

Regionalization of the stretch reflex in the human vastus medialis

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1 Title: **Regionalization of the stretch reflex in the human vastus medialis**

2 Running title: **Regionalization of the human stretch reflex**

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24 **KEY POINTS SUMMARY:**

- 25 • Regionalization of the stretch reflex, i.e.: the notion that the activation of 1a afferents from a
26 muscle region influences only the activation of motor units in the same region, has been
27 demonstrated previously in animals but not in humans.
- 28 • Mechanical stretches applied to regions of vastus medialis as close as 10 mm apart resulted in
29 recruitment of motor units localized topographically with respect to the location of the
30 mechanical stretch.
- 31 • Stretch reflexes are regionalized in the human vastus medialis.
- 32 • The human spinal cord has the neuromuscular circuitry to preferentially activate motoneurons
33 innervating muscle fibres located in different regions of the vastus medialis.

34

35 **ABSTRACT:**

36 The localization of motor unit territories provides an anatomical basis to suggest that the CNS may have
37 more independence in motor unit recruitment and control strategies than what was previously thought.
38 In this study, we investigated whether the human spinal cord has the neuromuscular circuitry to
39 independently activate motor units located in different regions of the vastus medialis.

40 Mechanical taps were applied to multiple locations in the vastus medialis (VM) in nine healthy individuals.
41 Regional responses within the muscle were observed using a grid of 5×13 surface EMG electrodes. The
42 EMG amplitude was quantified for each channel, and a cluster of channels showing the largest activation
43 was identified. The spatial location of the EMG response was quantified as the position of the channels
44 in the cluster. In a subset of 3 participants, intramuscular recordings were performed simultaneously with
45 the surface EMG recordings.

46 Mechanical taps resulted in localized, discrete responses for each participant. The spatial location of the
47 elicited responses was dependent on the location of the tap ($P < 0.001$). Recordings with intramuscular
48 electrodes confirmed the regional activation of the VM for different tap locations.

49 Selective stimulation of 1a afferents localized in a region of the VM results in reflex recruitment of motor
50 units in the same region. These findings suggest that the human spinal cord has the neuromuscular
51 circuitry to modulate spatially the motoneuronal output to vastus medialis regions, which is a
52 neuroanatomical prerequisite for regional activation.

53 **KEYWORDS:** Motor unit; stretch reflex; EMG; quadriceps; spinal cord; neuromechanics

54 **ABBREVIATIONS LIST:** CNS: Central Nervous System; VM: Vastus Medialis; HDsEMG: High-Density
55 surface Electromyography; EMG: electromyographic; MVC: Maximal Voluntary Contraction; CoV:
56 Coefficient of Variation.

57 **INTRODUCTION:**

58 Motor units, consisting of the motoneurone and the muscle fibres it innervates, provide the
59 conduit for the central nervous system (CNS) to convert neural signals into forces. It has been known for
60 years that motoneurone soma size is a main determinant of motor unit recruitment order, with smaller
61 motoneurons activated before larger ones (Henneman, 1957), and that motor units within individual
62 muscles and across synergistic muscles share a common command from the CNS (De Luca & Erim, 1994;
63 Laine *et al.*, 2015). However, the existence of motor units with localized territories in humans suggests
64 that the CNS may have more independence in motor unit recruitment and control strategies than
65 previously thought. For example, most (Buchthal *et al.*, 1959; Gootzen *et al.*, 1992; Vieira *et al.*, 2011;
66 Gallina & Vieira, 2015), 70% (Harris *et al.*, 2005) or up to half (Héroux *et al.*, 2015) of the motor units in
67 human muscles have localized territories, meaning that motoneurons innervate muscle fibres clustered
68 in limited muscle regions. While the functional implication of this anatomical structure is currently
69 unknown, it constitutes a basis for the CNS to distribute neural inputs to motoneurons based on the
70 location of the muscle fibres they innervate. This was suggested as a possible mechanism for the CNS to
71 take advantage of heterogeneous muscle architecture (Windhorst *et al.*, 1989; Vieira *et al.*, 2011), and for
72 changes in motor control strategies in the presence of pain (Tucker *et al.*, 2009); yet, there is currently no
73 evidence for the existence of such selective motor unit recruitment in humans.

74 The regionalization of the stretch reflex requires that motor units located in different muscle
75 regions can be selectively recruited by the human spinal cord. Due to its complex architecture, the vastus
76 medialis (VM) offers a good opportunity to study the regionalization of the stretch reflex in humans. The
77 VM has a distributed insertion along 40-60% of the medial side of the patella (Holt *et al.*, 2008) and on the
78 common quadriceps tendon (Smith *et al.*, 2009). Proximal-to-distal differences were observed in the
79 orientation of VM muscle fibres (Smith *et al.*, 2009; Gallina & Vieira, 2015), pennation angle (Blazevich *et*

80 *al.*, 2006; O'Brien *et al.*, 2010), thickness (Blazevich *et al.* 2006; O'Brien *et al.* 2010) and fibre length
81 (O'Brien *et al.*, 2010). Because of these regional differences in muscle architecture and insertion, muscles
82 fibres located in the proximal or distal VM exert forces directed proximally or medially on the patella (Lin
83 *et al.*, 2004) despite the absence of clear anatomical compartmentalization (Smith *et al.*, 2009). Also,
84 motoneurons supplying the human VM innervate muscle fibres confined to limited regions within the
85 muscle (Gootzen *et al.*, 1992; Gallina & Vieira, 2015), which is a neuro-anatomical prerequisite for regional
86 activation. Regionalization of the stretch reflex may be a mechanism the CNS uses to coordinate the
87 activation of VM regions with different mechanical properties.

88 In this study, we investigated whether motoneurons innervating muscle fibres located in
89 different regions of the human VM can be independently recruited at the spinal level. Regional activation
90 of 1a afferents localized in different VM regions was produced through muscle taps and the spatial
91 location of the recruited motor units was investigated using a grid of surface electromyographic
92 electrodes (Vieira *et al.*, 2011) and confirmed with intramuscular fine-wire recordings. It was
93 hypothesized that stretch reflexes within the VM are localized and vary systematically with the location
94 of the tap. If localized stretch of muscle fibres results in regional activation within the muscle, this would
95 demonstrate that motor units located in different regions can be independently recruited at the spinal
96 level.

97 **METHODS:**

98 **Participants**

99 The screening process consisted of applying taps along the VM insertion on the patella and visually
100 assessing whether muscle twitches were elicited. Reflex contractions were observed in all screened
101 individuals but some reported discomfort due to the large amount of force needed to elicit the reflex and
102 were not further tested. Nine individuals participated in this study (1 female; 24 - 53 years old). All

103 participants signed a written informed consent form. The study conformed to the standards set by the
104 latest revision of the *Declaration of Helsinki* and was approved by the University of British Columbia
105 Clinical Research Ethics Board.

106 **High-Density surface Electromyography**

107 Placement of the High-Density surface Electromyography (HDsEMG) grid was guided by anatomical
108 references. The medial and lateral boundaries of the VM were identified with ultrasound imaging
109 (LogicScan 64 LT-1T, Telemed, Vilnius, Lithuania) and were marked on the skin. The innervation zone of
110 VM was located using a linear electrode array (16 silver bar electrodes, 10mm inter electrode distance,
111 OTBioelettronica, Torino, Italy) moved over different regions of the muscle along muscle fibres while the
112 participants maintained a low-force isometric knee extension contraction. The innervation zone,
113 identified by the bi-directional propagation of the bipolar action potentials observed in consecutive
114 channels, was marked on the skin. Similar to a previous study (Gallina & Vieira, 2015), the innervation
115 zone of fibre groups was found to be oriented diagonally across the VM (fig. 1). The HDsEMG grid (semi-
116 disposable adhesive matrix; OTBioelettronica, Torino, Italy) consisted of 64 electrodes arranged in 5
117 columns and 13 rows (an electrode missing in one of the corners), spaced by 8 mm with a total area
118 covered by the electrodes of 3072 mm² (96×32 mm). The grid was placed proximally to the innervation
119 zone with the long axis of the grid (columns) parallel to it. The distal column of electrodes (column 1) was
120 placed approximately 5 mm from the estimated location of the innervation zone and the medial row of
121 electrodes (row 1) was close to the medial border of the VM muscle (fig. 1). Bi-adhesive foam held the
122 grid in place and conductive paste (Ten20, Weaver and Co., Aurora, CO, USA) ensured good electrical
123 contact between the skin and electrodes. Two surface electrodes (20×35 mm; conductive hydrogel;
124 Kendall, Covidien, Mansfield, MA, USA) were placed on the medial side of the knee as reference
125 electrodes.

126 **Intramuscular recordings**

127 As differences in motor unit territory estimates in the medial gastrocnemius were recently observed when
128 assessed using HDsEMG versus intramuscular recordings (Vieira *et al.*, 2011; Héroux *et al.*, 2015),
129 intramuscular multiunit electromyographic (EMG) signals were recorded in three participants together
130 with HDsEMG. Custom-made electrodes consisted of two 0.05 mm insulated stainless steel wires
131 (California FineWire, Grover Beach, CA, USA) wound together and inserted via a 1.75 inch 25 gauge
132 hypodermic needle (EXEL International Medical Products, St Petersburg, FL, USA). The threaded wires
133 were folded back to create one 4 mm barb and one 10 mm barb, with the insulation removed from the
134 distal 5 mm (longer barb) or 2 mm (shorter barb) to form the recording sites. The large exposed areas of
135 the wires were chosen in order to favour multi-unit EMG recordings. Three wire electrodes were inserted
136 under ultrasound guidance at a ~10 mm depth along the 3rd column of HDsEMG electrodes at rows 3, 7
137 and 11. A surface electrode was placed over the lateral femoral epicondyle and served as the ground for
138 fine-wire electrode recordings.

139 **Protocol**

140 Participants sat in the chair of a Biodex dynamometer (System 4 Pro, Biodex Medical Systems, Shirley, NY,
141 USA) with their lower leg strapped to the knee attachment at 80 degrees knee flexion. Taps were manually
142 applied using a custom-made hammer with a load cell embedded (Force-Displacement Transducer FT 10,
143 Grass Instrument Co., Quincy, Mass, USA). The head of the hammer was a plastic cone with a rounded tip
144 (5 mm diameter). Taps were applied orthogonally to the skin over the VM muscle fibres by the same
145 investigator in all participants while monitoring the tap force and the EMG response on a computer
146 screen. Taps were first applied close to the distal insertion of the most distal fibres of the VM, and then
147 moving proximally following the patellar edge until a clear response was observed (L1, fig. 1). The other
148 locations (5 locations maximum) were identified by applying taps progressively more proximally along the
149 edge of the patella in steps of 10 mm until no responses could be observed (fig. 1). For each location, taps
150 were applied starting at the edge of the patella, and then moving away from the patella along the muscle

151 fibres. The location that provided the largest EMG responses while minimizing artefacts was marked on
152 the skin (fig. 1). Thirty taps were applied to each location with varying input force to obtain a range of
153 reflex response amplitudes; surface EMG activity was carefully monitored online to ensure that the VM
154 was at rest when taps were applied. Following muscle taps, participants who took part in the validation
155 with intramuscular EMG recordings were asked to perform three isometric maximal voluntary
156 contractions (MVC) with verbal encouragement. Tap locations were marked and measured on a
157 coordinate system referenced to the centre of the patella.

158 Surface EMG signals were collected in monopolar configuration using an HDsEMG amplifier (128-channel
159 EMG-USB; OTBioelettronica, Torino, Italy). Signals were amplified ($\times 500$ - 1000), filtered (band-pass 10-
160 750 Hz) and digitized at 2048 Hz using a 12 bit A/D converter. Differential fine-wire EMG signals were
161 filtered (band-pass 30–6000 Hz; NL 134 and NL 844, Digitimer, Garden City UK), amplified ($\times 1000$; NL 820
162 A and NL 844, Digitimer, Garden City UK) and then A/D converted at 20 kHz (Power 1401 with Spike2
163 software, Cambridge Electronic Design, Cambridge, UK). The force signal was amplified ($\times 100$), low-pass
164 filtered (10 KHz) and simultaneously digitized by the two acquisition systems used for EMG recordings;
165 the force signal collected at 20kHz was used for analysis.

166 **Data analysis**

167 All data analysis was performed in Matlab R2013b (The MathWorks, Inc., Natick, MA, USA). EMG signals
168 were band-pass filtered (dual-pass Butterworth, 4th order for each direction; surface: 20-400 Hz;
169 intramuscular: 300-2000 Hz) before analyses. Tendon taps were identified using the force measured with
170 the force transducer placed in the hammer. The timing of each tap was identified as the first data point
171 after which the force signal reached 5% of the peak force amplitude (fig. 2). Epochs from 50 ms before to
172 450 ms after each tap were analyzed. Surface EMG channels showing artefacts or predominantly power
173 line interference, as determined by visual inspection (less than 10% of the channels; range: 0-7 channels),
174 were replaced by the linear interpolation of the four adjacent channels. The onset of the response and

175 the occurrence of action potential propagation along the rows of the electrode grids were used to
176 distinguish the presence of a spinal reflex from mechanical artefact. Only taps that resulted in clear
177 negative peaks delayed by 1-3 ms in channels progressively more proximal along the VM fibres (further
178 away from the neuromuscular junction) were included in the analysis (fig. 2). Because mechanical taps
179 were applied in a range of forces (see below), no muscle activation in response to the tap was observed
180 in 22% of the trials across all locations. These trials were excluded from all analyses.

181 For each channel, the magnitude of the surface EMG response was calculated as the amplitude of the
182 largest negative peak occurring 15-45 ms after the tap. Artefacts due to the tap could sometimes be
183 observed superimposed on the EMG response in columns 1 and 2 and these columns were excluded from
184 the analysis for all participants. An example of surface EMG signals can be observed in fig. 3 top rows.
185 For each of the taps where an EMG response was observed, the amplitude values of columns 3-5 of each
186 row were averaged obtaining an array of 13 values (an example is shown in fig. 1 above the EMG grid).
187 Thus, in each single tap location, up to 30 arrays of amplitude values representing the distribution of the
188 reflex response along the columns of the grid were established.

189 Figure 4 shows the amplitude range of the responses to the manually-evoked taps from the intramuscular
190 recordings of a representative subject. A consistent spatial localization of the response is observed despite
191 the difference in amplitude. As muscle thickness (Blazevich *et al.*, 2006) and the amount of skin/fat tissues
192 (Botter *et al.*, 2011) changes across the VM, applying the same input force across the tap locations does
193 not necessarily ensure similar muscle spindle activation. To ensure that the localization of the stretch
194 reflex across tap locations was not influenced by input force or amplitude of the EMG responses, three
195 separate analyses were conducted: all trials (all mechanical taps), trials matched for input (force-matched)
196 or trials matched for output (EMG amplitude-matched). Force or EMG amplitude matching across
197 different tap locations was done separately. For the force-matched analysis, the largest five taps in each
198 location were selected and the input force values were averaged. The lowest of the average values among

199 locations was chosen as reference. For each location, the 5 trials with input force closest to this reference
200 value were selected and included in the analysis. For the EMG amplitude-matching analysis, selection of
201 the trials was done following the same procedure but trials were matched for amplitude of the EMG
202 responses instead of force input. For the force-matched and amplitude-matched responses, the
203 coefficient of variation (CoV) was calculated for each participant as the standard deviation divided by
204 mean across tap locations and expressed as a percentage. This index was used to describe the variability
205 of force and EMG amplitude across tap locations to verify that the matching was effective.

206 In all analyses, the amplitude distributions of the selected trials (5 out of 30 for force-matched and
207 amplitude-matched analyses) or of all trials were averaged for each location, resulting in one array of 13
208 amplitude values per tap location. Position, amplitude, size and latency of the responses were identified
209 for each averaged distribution as follows: a cluster of channels with amplitude larger than 40% of the
210 maximal value of the 13 channels were identified (threshold determination detailed below; fig. 5), and: i)
211 the size of the active region within the VM was calculated as the number of channels included in the
212 cluster; ii) the localization of the EMG response was described as the barycentre of the channels,
213 calculated as

$$214 \quad \text{barycentre} = \frac{\sum ARV_{ch} POS_{ch}}{\sum ARV_{ch}}$$

215 with ch being each channel in the cluster, ARV being their Average Rectified Value (measure of amplitude),
216 POS being their position in the array. The channels in the cluster were used to estimate the latency of the
217 response, calculated as the average timing between the onset of the tap and the negative peak of each of
218 the channels.

219 For intramuscular recordings, the same taps analyzed in the all-trials surface EMG analysis for locations 1,
220 3 and 5 were included in the analysis. The amplitude of the response for each wire was calculated as the
221 root mean square value in the 10-50 ms window after tap onset. The amplitude of the baseline noise (root
222 mean square value of an epoch 10-50 ms before the tap) was subtracted from the amplitude of the

223 response. For each tap, the amplitude of the EMG response in each wire was expressed as a percentage
224 of root mean square value measured during isometric maximal knee extension (maximal value of 50 ms
225 epoch calculated with 45 ms overlapping windows). For each tap location, the normalized amplitude
226 measured in each of the three intramuscular electrodes was averaged across the thirty taps resulting in a
227 matrix of 3 participants \times 3 tap locations \times 3 fine-wire locations.

228 The 40% threshold for the surface EMG analysis was chosen based on the concurrent analysis of the
229 surface and intramuscular EMGs in the subset of three experiments where both EMGs were collected.
230 For each intramuscular recording location, the surface EMG amplitude distribution averaged over all trials
231 was compared to the intramuscular recordings. As seen in fig. 3 (bottom rows) no EMG responses were
232 observed in the wires other than the wire corresponding to the location of the mechanical tap. This
233 indicated that the low level EMG activity registered by the surface electrodes located above the
234 intramuscular wires with no EMG activity was not a response to the mechanical tap but was due to volume
235 conduction or crosstalk. Therefore, a series of thresholds from 5 to 95% of the peak value of the surface
236 EMG amplitude distribution were tested for each tap location (3 participants with 3 tap locations each).
237 The lowest threshold that excluded surface EMG channels placed above the intramuscular EMG locations
238 that exhibited no activity was selected for each location. The average threshold value across all 9 tap
239 locations (40%; 38.8% rounded up to the closest 5%, range: 25-50%) was used to analyze all data from the
240 HDsEMG. This threshold value is more conservative than the 70% used in other studies (Vieira *et al.*, 2010)
241 and will lead to larger estimated regions of active muscle fibres.

242

243 **Statistical Analysis**

244 Statistical analyses were performed using SPSS v. 22 (IBM Inc., Armonk, NY, USA). When data were not
245 normally distributed (Shapiro-Wilk test), non-parametric statistics were used. To verify that the input
246 force or amplitude of the response were effectively matched in the corresponding analyses, Friedman

247 tests were run to assess the effect of *Tap location* on input force (force-matched) or EMG amplitude of
248 the response (amplitude-matched); the variability of input force and EMG amplitude values across
249 locations was also verified using the CoV. To investigate the regionalization of the stretch reflexes within
250 the VM, the number of channels in the cluster was used as a measure of size of the active area within the
251 VM and the barycentre of the channels in the cluster was used as a measure of spatial localization of the
252 active area within the VM. The effect of ‘Tap location’ on the number of channels in the cluster was tested
253 using the Friedman test. The effect of ‘Tap location’ on the barycentre of the channels in the cluster was
254 tested using ANOVA with repeated measures, performed separately for force-matched, amplitude-
255 matched and all-trial analyses. Separate analyses were run to avoid violations of the assumption of
256 independent observations for the ANOVA test. As reflexes from locations 5 and 4 were not observed in
257 some participants, only locations 1, 2 and 3 were compared (additional locations are shown in fig. 6).
258 Post-hoc decompositions of main effects were performed using paired Student T-tests with Bonferroni
259 correction; for each pair of locations, effect sizes were calculated as:

260
$$d = \frac{mean}{SD}$$

261 where *mean* and *SD* are the mean and standard deviation of the difference between the groups. Results
262 from the validation with intramuscular electrodes are reported as the average across participants. Data
263 are reported as mean and standard deviation unless specified otherwise. The statistical significance was
264 set at $P < 0.05$.

265

266 **RESULTS:**

267 Localized muscle twitches could be visually observed in all participants (video: Muscle twitches in
268 response to mechanical taps). Reflexes were observed in the surface EMG signals as a single burst of
269 activity (fig. 3), with a mean latency of the largest negative peak of approximately 29 ms (force-matched:
270 29.2 ± 3.6 ms; amplitude-matched: 28.9 ± 3.6 ms; all-taps: 28.9 ± 3.4 ms). No medium- or long-latency
271 EMG responses to the taps were observed. No muscle activation was observed before applying the
272 mechanical taps (average rectified value calculated on a 100 ms window 50 ms before the taps, mean:
273 3.6 ± 0.9 μ V).

274 There was no difference in force or EMG response amplitude across tap locations for the trials selected
275 based on these measures, respectively, confirming that the matching was effective (force-matched: $P =$
276 0.89 , $CoV = 3.4 \pm 1.8\%$ across participants, 25th-75th percentiles: $13.3 - 22.7$ N; EMG amplitude-
277 matched: $P = 0.36$; $CoV = 9.6 \pm 5.4\%$, $57 - 166$ μ V; $N=5$ taps in each location). The response to a tap
278 always consisted in a single area of activity within the VM. The size of this active area spanned only few
279 channels for all tap locations (fig. 5) with the median being 5 channels irrespective of the tap location for
280 any analysis (all-trials: $P=0.14$, 25th–75th percentiles: $4.5-6$; force-matched: $P = 0.07$, $5-6$; amplitude-
281 matched: $P = 0.08$, $4-6$). All participants showed responses when taps were applied in the distal region
282 of the muscle (locations 1-3 in fig. 1). Taps applied to locations 4 and 5 elicited EMG responses in 8 and 5
283 participants, respectively (fig. 6). The location of the tap influenced the localization of the EMG
284 response on the grid (all-trials: $P < 0.001$, $F(2,16) = 45.5$; amplitude-matched: $P < 0.001$, $F(2,16) = 51.5$;
285 force-matched: $P < 0.001$, $F(2,16) = 60.5$; Fig. 6). For all analyses, post-hoc testing revealed that each
286 location was different from the other two (all $P < 0.01$; $t > 3.6$), resulting in large effect sizes ($d > 1.2$).
287 Taps applied more proximally along the patella resulted in more proximal responses within the VM than
288 taps applied more distally. This localization of the response was confirmed by the intramuscular EMG
289 recordings (fig. 3). Taps applied more proximally along the patella resulted in more proximal responses

290 within the VM than taps applied more distally. This localization of the response was confirmed by the
291 intramuscular EMG recordings (Fig. 3). Taps applied to the distal location (location 1) resulted in larger
292 EMG responses in the intramuscular wire placed distally in the VM (7.5% MVC distal; 0.7% MVC middle;
293 0.1% MVC proximal). Similar patterns of localized responses were observed for taps applied to the
294 middle (0.1%MVC distal; 11.8% MVC middle; 0.4% MVC proximal) and proximal location
295 (0.9%MVCdistal; 2.3%MVCmiddle; 9.1%MVC proximal). Responses for each participant are presented in
296 Table 1.

297

298 **DISCUSSION:**

299 The regionalization of the stretch reflex observed in this study implies that the human spinal cord
300 can independently recruit motoneurons innervating muscle fibres located in different regions within the
301 VM. As regional recruitment was observed in response to the activation of 1a afferents localized in regions
302 separated by only 10 mm, it follows that the human spinal cord has the circuitry to control motoneuronal
303 output regionally based on motor unit location.

304 Mechanical taps applied to the VM muscle fibres and HDsEMG enabled the characterization of
305 the spatial relation between regional stimulation of 1a afferents and location of the motor units recruited
306 by the spinal cord. Mechanical taps were used to activate muscle spindles located in different regions of
307 the VM. Although techniques such as the Hoffman reflex enable a fine control of the input and consistency
308 across trials and conditions (McNeil *et al.*, 2013), mechanical taps can target muscle spindles located in
309 different regions within large, flat muscles. HDsEMG was used to investigate the localization of the EMG
310 response within the VM. As the surface EMG amplitude peaks above the active motor units and decreases
311 with distance from the active muscle fibres (Roeleveld *et al.*, 1997), surface EMG amplitude distribution
312 obtained with HDsEMG provides information on the position of the active motor units within a muscle
313 (Roeleveld *et al.*, 1997; Vieira *et al.*, 2011; Gallina & Vieira, 2015; Gallina *et al.*, 2016). Spatial localization

314 was confirmed in a subset of participants using multiple intramuscular recordings, validating the findings
315 of the HDsEMG as localized activation was observed using both HDsEMG and intramuscular electrodes.
316 Using a threshold based on the intramuscular recordings, this study identified active areas spanning 5
317 channels of the grid (median value) in response to the mechanical taps. The 40% threshold we used is
318 more conservative than the threshold value previously utilized to identify regional activation in simulated
319 EMG signals (Vieira *et al.*, 2010). Similar spatial localization for different tap location was also observed
320 when data were analyzed using the 70% threshold (analysis not reported), although the active muscle
321 region was smaller (3 channels, median value). Regardless of the threshold used, the present results imply
322 that the active VM region in response to mechanical taps is not larger than 5 channels. This value,
323 however, may be an overestimation and further experimental validation is needed to determine the
324 threshold that accurately defines the contracting muscle region. Regardless, there are similarities
325 between the regional activation observed in the current and a previous study employing selective,
326 intramuscular stimulation (Gallina *et al.*, 2016). Previous research on the relationship between surface
327 EMG amplitude distribution and active fibres (Roeleveld *et al.*, 1997) and the results of the intramuscular
328 recordings in this study strongly suggest that activation in response to the mechanical tap was regionalized
329 within the vastus medialis.

330 Taps applied to muscle fibres in specific VM regions resulted in EMG reflex responses
331 preferentially observed in some channels of the electrode grid and in a single intramuscular site. In
332 addition, mechanical taps applied to muscle fibres in different VM locations resulted in EMG responses
333 localized in different regions within the muscle. This indicates that the excitation of muscle spindles of a
334 limited region of the muscle does not result in reflex activation of the whole muscle, but instead the reflex
335 is confined to a specific region. Localized activation of the VM to mechanical taps was observed regardless
336 of which taps were included in the analyses (force-matched, amplitude-matched, all-taps), strongly
337 supporting the main results of this study. The localization of the stretch reflex implies regionalization at

338 three levels of the spinal circuitry: preferential response of 1a afferents located in different regions of the
339 target muscle, specific connection of these afferents to motoneurons innervating the same muscle
340 region in the spinal cord, and motoneurons innervating fibres confined in a region of the muscle
341 (Windhorst *et al.*, 1989). Regional response of 1a afferents was demonstrated in animals (Cameron *et al.*,
342 1981) and humans (McKeon *et al.*, 1984), where mechanical stimuli applied to regions of a muscle were
343 shown to result in discharges of 1a afferents from those regions only. Our results support previous
344 observations suggesting that motoneurons innervate VM muscle fibres confined to limited regions of the
345 muscle (Gootzen *et al.*, 1992; Gallina & Vieira, 2015). The localization of the stretch reflex was shown in
346 cats (Cohen, 1953; Bilotto *et al.*, 1982; Eng & Hoffer, 1997) but not in humans (McKeon *et al.*, 1984).
347 Differences in the results between our study and the one by McKeon and colleagues (McKeon *et al.*, 1984)
348 may be related to differences in the architecture between the two muscles, e.g.: single long tendon vs.
349 flat insertion along the patellar edge. To our knowledge, this is the first evidence for the regionalization
350 of the stretch reflex in humans. Our results further reveal that this regionalization is distributed quite
351 finely, as clearly separated responses could be observed for locations as close as 10 mm apart. Similarly
352 to the observations for directional preference of motor unit activation in biceps brachii and deltoid
353 (Herrmann & Flanders, 1998), our results indicate that stretch reflexes can be elicited continuously across
354 the VM rather than clustering in anatomically-defined neuromuscular compartments (e.g.: VM longus or
355 obliquus, (Smith *et al.*, 2009)).

356 The regionalization of the stretch reflex may potentially modulate the motor output of large,
357 structurally complex muscles such as the VM. For instance, selective stretch reflexes may be useful in the
358 case of perturbations that result in preferential stretch of a muscle region, such as sudden directional
359 translation of the patella or tibio-femoral rotation which may occur especially in certain activities or
360 sports. The current study indicates that the human spinal cord has the neuromuscular circuitry to
361 modulate spatially the motoneuronal output to vastus medialis regions based on regional afferent

362 feedback. It has been suggested that regionalization of afferents and efferents may be used by the central
363 nervous system to shape patterns of activation in order to optimize muscle performance (Windhorst *et*
364 *al.*, 1989). The abundance and distribution of muscle spindles in human muscles was suggested to be
365 functionally useful to detect regional changes in length within the muscle and locally regulate the
366 motoneuronal output (Windhorst *et al.*, 1989). Indeed, while the synaptic input is largely shared across
367 motor units both within single muscles and between synergists (De Luca & Erim, 1994; Laine *et al.*, 2015),
368 the common drive between motor units tends to be lower in muscles with a higher density of spindles (De
369 Luca *et al.*, 2009). This suggests that afferent proprioceptive information from muscle spindles may
370 promote more independent motor unit firing patterns. The current study adds that the spinal cord has
371 the circuitry to spatially organize the 1a stretch response within a muscle. Furthermore, this study shows
372 that the human spinal cord has the neuromuscular circuitry to preferentially drive motor units localized
373 in different muscle regions. This constitutes a neuroanatomical substrate for reports of region-specific
374 motor unit recruitment (Herrmann & Flanders, 1998; Butler & Gandevia, 2008) and inhomogeneous
375 alteration of motor unit recruitment and firing rate in the condition of experimental pain (Tucker &
376 Hodges, 2009; Tucker *et al.*, 2009). Future studies should investigate whether regional activation of
377 afferents during voluntary contractions can alter motor unit recruitment strategies.

378 Overall, our results showed that mechanical stimulation of 1a afferents localized as close as 10
379 mm apart within the human VM resulted in regional recruitment of motor units whose location was
380 organized topographically with respect to the stimulus location. This indicates that the human spinal cord
381 has the neuromuscular circuitry to preferentially modulate the neural drive directed to motor units
382 residing in different muscle regions, which is a neuroanatomical prerequisite for regional activation of
383 skeletal muscles.

384

385 **ADDITIONAL INFORMATION:**

386 Competing Interests: The authors declare no competing interests.

387 Author contributions: Conception and design of the experiments: AG, JSB, SJG; Collection, assembly,
388 analysis and interpretation of data: AG, TI, SJG; Drafting the article or revising it critically for important
389 intellectual content: AG, JSB, TI, SJG. All authors approved the final version of the manuscript. All
390 persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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396

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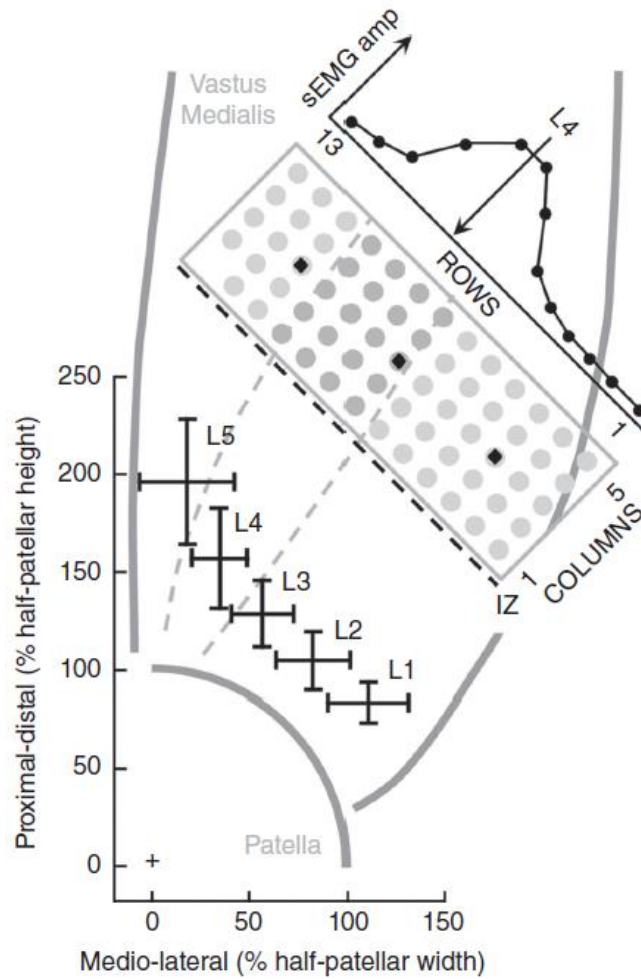
460 **TABLE:**

461 Table 1: Intramuscular EMG activation (%Maximal Voluntary Electrical activation) in response to
 462 mechanical taps. Rows identify different tap locations (DISTAL – location 1; MIDDLE – location 3;
 463 PROXIMAL – location 5); columns represent the three wires placed distally (D), middle (M) and proximally
 464 (P). Each row depicts the EMG responses in the three muscle locations for the same mechanical
 465 stimulation. For each participant, the wire with expected largest response (gray) recorded amplitude
 466 higher than the other two muscle regions in the same row

	PARTICIPANT 1			PARTICIPANT 2			PARTICIPANT 3		
	D	M	P	D	M	P	D	M	P
DISTAL	3.7	2.0	0.2	1.0	0.0	0.0	17.8	0.1	0.0
MIDDLE	0.1	12.8	0.3	0.0	0.4	0.0	0.0	22.2	0.9
PROXIMAL	0.3	2.4	8.1	0.9	0.8	1.4	1.6	3.5	17.8

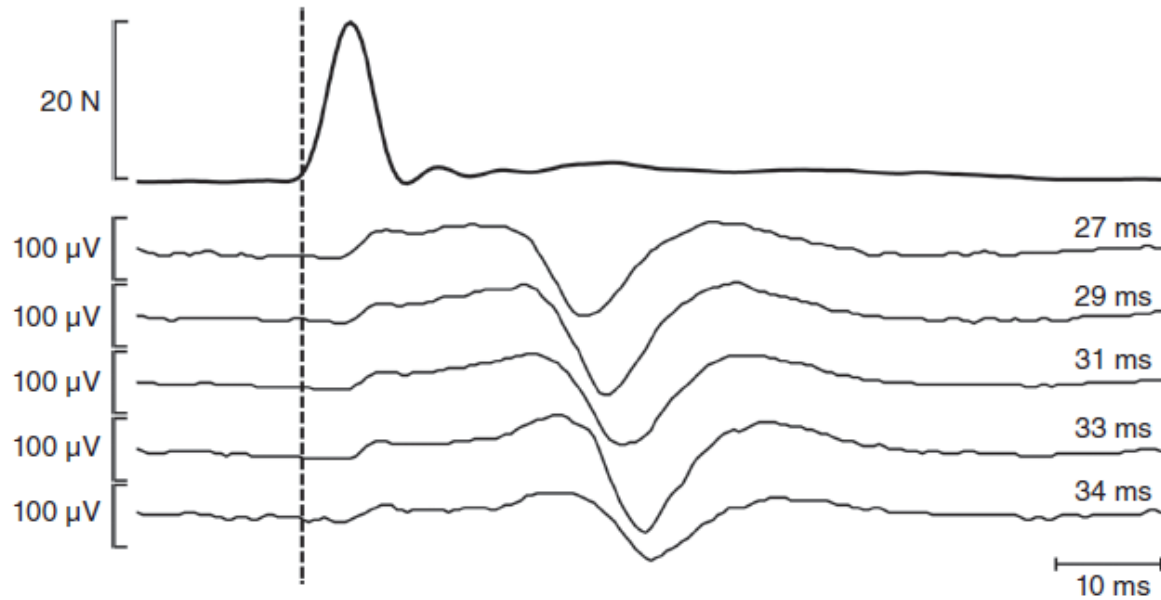
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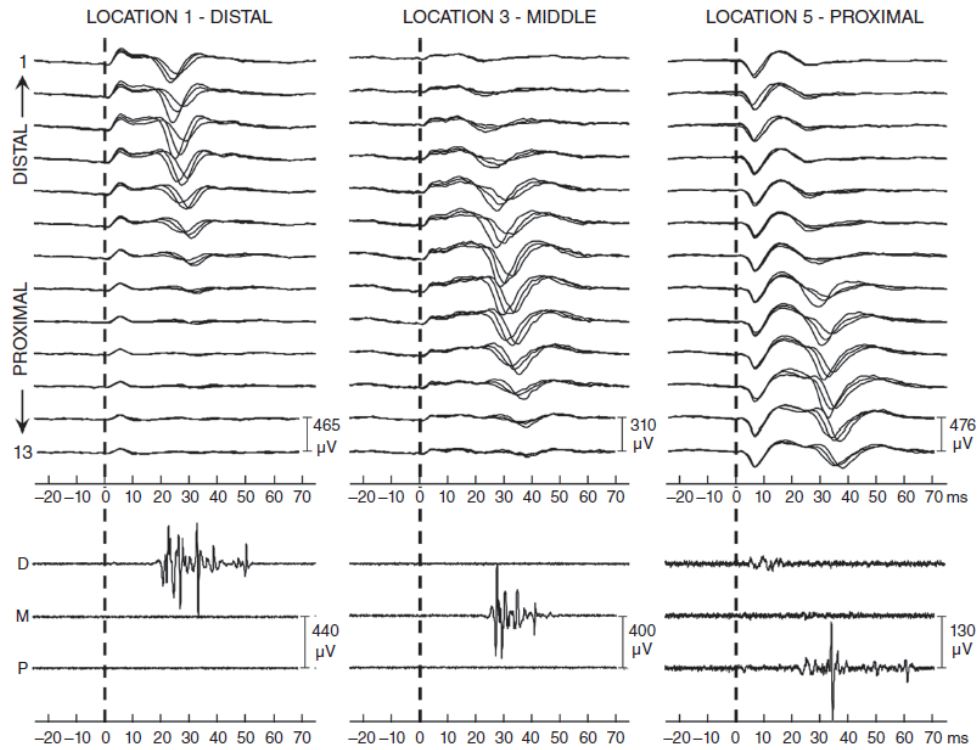
471 Fig.1: Experimental setup. The innervation zone is illustrated as a dashed black line. Black crosses identify
 472 the average location of the tendon taps (mean and standard deviation) across participants. The surface
 473 EMG amplitude plot on top of the electrode grid represents the expected spatial distribution of the
 474 response to the tap of location 4. The target location for the intramuscular wires is indicated by the three
 475 black diamonds.



476

477 Fig.2: Identification of a surface EMG response. The force signal used to determine the tap onset is
 478 depicted on top. EMG signals from five channels along the muscle fibre orientation are plotted in the
 479 channels below; numbers on the right side of the plot are latency estimates of the negative peak of the
 480 action potential. Physiological action potential propagation latencies ensured the distinction of artefacts
 481 from EMG reflexes.

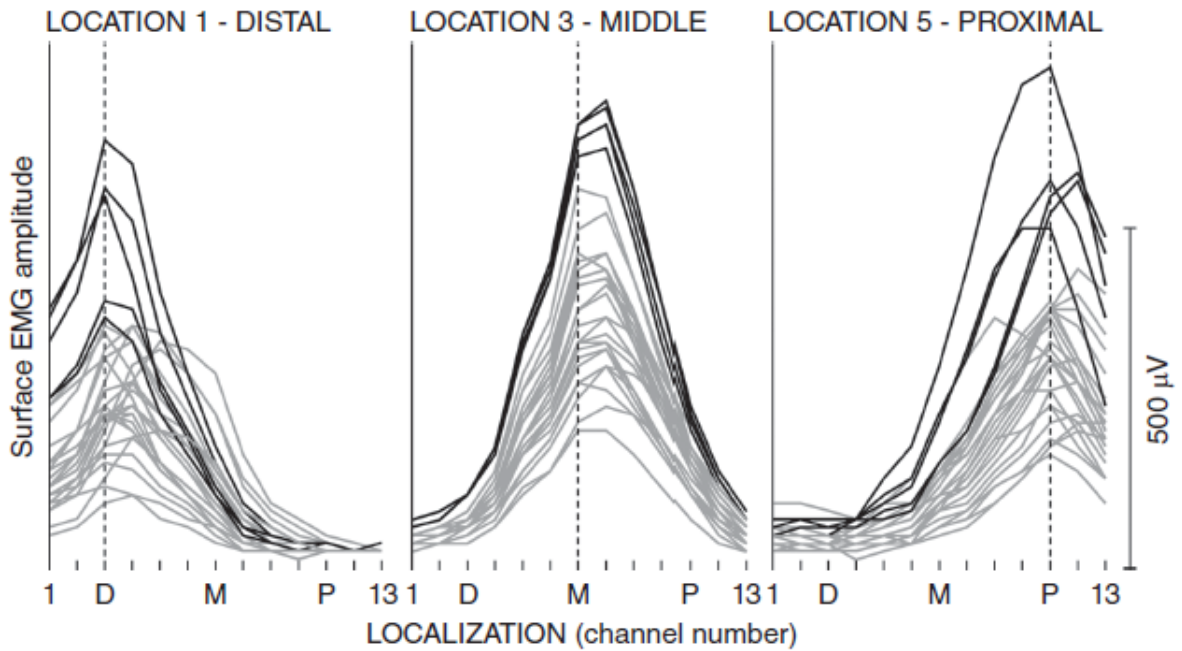
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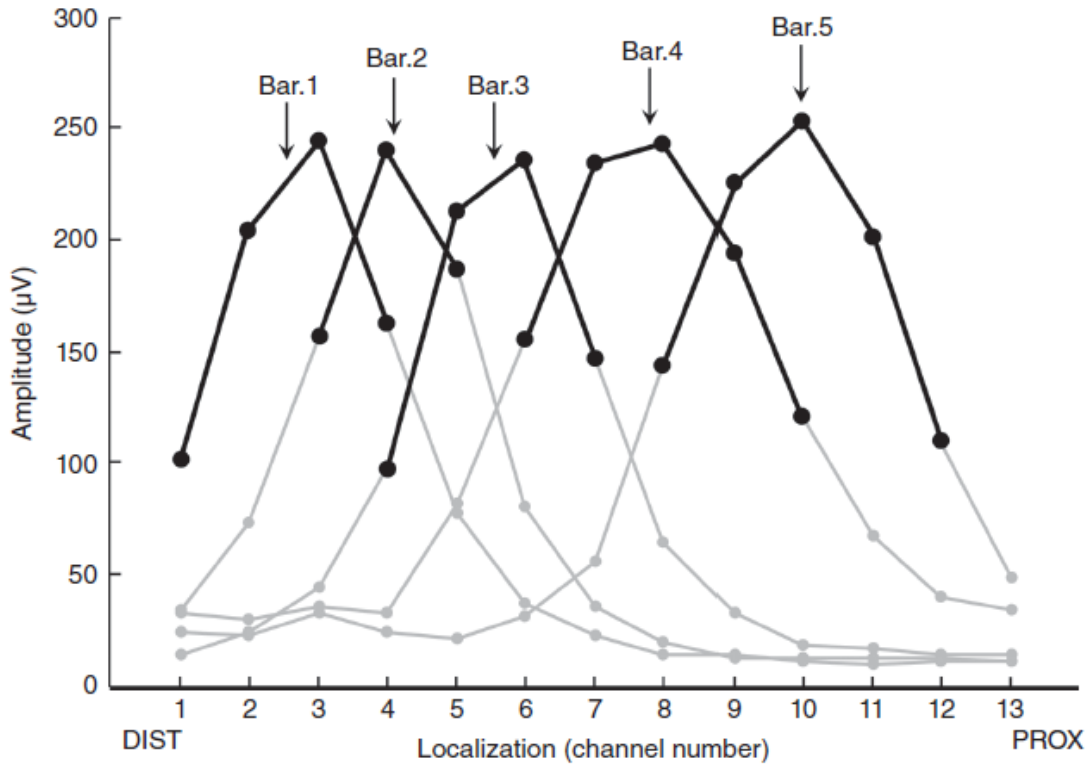
484 Fig.3: Example of responses to taps of location 1 (left panels), 3 (middle panels), and 5 (right panels) for
 485 participant 3 (Table 1). Surface EMGs channels (top panels) are organized from distal (ch.1) to proximal
 486 (ch.13); each row shows EMG signals from three channels placed along the approximate fibre orientation.
 487 For intramuscular EMG signals (bottom panels), the top signals was collected from the wire inserted in
 488 the distal region of the VM, the bottom ones from the most proximal.

489



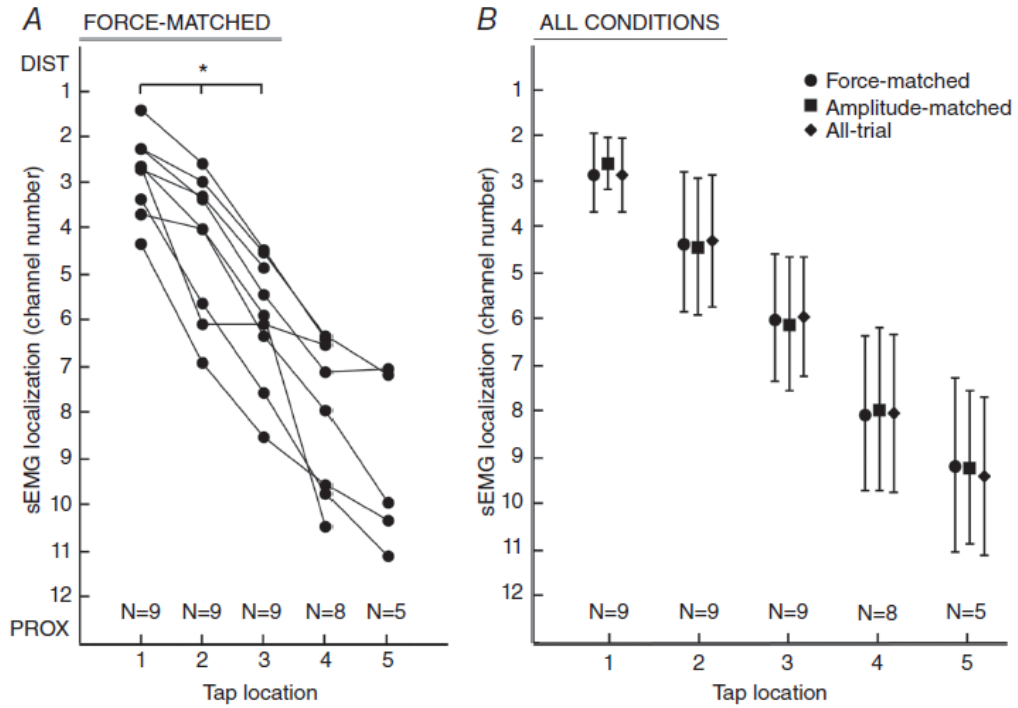
490

491 Fig.4: Responses to individual mechanical taps in three locations for a representative participant. The
 492 location of the proximal (P), middle (M) and distal (D) intramuscular wires are depicted. Black lines
 493 identify the five responses with highest amplitude; for each location, the spatial location of the response
 494 is similar across taps. The location of the intramuscular wire in relation to the HDsEMG grid is presented
 495 with dashed line in each panel.



496

497 Fig.5: Identification of size and location of EMG responses across the grid. Each gray line is the amplitude
 498 distribution calculated from five responses, matched for amplitude across locations. The arrows identify
 499 the barycentre (Bar) of the channels above the threshold (black circles) for taps of locations 1 to 5.



500

501 Fig.6: A) Effect of tap location on the localization of the response, force-matched condition. Lines depict
 502 the position of the responses on the grid for individual participants. * P < 0.001. B) Localization of the
 503 responses for force-matched, amplitude-matched and all-trial conditions.

504