

Preferential distribution of nociceptive input to motor neurons with muscle units in the cranial portion of the upper trapezius muscle

Dideriksen , Jakob; Holobar, Ales; Falla, Deborah

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5 **PREFERENTIAL DISTRIBUTION OF NOCICEPTIVE INPUT TO MOTOR**
6 **NEURONS WITH MUSCLE UNITS IN THE CRANIAL PORTION OF THE UPPER**
7 **TRAPEZIUS MUSCLE**

8
9 Jakob L. Dideriksen¹, Ales Holobar², Deborah Falla³

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12
13 ¹ SMI, Department of Health Science and Technology, Aalborg University, Aalborg,
14 Denmark.

15 ² Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor,
16 Slovenia

17 ³ School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental
18 Sciences, University of Birmingham, Birmingham, UK.

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26
27 **Address for correspondence:**

28 Prof. Dr. Deborah Falla
29 School of Sport, Exercise and Rehabilitation Sciences,
30 College of Life and Environmental Sciences,
31 University of Birmingham,
32 Birmingham, B15 2TT, UK.
33 Tel: +44 (0)121 415 4220
34 Email: d.falla@bham.ac.uk

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39 **ABSTRACT**

40 Pain is associated with changes in the neural drive to muscles. For the upper
41 trapezius muscle, surface EMG recordings have indicated that acute noxious
42 stimulation in either the cranial or the caudal region of the muscle leads to a relative
43 decrease in muscle activity in the cranial region. It is however not known if this
44 adaption reflects different recruitment thresholds of the upper trapezius motor units in
45 the cranial and caudal region or a non-uniform nociceptive input to the motor units of
46 both regions. This study investigated these potential mechanisms by direct motor unit
47 identification. Motor unit activity was investigated with high-density surface EMG
48 signals recorded from the upper trapezius muscle of 12 healthy volunteers at baseline,
49 control (intramuscular injection of isotonic saline), and painful condition (hypertonic
50 saline). The EMG was decomposed into individual motor unit spike trains. Motor unit
51 discharge rates decreased significantly from control to pain conditions by 4.0 ± 3.6
52 pps in the cranial region but not in the caudal region (1.4 ± 2.8 pps; not significant).
53 These changes were compatible with variations in the synaptic input to the motor
54 neurons of the two regions. These adjustments were observed irrespective of the
55 location of noxious stimulation. These results strongly indicate that the nociceptive
56 synaptic input is distributed in a non-uniform way across regions of the upper
57 trapezius muscle.

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63 **New and Noteworthy:** By evaluating adjustments in the behavior of motor units
64 located in different regions of the upper trapezius to experimentally induced pain, we
65 observed differential changes depending on the region of the muscle which were not
66 dependent on pain location. These findings indicate that nociceptive synaptic input is
67 distributed in a non-uniform way across regions of the muscle suggesting a fixed
68 response to muscle pain possibly with the aim of protecting the more sensitive muscle
69 region.

70 INTRODUCTION

71

72 Pain is associated with changes in the neural drive that muscles receive. In
73 single motor units, this has been extensively observed as a decrease in the discharge
74 rates (Sohn et al., 2000; Farina et al., 2004, 2005a; Hodges et al., 2008; Tucker et al.,
75 2009) or as de-recruitment of motor units (Tucker et al., 2009; Hug et al., 2013;
76 Minami et al., 2013) for the painful muscle. In such cases, maintenance of the net
77 motor output (e.g. a similar movement/position) is achieved by redistribution of the
78 activity to other motor neurons or to other muscles (Tucker et al., 2009; Hug et al.,
79 2013, 2014; Minami et al., 2013). The pain-related reduction in motor unit excitability
80 observed during experimentally induced muscle pain is likely due to a combination of
81 reflex mechanisms mediated by small diameter muscle afferents and reduced
82 supraspinal drive to the muscle (Farina et al., 2004), which may be a protective
83 mechanisms for minimizing the activity of the painful muscle (Lund et al., 1991;
84 Hodges and Tucker, 2011).

85 Previous studies that used multi-channel (high-density) surface EMG in the
86 upper trapezius muscle before and during experimentally induced muscle pain while
87 maintaining a steady 90 degree shoulder abduction position provided evidence of a
88 relatively greater reduction in muscle activity in the cranial compared to caudal region
89 of the muscle (Madeleine et al., 2006; Falla et al., 2009). The trapezius muscle acts as
90 an accessory muscle during shoulder abduction (Mathiassen et al., 1995) (deltoid is
91 the primary agonist). In this way, the arm position was not directly affected by
92 trapezius muscle pain, enabling a meaningful comparison between the muscle activity
93 with and without pain. The observed redistribution of the activity within the trapezius
94 muscle may be explained by two different underlying mechanisms. The first
95 possibility is that noxious stimulation of the muscle involves a uniform inhibition

96 across all motor units. Motor units of the caudal region are generally recruited before
97 those in the cranial region (Holtermann and Roeleveld, 2006) and consequently have
98 higher discharge rates at a given contraction level (Falla and Farina, 2008). Therefore,
99 a uniform distribution of inhibition across all motor units innervating the muscle will
100 lead to a higher chance of de-recruitment of cranial motor units, since the excitation
101 levels for these units is closer to their recruitment thresholds. In this way, the
102 compound activity in the cranial region would exhibit a greater relative reduction of
103 EMG amplitude. An alternative explanation for these observations is that the behavior
104 of motor units (i.e. discharge and recruitment patterns) of the two regions is adjusted
105 in different ways, as a result of non-uniform projections of nociceptive afferents.

106 Despite the fact that results based on the interference EMG indicate certain
107 adjustments to pain, the compound muscle activity cannot reveal details on the
108 underlying adjustments in motor unit behavior. For example, if a decline in motor unit
109 discharge rates (expected to decrease the EMG amplitude) occurs concurrently with
110 the recruitment of motor units (expected to increase the EMG amplitude), the EMG
111 amplitude may not change substantially. For this reason, the primary aim of this study
112 was to investigate changes in the behavior of motor units located in two regions
113 (cranial and caudal) of the upper trapezius muscle following the injection of
114 hypertonic saline (experimental muscle pain).

115 Interestingly, the adjustments to noxious stimulation of the upper trapezius
116 was confirmed to be independent of the location of the painful stimulus (Falla et al.,
117 2009). In this way, the greatest reduction of EMG amplitude occurred in the cranial
118 region, even when nociceptive afferents in the caudal region were stimulated.

119 Assuming that adjustments to pain aim to reduce the activity of the painful region
120 (Lund et al., 1991; Hodges and Tucker, 2011), this strategy appears suboptimal since

121 the activity in the affected region is not reduced to the highest possible degree. For
122 this reason, a secondary aim of the study was to investigate how the location of
123 noxious stimulation influences the changes in motor unit behavior across the two
124 muscle regions.

125

126 **METHODS**

127 *Subjects*

128 Twelve healthy volunteers (6 men; age: 26.5 ± 5.1 yrs; height: 173.2 ± 10.9
129 cm; weight: 65.6 ± 11.3 kg) participated in the study after providing written informed
130 consent. All participants were free of shoulder and neck pain, had no past history of
131 orthopaedic disorders affecting the shoulder or neck region and no history of
132 neurological disorders. All subjects were right hand dominant. The study was
133 conducted in accordance with the Declaration of Helsinki and approved by the local
134 ethics committee (N-200538).

135

136 *Procedure*

137 Subjects were comfortably seated in a chair with their back supported, knees
138 in 90° of flexion and feet flat on the ground. The subjects were asked to hold both
139 arms in 90° abduction for 60 s, with elbows fully extended and forearms pronated
140 with palms facing toward the ground. In this position, the load on the upper trapezius
141 is ~15-20% of the maximum voluntary contraction of the trapezius muscle
142 (Mathiassen et al., 1995). The task of shoulder abduction was selected since earlier
143 work had shown a redistribution of upper trapezius muscle activity in response to
144 noxious stimulation of the trapezius muscle (Falla et al., 2009). Two flexible bars
145 positioned on a board behind the subject extended horizontally over the subjects
146 shoulders to provide tactile position feedback. The bars also allowed the investigator

147 to monitor the subjects shoulder position during the 60 s contraction to ensure that the
148 subject did not move their arms in the transverse or coronal planes. Guides were also
149 placed behind and on the side of the subjects' head which were used by the
150 investigator to ensure the same position of the neck and head in all contractions.
151 Following a rest of 10 min, the subject repeated the sustained shoulder abduction
152 contraction after the injection of isotonic saline into the upper division of the right
153 trapezius muscle. Following a further 10-min rest the subject performed a final
154 sustained contraction following the injection of hypertonic saline into the right upper
155 trapezius.

156

157 *Experimental Muscle Pain*

158 Experimental muscle pain was induced by injection (27G cannula) of 0.4 ml
159 sterile hypertonic saline (5.8%) into the upper division of the trapezius muscle on the
160 right side. During each injection the subject was seated in a comfortable position. Half
161 of the subjects received the injection of hypertonic saline into the cranial region of the
162 upper trapezius and other half received the injection in the caudal region of the
163 muscle. The distribution of men and women was even for the two injection locations.
164 The cranial and caudal locations were defined as 15 mm cranial and 40 mm caudal to
165 the line between the acromion and the spinous process of the seventh cervical vertebra
166 respectively at an approximate depth of 1 cm. Isotonic saline (0.4 ml, 0.9 %) was used
167 as a control injection at the same location that the subject received the injection of
168 hypertonic saline.

169 The bolus was injected over a 10-s period. The isotonic saline injection was
170 given first however participants were blinded to each injection and were told that one
171 or both might be painful.

172

173 *Measures of Perceived Pain Intensity and Area*

174 Participants were asked to verbally rate their level of perceived pain intensity
175 on an 11 point numerical rating scale (NRS) anchored with “no pain” and “the worst
176 possible pain imaginable”. Pain intensity ratings were obtained immediately following
177 the injection and every 30 s until pain was no longer reported. For each trial, the peak
178 pain intensity and the duration of the pain were calculated. Participants documented
179 the area of pain on a body chart. Pain drawings were subsequently digitized
180 (ACECADD9000 + Taiwan) and pain areas were measured.

181

182 *Multi-channel surface EMG*

183 Prior to electrode placement, the main innervation zone location of the upper
184 trapezius along the seventh cervical vertebra (C7) - acromion line was identified with
185 an array of 8 electrodes (silver bars, 5-mm long, 1-mm diameter, 5-mm interelectrode
186 distance), as previously described (Farina et al., 2002).

187 During the experimental measures, surface EMG signals were detected with a
188 semi-disposable adhesive grid of electrodes (LISiN-OT Bioelettronica, Torino, Italy).
189 The grid consists of 13 rows and 5 columns of electrodes (1-mm diameter, 8-mm
190 interelectrode distance in both directions) with one electrode absent from the upper
191 right corner. The position corresponding to the missing electrode was used as the
192 origin of the coordinate system to define the electrode location. The subject’s skin
193 was prepared by gentle local abrasion using abrasive paste (Medic-Every, Parma, Italy)
194 and cleaned with water. The electrode grid was placed with the 4th row along the C7-
195 acromion line and with the most lateral electrode column 10-mm distant from the
196 innervation zone location (Figure 1). 30 µl of conductive gel was inserted into each

197 cavity of the grid to provide electrode-skin contact. A reference electrode was placed
198 around the right wrist.

199

200 [FIGURE 1 AROUND HERE]

201

202 The 51 bipolar channels were derived by subtracting EMG recordings from
203 two consecutive electrodes in direction of rows, amplified (128-channel surface EMG
204 amplifier, LISiN-OT Bioelettronica, Torino, Italy; -3dB bandwidth 10-500 Hz) by a
205 factor of 2000, sampled at 2048 Hz, and converted by a 12-bit analog-to-digital
206 converter.

207 The injections were performed lateral to the electrode grid (~ 10 mm) and
208 corresponded to the 2nd and 9th row of electrodes for the cranial and caudal locations
209 respectively (Figure 1).

210

211 *Surface EMG analysis*

212 The root mean square (RMS) of the EMG amplitudes was calculated for
213 each bipolar EMG channel , and the average EMG amplitude was defined as the
214 average RMS value across all channels. Next, the centroid of the EMG signals in the
215 medial-lateral and the cranial-caudal direction were calculated. First, the average
216 RMS values along one axis were calculated leaving five values (representing the 5
217 columns) for the medial-lateral direction and 12 values for the cranial-caudal
218 direction. The index of electrode number that divided these values into two parts of
219 50% of the sum of the RMS in that direction was defined as one coordinate for the
220 centroid. These procedures were repeated for each of the three contractions (control,
221 isotonic and hypertonic, respectively).

222 *High-density surface EMG decomposition*

223 Convolution Kernel Compensation (CKC) method, introduced in (Holobar and
224 Zazula, 2004, 2007) and validated in numerous previous studies (Farina et al., 2009;
225 Holobar et al., 2009, 2010, 2012; Marateb et al., 2011) was used to decompose the
226 acquired EMG signals into contributions of individual motor units. Once identified,
227 discharge times of individual motor units were dynamically tracked over entire EMG
228 signal, taking into account potential changes in shapes of motor unit action potentials,
229 such as those caused by small arm movements and fatigue (Holobar et al., 2009, 2010,
230 2012).

231

232 *Motor unit analysis*

233 Out of accurately identified motor units, only those discharging regularly
234 during the majority of first 60 s of the contraction were included in the analysis of
235 single motor unit behavior (Inclusion criteria: number of action potentials > 300;
236 coefficient of variation (CoV) for the inter-spike intervals < 45%). The discharge rate
237 characteristics were analyzed in four non-overlapping windows of 15 seconds
238 (starting immediately after the onset of the contraction).

239 All included motor units were divided into two groups based on their spatial
240 location. Cranial motor units were those identified from the first 6 rows of electrodes,
241 while motor units located from channels 7-12 were defined as caudal units. In the
242 cases where one motor unit was present in the channels of both regions, the channel at
243 which the amplitude of the action potential was highest determined the region. To
244 identify spike trains from the same motor unit across trials within each subject, the
245 correlation coefficient (averaged across all channels) and normalized root mean
246 square error (NRMSE) between the shapes of two action potentials recorded were

247 calculated for all potential pairs. First, all pairs of action potentials with correlation
248 coefficients below 0.92 were discarded. Next, the remaining pairs were manually
249 inspected by two experienced operators to determine the matching pairs of action
250 potential shapes.

251

252 [FIGURE 2 AROUND HERE]

253

254 The common synaptic input to groups of motor neurons in different frequency
255 bands was analyzed using coherence between groups of motor units spike trains
256 (Negro and Farina, 2012; Farina et al., 2014). Specifically, the coherence was
257 calculated between cumulative spike trains (CST), that were defined as the algebraic
258 sum of a subset of the motor unit spike trains. Unlike the single motor unit analysis,
259 all reliably identified motor unit spike trains were included, and duration of each CST
260 spanned the entire 60 s duration of the contraction. In the coherence spectrum, the
261 peak coherence in the delta (0-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands were
262 calculated. Coherence analysis was used to estimate the differences in the motor
263 neuron input with and without (control and isotonic conditions) pain in the two
264 following ways:

265 First, the common input across the two regions (and thus the whole upper
266 trapezius) was estimated. To this end, two CSTs were generated from the highest
267 possible number of motor unit spike trains of each region for each trial. This number
268 was equivalent to that of the region with the lowest number of identified motor unit
269 spike trains, so both CSTs contained the same number of motor units. In the cases
270 where an unequal number of motor units were identified across the regions, all
271 possible combinations of motor unit spike trains in the CST with the most spike trains

272 were used. The final estimate of the coherence spectrum was the average of the
273 spectrum obtained in each combination.

274 Next, the common input to each of the two regions was estimated. To this end,
275 two CSTs were generated from all the spike trains identified in each region for each
276 trial. The number of motor units per CST was the same, so if e.g. five motor unit spike
277 trains were identified in one region in one trial, two CSTs consisting of two motor
278 unit spike trains each, was generated. All possible combinations of motor units in the
279 two CSTs were used and the final estimate of the coherence spectrum was the average
280 of the spectrum obtained with each combination. In both cases, the CST-CST
281 coherence was estimated using Welch's averaged periodogram method in 10-s semi-
282 overlapping windows. Only coherence spectra based on CSTs consisting of at least
283 two motor unit spike trains with a combined rate of 20 pps throughout the contraction
284 was included for further analysis. The significance level for the coherence was
285 estimated using the method described by (Rosenberg et al., 1989).

286

287

288 *Statistical Analysis*

289 Data distributions were first checked with the Shapiro-Wilk normality test. All
290 data were normally distributed. One-way ANOVA were applied to parameters of pain
291 intensity, duration and area of pain with injection (hypertonic cranial, hypertonic
292 caudal, isotonic) as a factor and significant differences revealed by ANOVA were
293 followed by post-hoc Student-Newman-Keuls (SNK) pair-wise comparisons. The
294 average RMS of the EMG and the location of its centroid, as well as motor unit
295 discharge rates and the CoV for the inter-spike intervals across conditions (control,
296 isotonic, hypertonic) were analyzed using paired t-tests. The decline in the discharge
297 rate from the beginning (first 15 s) to the end of contractions (last 15 s) and the

298 coherence values in the same frequency bands in the painful vs. non-painful
299 conditions (baseline and isotonic) were analyzed using student's t-test. Results are
300 reported as mean and standard deviation (SD) in the text and standard error (SE) in
301 the figures. Statistical significance was set at $P < 0.05$.

302

303 **RESULTS**

304 *Sensory characteristics*

305 Peak pain intensity was greater following the injection of hypertonic (caudal:
306 4.3 ± 1.8 , cranial: 4.8 ± 1.6) compared to isotonic saline ($F = 19.7$, $P < 0.0001$; Figure
307 3). No difference in peak pain intensity was identified for the hypertonic saline
308 injections given at the two locations (SNK: $P > 0.05$).

309 Pain duration and area were not dependent on the location of the hypertonic
310 saline injection (Figure 3). The isotonic saline injection produced lower scores on all
311 measured pain parameters compared to the hypertonic saline injections ($P < 0.05$).

312

313 [FIGURE 3 AROUND HERE]

314

315 *Surface EMG variables*

316 The average amplitude of the surface EMG across all channels was
317 significantly lower after the injection of hypertonic saline ($58.1 \pm 26.8 \mu\text{V}$) compared
318 to the control condition ($74.9 \pm 38.7 \mu\text{V}$; $p=0.011$) and following the injection of
319 isotonic saline ($67.6 \pm 29.9 \mu\text{V}$; $p=0.008$). The difference in EMG amplitude from the
320 control to the isotonic condition was not statistically significant ($p=0.07$). The
321 reduction in mean EMG amplitude from the control to hypertonic condition (22%)
322 was similar to those previously observed in similar conditions (approximately 20-25%

323 (Madeleine et al., 2006; Falla et al., 2009)): The centroid of the surface EMG across
324 the channels (expressed in units of electrode number) did not change in the medial-
325 lateral direction (control: 3.00 ± 0.04 , isotonic: 3.01 ± 0.03 , hypertonic: 3.01 ± 0.03 ;
326 $p > 0.58$). However, following the injection of hypertonic saline, the centroid in the
327 cranial-caudal direction (6.64 ± 0.25) was located more caudally compared to the
328 control (6.31 ± 0.47 ; $p = 0.04$) and the isotonic (6.35 ± 0.40 ; $p = 0.05$) conditions. This
329 migration of the centroid did not depend on injection location (cranial: 6.55 ± 2.22 ;
330 caudal: 6.72 ± 2.69 ; $p = 0.16$).

331

332 *Motor unit behavior*

333 Across all trials, the spike trains of 199 single motor units were discriminated.
334 Of these, 127 single motor unit action potentials discharged regularly throughout the
335 contraction (cranial: 73 (mean per subject: 6.1 ± 2.7), caudal: 54 (mean per subject:
336 4.5 ± 4.0)). Of these, the trains of action potentials of eight caudal motor units (in seven
337 subjects) and 14 cranial motor units (in eight subjects) were reliably identified across
338 each of the three condition (baseline, isotonic and hypertonic; see Figure 2 for
339 examples). The average correlation coefficients of these pairs was 0.96 ± 0.01 and the
340 average NRMSE was $30.7 \pm 15.3\%$. Figure 4 summarizes the discharge
341 characteristics of these motor units. In both regions of the muscle, there was no
342 difference between initial motor unit discharge rates in the control condition and when
343 isotonic saline was injected. For the cranial motor units, however, the discharge rates
344 declined significantly in the presence of pain (control vs. hypertonic: 4.0 ± 3.6 pulses
345 per second (pps) ($p = 0.02$), isotonic vs. hypertonic: 4.2 ± 3.9 pps ($p = 0.02$); Figure 4A).
346 More modest and non-significant declines (1.4 ± 2.8 pps and 1.0 ± 3.6 pps; Figure 4B)
347 were observed for the caudal motor units across the same conditions. In fact, the

348 discharge rates for 3 of the 8 motor units were higher after the injection of hypertonic
349 saline compared to the control condition. There was no difference in the CoV for the
350 inter-spike intervals across conditions for motor units of the two regions (cranial:
351 $24.1 \pm 9.6\%$ (control), $21.6 \pm 7.7\%$ (isotonic), $25.7 \pm 10.2\%$ (hypertonic); caudal:
352 $27.7 \pm 6.7\%$ (control), $29.1 \pm 9.0\%$ (isotonic), $27.1 \pm 7.6\%$ (hypertonic)). The injection
353 location did not affect the motor unit discharge rates during the painful condition as
354 illustrated in Figure 4C. The trend described above, i.e. that the discharge rates of the
355 cranial units were lower than those of the caudal region, was maintained despite
356 different regions of noxious stimulation of the trapezius muscle.

357

358 [FIGURE 4 AROUND HERE]

359

360 The differences in average motor unit discharge rate across conditions for the
361 two regions were confirmed when analyzing all motor units, irrespective of whether
362 they were identified in more than one condition. There was little difference between
363 the discharge rates during the first 15 s of the contractions for the control and isotonic
364 conditions (cranial: 16.5 ± 3.8 pps (control) and 16.3 ± 3.3 pps (isotonic); caudal:
365 17.0 ± 3.8 pps (control) and 16.6 ± 2.3 pps (isotonic)), while the injection of hypertonic
366 saline implied a significant reduction ($p=0.004$) for cranial motor units (11.5 ± 3.6 pps)
367 than for caudal motor units (14.0 ± 2.3 pps). Unlike the discharge rates for the motor
368 units present across all trials (Figure 4), these results may have been biased towards
369 lower values by motor units recruited or de-recruited in the painful condition, as the
370 excitability of such motor units is expected to be lower than those active in all
371 conditions.

372 A total of 20 motor units (cranial: 4; caudal: 16) were identified in both the
373 control and isotonic conditions, but not with hypertonic saline. The average discharge
374 rates of these units were lower than for those identified in all conditions (cranial:
375 14.4 ± 4.5 pps; caudal: 14.6 ± 2.9 pps). Conversely, 25 motor units (cranial: 7; caudal:
376 18) identified in the trial with pain were not present in either of the two trials without
377 pain (discharge rates: cranial: 11.2 ± 3.6 pps; 13.8 ± 4.2 pps).

378 Throughout the duration of the contraction, discharge rates tended to decrease
379 more for the caudal motor units (control: $-17.8 \pm 7.2\%$ ($p < 0.001$), isotonic: -16.2 ± 8.8
380 % ($p = 0.001$)) than for the cranial motor units (control: $0.2 \pm 12.8\%$ ($p = 0.96$), isotonic:
381 $-9.0 \pm 9.1\%$ ($p = 0.03$)). Similarly, during pain a higher, but not statistically significant
382 change in the discharge rates was observed for the caudal region (cranial: $3.5 \pm 11.2\%$
383 ($p = 0.41$), caudal: $-9.9 \pm 12.2\%$ ($p = 0.05$)). These observations were confirmed when
384 considering all motor units (cranial, average values: -5.5% (control), -6.5% (isotonic),
385 3.4% (hypertonic); caudal, average values: -20.0% (control), -17.2% (isotonic), -
386 12.5% (hypertonic)).

387

388 *Coherence between cumulative spike trains*

389 The common input to motor neuron innervating muscle fibers across the two
390 muscle regions with and without pain were estimated from eight subjects each
391 (equivalent to 16 trials in which the CSTs fulfilled the inclusion criteria). The average
392 number of motor units per CST was 3.0 ± 0.8 and the rate of spikes in each CST was
393 similar for the two conditions (no pain: 39.4 ± 16.8 pps; pain: 38.2 ± 16.2 pps). Across
394 the three frequency bands, the peak coherence did not change with pain (Figure 5A),
395 indicating that the degree of common synaptic input to the entire upper trapezius was
396 unaffected. The peak coherence was significant in 10/16 trials (no pain: 5, pain: 5) for

397 the delta band, 8/16 trials (no pain: 3, pain: 5) for the alpha band, and in 5/16 trials (no
398 pain: 3, pain: 2) for the beta band.

399 The common input to motor neurons innervating muscle fibers in the cranial
400 region was estimated in 5 trials with pain (mean number of motor unit spike trains per
401 CST: 2.2; mean CST rate: 33.1 ± 8.0 pps) and in 13 trials without pain (mean number
402 of motor unit spike trains per CST: 2.4; mean CST rate: 31.2 ± 8.6 pps). For the cranial
403 region these numbers were 5 trials with pain (mean number of motor unit spike trains
404 per CST: 2.2; mean CST rate: 32.3 ± 6.1 pps) and in 10 trials without pain (mean
405 number of motor unit spike trains per CST: 2.4; mean CST rate: 29.6 ± 8.3 pps). In all
406 cases, the range for number of motor unit spike trains per CST was 2-4. Figure 5B
407 shows the change in average coherence in each region from no pain to pain in all
408 included trials. Overall, 59% of the coherence peaks in all included trials had
409 significant peaks (fewest significant peaks occurred in the beta band (<40%)).
410 Following pain, the average coherence in the delta band increased for both regions.
411 Accordingly, the percentage of trials with significant delta band coherence peaks
412 increased with pain by 10% (cranial) and 6.2% (caudal) respectively. In contrast, the
413 common input across motor neurons innervating muscle fibers in both regions tended
414 to decrease (Figure 5A), which suggests that the low-frequency input to these two
415 groups of motor neurons are under some level of independent control. For the alpha
416 band, pain tended to decrease the common input for cranial motor neurons, but to
417 increase the common input for the caudal motor neurons. Similarly, the percentage of
418 trials with significant coherence peaks increased for caudal motor units (33.8%), but
419 decreased for the cranial region (-20%). Finally, for the beta band, no substantial
420 changes during pain occurred. The within-trial variability in the peak coherences was

421 highest for the delta band (average standard deviations: delta: 0.08, alpha: 0.03, beta:
422 0.02).

423

424 [FIGURE 5 AROUND HERE]

425

426 **DISCUSSION**

427 In this study we investigated the adjustment in the behavior of motor units
428 located in different regions of the upper trapezius muscle to experimentally induced
429 pain. As reported in previous studies, and confirmed in the current study, the
430 amplitude of the surface EMG in the cranial region exhibited a larger decline relative
431 to that of the caudal region in response to pain (Madeleine et al., 2006; Falla et al.,
432 2009). To explain the underlying mechanisms for this observation, the study had two
433 aims: 1) To investigate whether these changes in EMG amplitude reflect uniform or
434 non-uniform adjustments across the motor units of the two regions, and 2) to
435 investigate whether the nature of the adjustments to pain across the two regions
436 depends on pain location.

437 With regards to the first aim, we found that the discharge rates of motor units
438 located in cranial region decreased by approximately 4 pps during pain (Figure 4).
439 This is approximately equivalent to 25% of the discharge rate in the control condition.
440 Conversely, the discharge rates of motor units located in the caudal region did not
441 change in response to pain. In comparison, the decreases in motor unit discharge rate
442 reported in other muscles for similar levels of acute pain (VAS: 3-6) are in the range
443 7-13% (Sohn et al., 2000; Farina et al., 2004; Hodges et al., 2008). Careful
444 examination of the results shown in these studies did not reveal indications of non-
445 uniform motor unit inhibition, except to some degree for the medial gastrocnemius

446 (see Figure 5B in (Hodges et al., 2008)). We believe that the observed difference in
447 the adjustment of the discharge rate among motor units of the two regions of the upper
448 trapezius can only be explained by that the nociceptive input affects the motor
449 neurons innervating the different muscle regions in different ways.

450 This observation was confirmed by the coherence analysis, that indicated that
451 adjustments in the motor unit behavior was not driven by changes in the common
452 synaptic input to the motor neurons innervating the two regions following pain
453 (Figure 5A). Instead, the synaptic input to the motor neurons innervating muscle
454 fibers in one of the regions changed in different ways (Figure 5B). Specifically, the
455 input in the alpha band increased for caudal motor units but decreased for cranial
456 motor units. This input has been associated to muscle-stretch sensitive feedback
457 (Lippold, 1970; Christakos et al., 2006; Erimaki and Christakos, 2008). This suggests
458 that the decrease in motor unit discharge rate in the cranial region may in part be due
459 to an increase in pre-synaptic inhibition of type Ia input mediated by the nociceptive
460 input. These changes, however, may also have been affected by differences in the
461 fatigue-related changes in discharge rate across regions (larger decreases for caudal
462 motor units) and conditions (smaller decreases during pain), as the CSTs spanned the
463 entire duration of the contraction.

464 When analyzing common motor neuron input using CST-CST coherence,
465 several methodological issues deserve consideration. The principle underlying the
466 analysis is that single motor unit spike trains are heavily influenced by synaptic noise
467 (independent motor unit input). For this reason, correlation between single motor unit
468 spike trains provide a poor basis for estimating the common synaptic input to the
469 motor neuron population, that is the effective neural drive to the muscle (Farina et al.,
470 2014). However, when spike trains from multiple motor units are considered (CSTs)

471 this bias will be reduced and the strength of the common input can be identified
472 (Negro and Farina, 2012; Farina et al., 2014). If common input is present at a given
473 frequency the CST-CST coherence will converge to 1 when a sufficiently high
474 number of motor units are included in each CST. This implies that in order to
475 compare the strength of the common input across two conditions in a meaningful way,
476 the appropriate number of motor units per CST must be higher than one (to reduce
477 influence of synaptic noise), but below the number at which the coherence converges.
478 This upper limit has been estimated to 3-6 motor units, but is likely to vary across
479 muscles and across different conditions (Negro and Farina, 2012; Farina et al., 2014).
480 In the current study, we used 2-3 motor units per CST with comparable numbers of
481 action potentials per second. Based on the above considerations, we believe that this
482 number enables not a complete, but a substantial reduction of the bias due to synaptic
483 noise with respect analyzing the correlation between single motor units. Furthermore,
484 it is unlikely that this number involved convergence of the coherence values, which
485 would disable a meaningful comparison between the two conditions (pain/no pain).

486 A total of 45 of motor units were identified either only with or only without
487 pain. For a number of reasons discussed in detail below, this may, at least in part,
488 reflect motor units that were either recruited or de-recruited following the painful
489 injection. A large proportion of these motor units (76%) were located in the caudal
490 region of the muscle. Although this observation does not prove a higher rate of
491 recruitment/de-recruitment among the caudal motor units compared to the cranial
492 motor units, it suggests that a substantial degree of recruitment/de-recruitment
493 occurred for these motor neurons. If so, this is surprising, since the motor units in this
494 region exhibited little adjustment to pain in their discharge rate, which would suggest
495 an unchanged input to those motor neurons. This could suggest that although the

496 average synaptic input to these motor units did not change, there was a large
497 variability in the effect of nociception on the single motor neuron (it may be inhibited
498 to a degree where it is no longer active or excited to a degree where it becomes active
499 or increases its discharge rate). The mechanism(s) underlying this adjustment are not
500 clear, but type III/IV afferents (the nerve fiber types carrying nociceptive feedback)
501 have been shown to excite as well as to inhibit motor neurons (Kniffki et al., 1981).

502 With regards to the second aim (dependence of the adjustment on pain
503 location), there was no difference between the motor unit discharge rates within the
504 individual muscle region when hypertonic saline was injected in the same or in the
505 other region (Figure 4C). This indicates that the underlying neural mechanisms
506 reflected in the relative decrease of the amplitude of the EMG signal in the cranial
507 region are similar in the two conditions. Furthermore, this observation implies that
508 when pain was induced in the caudal region of the muscle, the net activity of the
509 trapezius was redistributed to be concentrated in that area. According to a recent
510 theory of motor adaptation to pain (Hodges and Tucker, 2011), muscle activity is
511 redistributed (within or across muscles) to minimize activity of the painful region with
512 the aim of “protecting” the painful area. As described above, however, the
513 adjustments to experimentally induced muscle pain in the caudal region of the upper
514 trapezius did not optimally protect the caudal region. This suggests that, when the
515 upper trapezius muscle is painful, the adjustment aims always to preferentially
516 minimize activation of the cranial region. The functional advantage underlying this
517 strategy, however, cannot readily be identified based on the current results. The
518 fascicles of cranial region attach to the lateral part of the clavicle, while fascicles in
519 the caudal region attach along the superior border of the scapula (from the acromion
520 along the scapula ridge) (Johnson et al., 1994). In this way, during 90 degree shoulder

521 abduction both regions contribute to upwards rotation of the clavicle and scapula with
522 comparable moment arm lengths. The physiological cross-sectional area and thus the
523 maximum force, however, is higher for the fascicles in the caudal region (Johnson et
524 al., 1994), which implies that reducing their activity less than those in the cranial
525 region may have a smaller mechanical impact on the stabilizing actions of trapezius
526 muscle during arm abduction. Alternatively, the decrease in muscle activity of the
527 cranial region may be explained by the fact that this region has higher pain sensitivity
528 (Binderup et al., 2010).

529 The identification of spike trains from the same motor unit across trials was
530 based on the similarity of the morphology of the MUAP (temporal and spatial
531 “fingerprint”; see Figure 2 for examples). The temporal morphology (shape of the
532 MUAP) does not change in the presence of experimentally induced pain (Farina et al.,
533 2005b), and, although severe muscle fatigue can induce substantial changes in the
534 MUAP shape (Dimitrova and Dimitrov, 2003), contractions which are equivalent to
535 ~20% MVC sustained for 60 seconds are unlikely to induce such levels of fatigue in
536 young, healthy individuals. In addition, in this study the gradual changes in MUAP
537 shapes were tracked and compensated for by CKC method (see Methods section).
538 Accordingly, the maximum decline in discharge rate (a typical indicator of muscle
539 fatigue) was below 20%, whereas it may be as high as 50% with severe fatigue
540 (Bigland Ritchie et al., 1983; Enoka et al., 1989). The spatial morphology
541 (distribution of the MUAP across the electrode grid) indicates the position of the
542 muscle fibers of the motor unit within the muscle and is thus unaffected by pain and
543 fatigue, as long as the arm position is maintained. These considerations support the
544 assumption that the activity of the motor units which were identified across all tasks
545 was accurately classified.

546 Classically, motor unit spike trains have been identified using highly selective
547 fine-wire intramuscular EMG electrodes. A well-known risk related to such
548 recordings in multi-trial experiments is that small movements of the electrode within
549 the muscle may change the location of the muscle fibers with respect to the recording
550 site. In this way, such changes can change the MUAP morphology, making it
551 impossible to recognize the same motor unit across two trials even though it remains
552 active. Since the high-density surface EMG grid covers a large proportion of the
553 muscle, the risk of failing to detect the spike trains due to such factors is eliminated.
554 Instead, the most likely reason for false negative identifications of a single motor unit
555 in one trial was related to the procedure for matching MUAP shapes across trials. This
556 procedure was relatively conservative (automatic pre-selection by strict inclusion
557 criteria and manual selection by two operators) to minimize the risk of false
558 detections. Nevertheless, it remains likely that motor units detected in only one
559 condition and not in others to some degree reflected actual recruitment/de-recruitment
560 of that unit. Furthermore, this is compatible with the observation that these motor
561 units had lower discharge rates, which would normally be expected of the latest
562 recruited motor unit (De Luca et al., 1996).

563 In conclusion, this study confirmed that upper trapezius muscle activity
564 exhibits a relatively greater reduction in the cranial compared to caudal region of the
565 muscle in response to pain evoked in either of the two muscle regions (Falla et al.,
566 2009). Furthermore, by analyzing the behavior of single motor units we found
567 evidence that nociceptive input is non-uniformly distributed across the motor units of
568 the two regions. Specifically, motor units in the cranial region exhibited large declines
569 in their discharge rate, whereas caudal motor unit discharge rates were unaffected.
570 Finally, we found that the adjustments to pain were similar irrespective of the location

571 of pain, suggesting a fixed response to pain anywhere in the upper trapezius possibly
 572 with the aim of protecting the cranial region from overuse.

573

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577

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681 **Figure Legends**

682 **Figure 1.** (A) Schematic representation of the electrode grid positioned over the right
683 upper trapezius with the indication of the location of the injections of
684 isotonic/hypertonic saline into the cranial and caudal regions of the upper trapezius.
685 The rectangle on the right illustrates the spatial distribution of the innervation zone
686 across the electrode grid for all subjects (black: high probability; white: low
687 probability). Here, the white, dashed line represents the most common location for all
688 subjects.

689

690 **Figure 2.** Trains of motor unit (MU) action potentials for one representative subject in
691 the control condition (A) and with hypertonic saline (B). Based on the shape and the
692 spatial distribution, the action potential from motor unit #1 (bold black lines in A and
693 B) and from #4 (bold grey lines in A and B) were identified as coming from the same
694 motor units across the two conditions. The shape and spatial distribution of the action
695 potential of motor unit #1 are shown in panels C (control condition) and D (pain
696 condition). Similarly, the action potential for motor unit #4 is shown in E (control
697 condition) and F (pain condition). Motor units #2 and #3 were not the same across the
698 two conditions. Each line in C, D, E, F represents the estimated shape of the action
699 potential as from each bipolar recording in intervals of 40 ms (± 20 ms with respect to
700 identified discharge time). The correlation coefficient for the action potentials across
701 the two conditions was 0.95 for MU#1 and 0.96 for MU#4. The injections was
702 performed on the right (lateral) side of at the 2nd row of electrodes (cranial) and at the
703 9th row of electrodes (caudal).

704

705 **Figure 3.** Mean (\pm SE) pain intensity scores following the injection of isotonic saline
706 and hypertonic saline into the cranial and caudal region of the upper trapezius muscle.
707 No differences in peak pain intensity were observed for the injection of hypertonic
708 saline in the two locations.

709

710 **Figure 4.** Discharge rate characteristics for motor units identified in all three
711 conditions. Panels A and B show the average discharge rate for the first 15 s across
712 the three conditions (control: white; isotonic: grey; hypertonic: black) for motor units
713 located in the cranial and caudal regions, respectively. * indicates statistically
714 significant difference across conditions ($p < 0.05$). Panel C shows the motor unit
715 discharge rates during the first 15 s following the injection of hypertonic saline for
716 motor units located in the cranial and caudal regions, depending on injection location
717 (uni-colored: caudal; stripes: cranial). Each bar includes 10, 11, 11, and 15 motor
718 units (from left to right). For motor units of each region, there was no statistically
719 significant difference between injection location (cranial: $p = 0.65$; caudal: $p = 0.45$).

720

721 **Figure 5.** Common input to the motor neurons innervating cranial motor units and
722 motor neurons innervating caudal motor units with and without pain (A). The
723 boxplots represent 0.25, 0.5 and 0.75 quartiles (whiskers indicate full range) of the
724 peak coherence in the delta (0-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz). Panel B
725 shows the pain-evoked changes in peak coherence across the delta, alpha and beta
726 bands for CSTs consisting of spike trains from cranial motor units (dark grey) or from
727 caudal motor units (light grey). The dashed line in panel A indicates the level for
728 significant coherence. In panel B, the number “n” below/above each bar indicates the
729 number of trials included (significant coherence peaks).