

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
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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^{1,2}. In their review, Smith et al.³ 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*^{4,5} and cadaveric¹
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 directions⁶. Such differential, architectural
64 organisation has led to the consideration that fibres in distinct VM regions may contribute to
65 distinct functions^{2,6}. 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^{7,8}.

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 subjects⁹. More recently, Gallina and Vieira² 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.⁶ 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^{2,10}. 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 4th order Butterworth
143 filter (15-350 Hz cut-off frequencies). Filtered EMGs were decomposed into their constituent
144 trains of motor unit action potentials^{11,12}. 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 (4th order, 3 Hz cut-off frequency¹²) 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 ms¹³
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 contractions¹⁴.
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^{12,15}, decomposition results are
236 typically limited to the identification of a relatively small number of MUs¹⁶. Among the MUs
237 recruited, those with greater amplitude are more likely to be identified for a given, fixed
238 contraction level¹⁷. Results shown in Figure 3 suggest, indeed, different populations of MUs
239 were identified for 20% and 40% MVC. Moreover, corroborating previous findings^{14,18}, 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 activities¹⁹.

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 modulated²⁰. In agreement with previous studies^{13,21-23}, 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^{18,24}.
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^{13,21,22,25-27}). 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^{23,28} 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., deltoid²⁹ and trapezius³⁰). 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^{13,31,32}), 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 level³³, 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 levels³³.

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 territory³⁴. 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 small³⁵. 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 edge⁴ and to variations in fibre
298 orientation within the VM³, distinct VM regions may contribute differentially to the patellar,
299 proximo-medial translation⁶. 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 regions². Our current findings seem to support this hypothesis (Figure 4
303 and 5). Given the spectrum of orientations of VM fibres², the potentially weak, mechanical
304 linkage between VM fibres⁶ 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

316 **References**

- 317 1. Peeler J, Cooper J, Porter MM, Thliveris JA, Anderson JE. Structural parameters of the
318 vastus medialis muscle. *Clin Anat* 2005;**18**:281-289.
- 319 2. Gallina A, Vieira TM. Territory and fiber orientation of vastus medialis motor units: a
320 surface electromyography investigation. *Muscle Nerve* 2015;**52**:1057-1065.
- 321 3. Smith TO, Nichols R, Darle H, Donell ST. Do the vastus medialis obliquus and vastus
322 medialis longus really exist? A Systematic Review. *Clin Anat* 2009;**22**:183-199.
- 323 4. Holt G, Nunn T, Allen RA, Gregori A. Variation of the vastus medialis obliquus
324 insertion and its relevance to minimally invasive total knee arthroplasty. *J Arthroplasty*
325 2008;**23**(4):600-604.
- 326 5. Engelina S, Antonios T, Robertson CJ. Ultrasound investigation of vastus medialis
327 oblique muscle architecture: an in vivo study. *Clin Anat* 2014;**27**:1076-1084.
- 328 6. Lin F, Wan G, Koh JL, Hendrix RW, Zhang L. In vivo and noninvasive three-
329 dimensional patellar tracking induced by individual heads of quadriceps. *Med Sci Sports*
330 *Exerc* 2004;**36**:93-101.
- 331 7. Peeler J, Anderson JE. Structural parameters of the vastus medialis muscle and its
332 relationship to patellofemoral joint deterioration. *Clin Anat* 2007;**20**:307-314.
- 333 8. Sawatsky A, Bourne D, Horisberger M, Jinha A, Herzog W. Changes in patellofemoral
334 joint contact pressures caused by vastus medialis muscle weakness. *Clinical*
335 *Biomechanics* 2012;**27**:596-601.
- 336 9. Gootzen THJM, Vingerhoets DJM, Stegeman DF. A study of motor unit structure by
337 means of scanning EMG. *Muscle Nerve* 1992;**15**:349-357.
- 338 10. Merletti R, Rainoldi A, Farina D. Surface electromyography for noninvasive
339 characterization of muscle. *Exerc Sport Sci Rev* 2001;**29**(1):20-25.

- 340 11. Holobar A, Zazula D. Multichannel blind source separation using convolution kernel
341 compensation. *IEEE Transactions on Signal Processing* 2007;**55**:4487-4496.
- 342 12. Holobar A, Minetto MA, Botter A, Negro F, Farina D. Experimental analysis of
343 accuracy in the identification of motor unit spike trains from high density surface EMG.
344 *IEEE Transactions on Neural Systems and Rehabilitation Engineering* 2010;**18**:221-
345 229.
- 346 13. Contessa P, Adam A, De Luca CJ. Motor unit control and force fluctuation during
347 fatigue. *J Appl Physiol* 2009;**107**:235-243.
- 348 14. De Luca CJ, Hostage EC. Relationship between firing rate and recruitment threshold of
349 motoneurons in voluntary isometric contractions. *J Neurophysiol* 2010;**104**:1034-1046.
- 350 15. Holobar A, Farina D. Blind source identification from the multichannel surface
351 electromyogram. *Physiol Meas* 2014;**35**:143-165
- 352 16. Farina D, Negro F, Gazzoni M, Enoka RM. Detecting the unique representation of
353 motor-unit action potentials in the surface electromyogram. *J Neurophysiol*
354 2008;**100**:1223-1233.
- 355 17. Roeleveld K, Stegeman DF, Vingerhoets HM, Oosterom AV. The motor unit potential
356 distribution over the skin surface and its use in estimating the motor unit location. *Acta*
357 *Physiol Scand* 1997;**161**:465-472.
- 358 18. Heckman CJ, Enoka RM. Motor unit. *Compr Physiol* 2012;**2**:2629-2682.
- 359 19. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al.
360 American College of Sports Medicine position statement: quantity and quality of
361 exercise for developing and maintaining cardiorespiratory, musculoskeletal, and
362 neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med*
363 *Sci Sports Exerc* 2011;**43**:1334-1359.

- 364 20. Myers LJ, Erim Z, Lowery M. Time and frequency domain methods for quantifying
365 common modulation of motor unit firing patterns. *Journal of NeuroEngineering and*
366 *Rehabilitation* 2004;**1**(2).
- 367 21. Beck TW, Kasishe PR, Stock MS, DeFreitas J. Eccentric exercise does not affect
368 common drive in the biceps brachii. *Muscle Nerve* 2012;**46**:759-762.
- 369 22. Ye X, Beck TW, Wages NP. Influences of dynamic exercise on force steadiness and
370 common drive. *J Musculoskelet Neuronal Interact* 2014;**14**(3):377-386.
- 371 23. De Luca CJ, Erim Z. Common drive of motor units in regulation of muscle force. *Trends*
372 *in Neuroscience* 1994;**17**(7):299-305.
- 373 24. Farina D, Negro F, Muceli S, Enoka R. Principles of motor unit physiology evolve with
374 advances in technology. *Physiology* 2016;**31**:93-94.
- 375 25. Erim Z, Berg MF, Burke DT, De Luca CJ. Effects of aging on motor-unit control
376 properties. *J Neurophysiol* 1999;**82**:2081-2091.
- 377 26. Sauvage C, Manto M, Adam A, Roark R, Jissendi P, De Luca CJ. Ordered motor-unit
378 firing behavior in acute cerebellar stroke. *J Neurophysiol* 2006;**96**:2769-2774.
- 379 27. De Luca CJ, Erim Z. Common drive in motor units of a synergistic muscle pair. *J*
380 *Neurophysiol* 2002;**87**:2200-2204.
- 381 28. De Luca CJ, LeFever RS, McCue MP, Xenaxis AP. Control scheme governing
382 concurrently active human motor units during voluntary contractions. *J Physiol*
383 1982;**329**:129-142.
- 384 29. Brown JM, Wickham JB, McAndrew DJ, Huang XF. Muscles within muscles:
385 coordination of 19 muscle segments within three shoulder muscles during isometric
386 motor tasks. *Journal of Electromyography and Kinesiology* 2007;**17**:57-73.

- 387 30. Holtermann A, Roeleveld K, Mork PJ, Grönlund C, Karlsson JS, Andersen LL, et al.
388 Selective activation of neuromuscular compartments within the human trapezius
389 muscle. *Journal of Electromyography and Kinesiology* 2009;**19**:896-902.
- 390 31. Westgaard RH, De Luca CJ. Motor unit substitution in long-duration contractions of the
391 human trapezius muscle. *J Neurophysiol* 1999;**82**:501-504.
- 392 32. Herrmann U, Flanders M. Directional tuning of single motor units. *J Neurosci*
393 1998;**18**(20):8402-8416.
- 394 33. Holobar A, Farina D, Gazzoni M, Merletti R, Zazula D. Estimating motor unit discharge
395 patterns from high-density surface electromyogram. *Clinical Neurophysiology*
396 2009;**120**:551-562.
- 397 34. Roeleveld K, Stegeman DF, Vingerhoets HM, Oosterom AV. Motor unit potential
398 contribution to surface electromyography. *Acta Physiol Scand* 1997;**160**:175-183.
- 399 35. Gallina A, Ivanova TD, Garland SJ. Regional activation within the vastus medialis in
400 stimulated and voluntary contractions. *J Appl Physiol* 2016;**121**:466-474.
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

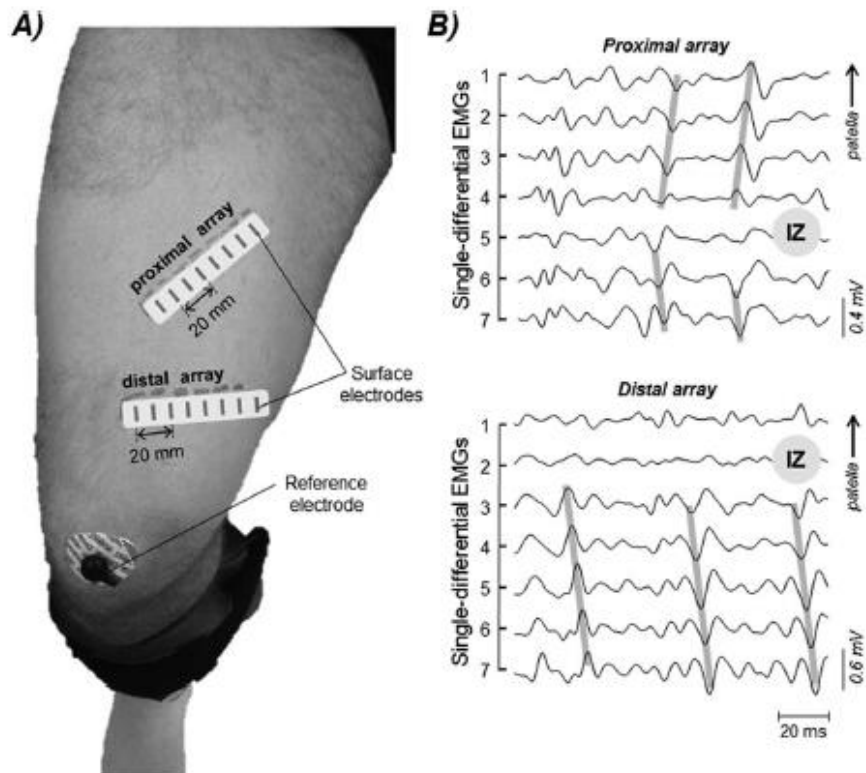
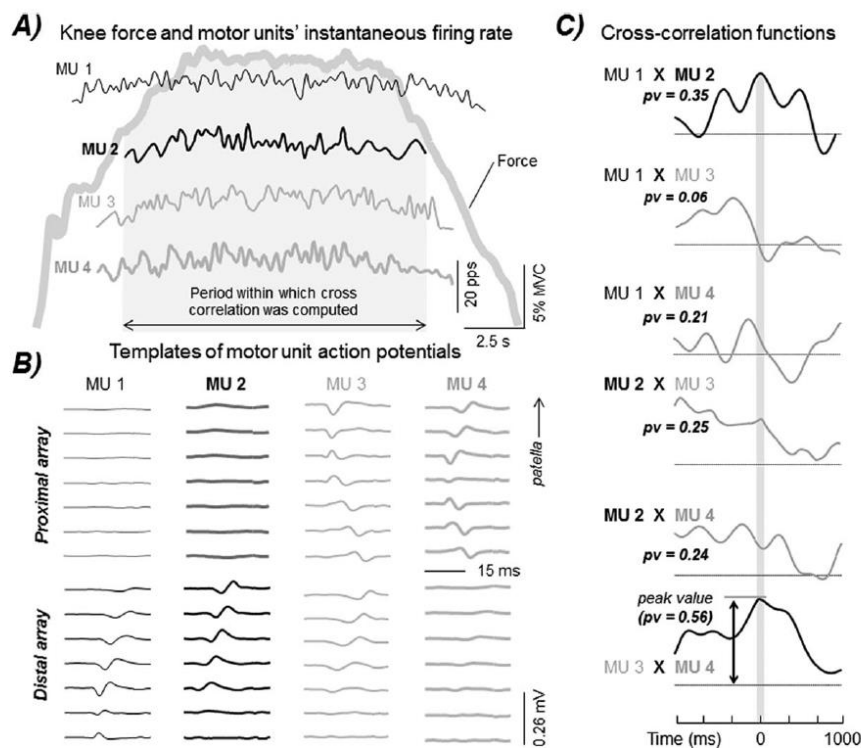


Figure 1: Electrode positioning and raw, differential electromyograms.

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.

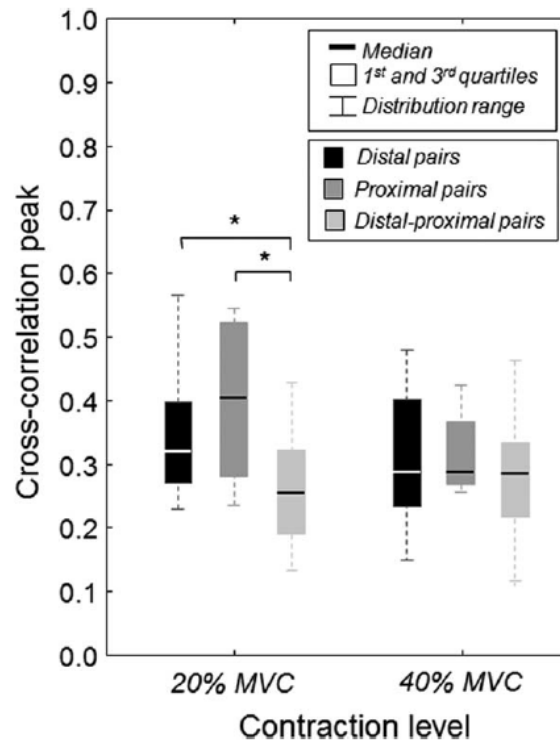


Figure 5: Cross-correlations of vastus medialis motor units.

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 ($P < 0.05$).