MU1, MU2, MU3 and MU4 were respectively 4.8, 12.0, 8.9 and 6.2% MVC. The spike-triggered, average representation of the action potential of each of the four decomposed motor units is shown in panel B. Note the action potentials of units decomposed proximally and distally

appear respectively predominantly in the proximal and distal arrays. *C*, shows the cross-correlation functions computed for the six possible combinations of pairs of proximal and distal motor units. The vertical, grey rectangle indicates the lag for which cross-correlation values were considered to compare how similarly the firing rate of pairs of motor units was modulated.



**Figure 5: Cross-correlations of vastus medialis motor units.**

The distribution of peak values of the cross-correlation functions computed for pairs of distal (black boxes), proximal (dark grey boxes) and proximo-distal (light grey boxes) motor units is shown separately for 20% and 40% MVC contraction levels. Horizontal traces, boxes and whiskers respectively denote the median value, the interquartile interval and the distribution range. Asterisk denotes statistical significance ( $P < 0.05$ ).