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# Pain-induced changes in motor unit discharge depend on recruitment threshold and contraction speed

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1	Pain-induced changes in motor unit discharge depend on
2	recruitment threshold and contraction speed
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28 ABSTRACT

29 At high forces, the discharge rates of lower and higher threshold motor units (MU) are influenced in a 30 different way by muscle pain. These differential effects may be particularly important for performing 31 contractions at different speeds since the proportion of lower and higher threshold MUs recruited varies 32 with contraction velocity. We investigated whether MU discharge and recruitment strategies are 33 differentially affected by pain depending on their recruitment threshold (RT), across a range of contraction 34 speeds. Participants performed ankle dorsiflexion sinusoidal-isometric contractions at two frequencies 35 (0.25Hz and 1Hz) and two modulation amplitudes [5% and 10% of the maximum voluntary contraction 36 (MVC)] with a mean target torque of 20%MVC. High-density surface electromyography recordings from 37 the tibialis anterior muscle were decomposed and the same MUs were tracked across painful (hypertonic 38 saline injection) and non-painful conditions. Torque variability, mean discharge rate (MDR), DR variability 39 (DRvar), RT and the delay between the cumulative spike train and the resultant torque output 40 (neuromechanical delay, NMD) were assessed. The average RT was greater at faster contraction velocities 41 (p=0.01) but was not affected by pain. At the fastest contraction speed, torque variability and DRvar were 42 reduced (p<0.05) and MDR was maintained. Conversely, MDR decreased and DRvar and NMD increased 43 significantly during pain at slow contraction speeds (p<0.05). These results show that reductions in 44 contraction amplitude and increased recruitment of higher threshold MUs at fast contraction speeds 45 appears to compensate for the inhibitory effect of nociceptive inputs on lower threshold MUs, allowing 46 the exertion of fast submaximal contractions during pain.

47 Keywords: Pain, hypertonic saline, motor unit, discharge rate, recruitment, neuromechanical delay

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#### 50 NEW & NOTEWORTHY

Pain induces changes in motor performance, motor unit recruitment and rate coding behavior that varies across different contraction speeds. Here we show that that pain reduces motor unit discharge rate and prolongs the neuromechanical delay at slow contraction speeds only. This new evidence suggests that there are differential nociceptive inhibitory effects across the motor unit pool, which allows fast submaximal contractions to be exerted despite the presence of pain.

#### 56 **INTRODUCTION**

57 The investigation of motor unit properties has helped to elucidate the main neural mechanisms 58 responsible for changes in motor function caused by pain. Previous research employing experimental pain 59 paradigms, such as intramuscular hypertonic saline injection, has commonly reported a decrease in the 60 discharge rate of lower threshold motor units during noxious stimulation of the muscle (12-14, 18, 25, 34, 61 36). This behavior is believed to be related to inhibitory mechanisms (i.e. group III-IV afferent inhibition) 62 (12, 13, 34, 36) acting on the motor neuron pool. More recent research has reported that nociception 63 induces differential adaptations across the motor unit pool, with inhibition of lower threshold units and 64 excitation of higher threshold units, presumably to unload the painful tissue while still maintaining the 65 exerted force (25). These findings suggest that higher threshold motor units are not inhibited by 66 nociceptive input and can compensate for the decrease in discharge rate of lower threshold units, allowing 67 the exertion of high submaximal forces in the presence of pain (25). This differential mechanism of 68 inhibition/excitation may be particularly relevant when the central nervous system (CNS) is required to 69 exert force at varying contraction speeds. Pain would presumably influence the activity of motor units at 70 varying speeds of contraction yet it should be possible, at least for a range of contraction speeds, to 71 maintain the same functional output.

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72 Contraction speed influences the activity of motor units in non-painful conditions, such that a greater 73 proportion of higher threshold motor units are recruited for contractions of increasing speed (7, 9). 74 Therefore, we expect that pain may affect motor unit discharge rate and recruitment differently during 75 contractions at low and high speeds since the proportion of lower and higher threshold motor units 76 recruited during these contractions is different. Nevertheless, there are no studies that have compared 77 the effect of pain on motor unit firing behavior across different contraction rates. We aimed to assess the 78 effect of experimental muscle pain, induced via intramuscular injection of hypertonic saline, on tibialis 79 anterior motor unit firing properties during isometric dorsiflexion contractions at different submaximal 80 contraction rates. We hypothesized that motor unit discharge rate and the delay between the 81 modulations in discharge rate and the resultant muscle force (also known as the neuromechanical delay 82 (7)) would be less affected during fast contractions due to an increased recruitment of higher threshold 83 motor units compensating for the influence of nociceptive inhibitory inputs on lower threshold motor 84 units.

#### 85 MATERIALS AND METHODS

The study was conducted between 14/05/2018 and 10/11/2018 at the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), University of Birmingham. All procedures were approved by the University of Birmingham ethical committee (approval number: 16-0934) and were conducted in accordance with the Declaration of Helsinki, except for registration in a public database. This study is reported according to STROBE guidelines (39).

Prior to the experiment, the participants were informed that the pain intensity could range from moderate
to severe. Informed written consent was obtained from all study participants before the experiment.
Fifteen volunteers participated [age 26 (3) years, nine males and six females]. Inclusion criteria were
healthy adults between 18 and 35 years old. Exclusion criteria were current or previous history of lower

95 limb pain, past history of orthopedic disorders affecting the leg, history of neurological disorders, known
96 bleeding disorders and taking anticoagulant medication.

97 The sample size was calculated based on an alpha level of 0.05, a power of 0.8, an effect size of (f) 0.36 98 (calculated from one-way repeated measures ANOVA results of previous data (12, 15) and a potential 20% 99 loss of data due to poor signal quality or participant withdrawal. The data from one participant had to be 100 removed from the analysis due to poor signal quality (motor units could not be tracked across all 101 conditions) and therefore the results are presented for 14 participants [age: 26 (3) years, eight males and 102 six females].

#### 103 Experimental muscle pain

104 Muscle pain was induced by injection (27-gauge cannula) of sterile hypertonic saline (0.5 ml, 5.8%) into 105 the tibialis anterior muscle, 10-mm distal to the third column of the electrode grid (see below). Isotonic 106 saline (0.5 ml, 0.9%) was used as a control injection at a similar location. The bolus of hypertonic or 107 isotonic saline solution was injected manually over a 10-s period. All participants performed isometric 108 ankle dorsiflexion under four conditions: baseline, isotonic, pain and post pain. Baseline and isotonic 109 conditions were randomized across participants and were always followed by pain and post pain 110 conditions as conducted previously (25). Therefore, the isotonic saline injection was administered before 111 the hypertonic saline injection, however, participants were advised that both injections may or may not 112 be painful. The first three conditions were each separated by 5-min rest. The contractions in the post pain 113 condition were performed 15 min after the cessation of pain.

All participants were asked to verbally rate their level of perceived pain intensity on an 11-point numerical rating scale (NRS) anchored with "no pain" and "the worst possible pain imaginable." Pain intensity ratings were obtained immediately after the injection and every 30 s until pain was no longer reported. By the end of the experiment, participants also marked the region where they felt pain on a body chart. 118 Task

119 Participants were seated with their trunk flexed in 30° (in relation to the horizontal plane) on the chair of 120 a Biodex System 3 dynamometer (Biodex Medical Systems). The subject's dominant leg (right for all 121 participants) was positioned over a support with the knee flexed to 160° (with 180° representing full knee 122 extension), and the foot fixed to a footplate (90° ankle joint angle). The lateral malleolus was aligned to 123 the center of rotation of the dynamometer in order to measure ankle dorsiflexion torque. A computer 124 monitor providing real-time feedback of the exerted dorsiflexion torque was positioned approximately 125 1.5 m away at eye level. At the beginning of the session, in the absence of pain, ankle dorsiflexion 126 maximum voluntary contraction (MVC) torgue was recorded three times, each separated by 2 min of rest. 127 The maximal MVC defined the submaximal torque level exerted by the participants in the subsequent 128 contractions. Following the MVC measurement, participants were given time to practice with the visual 129 feedback of their exerted torque (as seen on the computer monitor), by performing the same sinusoidal 130 contractions used in the main protocol (see below) in order to familiarize themselves with the task and 131 reduce the possibility that learning effects would affect the results. After 5 min of rest, participants were 132 asked to track sinusoidal torque trajectories at a frequency of 0.25 or 1 Hz and amplitudes of 5 or 10% 133 MVC with a mean torque level of 20%MVC (modulation in torque from 17.5%MVC to 22.5%MVC when the amplitude was set at 5%MVC and from 15%MVC to 25%MVC when the amplitude was set at 10%MVC). 134 135 Participants performed four sinusoidal contractions in all conditions (baseline, isotonic, pain and post pain) 136 with a 30s rest between contractions. The combination of sinusoidal frequencies and amplitudes were: 1) 137 0.25Hz and 5% amplitude, 2) 0.25Hz and 10% amplitude, 3) 1Hz and 5% amplitude and 4) 1 Hz and 10% 138 amplitude (Figure 1). These combined frequencies and amplitudes represented different contraction rates 139 (quantified as the product of the amplitude and frequency of the sinusoidal torque trajectory) with a rate 140 of change in torque (force derivative) of 7.9 %MVC/s, 15.7 %MVC/s, 31.4 %MVC/s and 62.8 %MVC/s,

respectively (Figure 1). Each contraction lasted 40s. The contraction order was randomized but the randomization order was kept constant across conditions.

143 *Electromyography* 

144 Surface electromyography (EMG) signals were recorded from the tibialis anterior and gastrocnemius 145 medialis muscles. Signals from the tibialis anterior muscle were recorded using a high-density, 64-channel 146 surface EMG electrode grid (OT Bioelettronica, Torino, Italy) consisting of 5 x 13 electrodes (1-mm 147 diameter, 8-mm interelectrode distance). The grid was located between the proximal and distal tendons 148 of the muscle, with the columns oriented parallel to the tibia (25). Signals from the gastrocnemius medialis 149 were recorded in bipolar mode with Ag-AgCl electrodes (Ambu Neuroline 720, Ballerup, Denmark; 150 conductive area 28 mm2), as reported previously (2). Signals were amplified and recorded (2048 Hz 151 sampling rate) using an OT Bioelettronica Quattrocento amplifier (16-bit analog-digital converter). The 152 EMG data were processed and analyzed offline using MATLAB 2020a (MathWorks, USA). Before further 153 processing, the 64 monopolar EMG channels (referenced at the lateral malleolus) were re-referenced 154 offline to form 59 bipolar channels in the presumed direction of the muscle fibers.

# 155 Motor unit decomposition and tracking

156 The HDEMG signals were decomposed into motor unit spike trains with a previously validated algorithm 157 based on blind source separation (29). The same individual motor units were followed across conditions 158 and contraction speeds in two different ways. Firstly, all contractions performed at each contraction rate 159 (i.e. 7.9 %MVC/s or 0.25Hz-5%MVC) were merged and decomposed together in order to follow the 160 behavior of the same motor units that were active during baseline, isotonic, pain and post conditions (26). 161 Secondly, contractions were also merged between different speeds in baseline, isotonic, pain and postpain conditions independently (i.e. 0.25Hz-5%MVC, 0.25Hz-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC 162 163 during baseline) in order to check the effect of contraction speed on the number of identified motor units.

164 Firing statistics (i.e. discharge rate and recruitment threshold) are only reported from units tracked with 165 the first approach (same velocity at different conditions). Only the motor units that were observed across 166 all conditions (baseline, isotonic, pain and post pain) were included in the analysis. There were some cases 167 in which motor units were recruited and de-recruited during the contractions (i.e., higher threshold motor 168 units in contractions with a modulation amplitude of 10% MVC). These units were kept in the analysis only 169 if they were present across all conditions. Each identified motor unit was then assessed for decomposition 170 accuracy with a validated metric (Silhouette), which represents the sensitivity of the decomposed spike 171 train (29). Only motor units with an accuracy >90% were included into the analysis. Moreover, further 172 examination of each spike train was performed visually by an experienced operator. Missing pulses 173 producing unphysiological firing rates i.e., inter-spike intervals >250ms, were manually and iteratively 174 included and the pulse train was re-estimated to correct the frequency profile (with the exception of 175 pauses seen in higher threshold units with continuous recruitment-de-recruitment). In cases where the 176 algorithm incorrectly assigned two or three pulses to what was likely only a single discharge time, the 177 operator removed this firing and the final pulse trains were re-estimated as presented previously (1, 4, 178 24).

# 179 Torque and motor unit analysis

The torque signal was low-pass filtered (15Hz) and then compared against the displayed torque target by cross-correlation and the mean squared error (MSE) in order to check the effect of torque tracking accuracy across all conditions and contraction rates. Mean torque, standard deviation of torque (SD torque), the coefficient of variation of torque (CoV torque) and, minimum and maximum torque were assessed in order to confirm the maintenance of the average torque target during the sinusoidal contractions (~20% MVC) and to check the effects of pain on the amount of torque modulation across contraction rates, respectively. Discharge times of the identified motor units were converted into binary 187 spike trains, and motor unit firing data was quantified as the inverse of the inter-spike interval 188 (instantaneous firing rate). Mean discharge rate was analyzed on the central part of the contraction after 189 the first sinusoid and before the last sinusoid at each contraction rate. Discharge rate variability was 190 quantified on the same region with the coefficient of variation of discharge rate (CoV discharge rate, SD 191 discharge rate/mean discharge rate \* 100). Maximum and minimum discharge rate values were calculated 192 as the maximum and minimum instantaneous discharge rates observed at the point which corresponded 193 with maximum and minimum torque values. Finally, motor unit recruitment threshold was defined as the 194 ankle dorsi-flexion torque (%MVC) at the time when the motor units began discharging action potentials. 195 After these analyses, individual motor unit discharge timings from all tracked motor units across 196 conditions were summed to generate a cumulative spike train as done previously (7, 28). The cumulative spike train and torque signals were filtered (4<sup>th</sup> order zero-phase Butterworth, 2Hz low-pass filter) and 197 198 cross correlated in order to quantify the neuromechanical delay, which is defined as the time delay (ms) 199 between the rise time of the motor unit action potentials and the resultant torque output (7). The 200 cumulative spike train and torque signals were divided into one-cycle time frames and the cross-201 correlation between the cumulative spike train and torque was computed for each time frame and then 202 averaged across all time frames. The time lag of the peak of the cross-correlation function provided an 203 estimate of the neuromechanical delay (7).

204 Interferential EMG

The EMG average rectified values (ARV) were obtained from the same region where motor unit activity was computed and were calculated as the mean of 50ms non-overlapping windows. ARV values were averaged across all channels of the electrode grid (59-bipolar channels). Coactivation was quantified as tibialis anterior ARV divided by gastrocnemius medialis ARV (6).

209 Statistics

210 Results are expressed as means and SD unless stated otherwise. Normality of the data was assessed with 211 the Shapiro-Wilk test and Sphericity was tested with the Mauchly test. Statistical significance was set at 212 p<0.05. Measures of torque matching accuracy, torque variability, the neuromechanical delay, motor unit 213 firing data and interference EMG (ARV for tibialis anterior and co-activation) were averaged for each 214 participant and assessed with three-way, repeated measures analysis of variance (ANOVA) with factors 215 condition (baseline, isotonic, pain and post-pain), frequency (0.25Hz and 1.0Hz) and sinusoidal amplitude 216 (5% and 10% MVC). These analyses were followed by pairwise comparisons with a Student-Newman-Keuls 217 (SNK) post hoc test when ANOVA was significant. Finally, linear regression analysis was applied to all motor 218 units identified during the contractions to assess the association between the difference in pain and 219 baseline discharge rate and baseline recruitment threshold ( $\Delta$  pain/baseline discharge rate vs. baseline 220 recruitment threshold).

#### 221 RESULTS

#### 222 Pain sensation

During the painful condition, pain lasted for the full set of contractions, reaching a peak intensity of mean (SD) 6.3 (1.6) out of 10, 60s after the hypertonic saline was injected, with a range between 4.5 (2.2) to 3.3 (1.5) points after the first and last contraction, respectively. All participants reported that pain was felt under the electrode grid, and two participants also experienced referred pain to the lateral malleoli and dorsal region of the foot. For the isotonic condition, participants experienced a peak pain of 0.1 (0.3) out of 10 immediately after the injection, but did not experience any pain during the contractions.

229 Torque variability and tracking accuracy

230 Mean torque was maintained across all conditions and contraction rates (p>0.08 in all cases). Moreover,

231 torque tracking accuracy did not vary across baseline, isotonic, pain and post-pain conditions (cross-

232 correlation condition effect: p=0.26,  $n^2=0.096$  and MSE condition effect: p=0.102,  $n^2=0.146$ ). However, 233 tracking accuracy was less at the highest frequency (1Hz, frequency effect: p<0.001) and lowest amplitude 234 (5% MVC, amplitude effect: p<0.001). Torque variability was significantly reduced during the pain 235 condition at the fastest contraction rates when calculated both in terms of SD torque (frequency x 236 condition interaction: p=0.007,  $\eta^2$ =0.267) and CoV torque (frequency x condition interaction: p<0.001, 237  $\eta$ 2=0.35). Finally, maximum torque decreased and minimum torque increased during pain at the fastest 238 contraction speed (frequency x condition interaction: p<0.01,  $\eta = 0.26$  and p=0.01,  $\eta = 0.24$ , respectively). 239 Mean values for measures of torque tracking accuracy and variability can be seen in **Table 1**.

240 Motor unit decomposition

241 When merging the different contraction rates in a single condition (i.e. 0.25Hz-5%MVC, 0.25Hz-10%MVC, 242 1Hz-5%MVC and 1Hz-10%MVC during baseline, pain, isotonic and post-pain conditions independently), 243 the number of identified motor units was dependent on both the frequency and amplitude of the 244 contraction, and for each subject, an average of 14 (7), 16 (7), 17 (7) and 18 (8) motor units could be identified for the contractions at 0.25Hz-5%MVC, 0.25Hz-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC, 245 246 respectively (frequency x amplitude interaction: p=0.038,  $\eta^2=0.29$ ). Most importantly, recruitment 247 threshold was not affected by contraction frequency nor amplitude as all motor units that were tracked 248 across the different contraction rates maintained their recruitment threshold in each individual condition 249 (13.8 (0.6) %MVC, 14.6 (0.2) %MVC, 14.0 (0.2) %MVC and 14.7 (0.3) %MVC at 0.25Hz-5%MVC, 0.25Hz-250 10%MVC, 1Hz-5%MVC and 1Hz-10%MVC, respectively, frequency x amplitude interaction: p=0.283, 251  $\eta$ 2=0.088). A total of 229 (24) motor units (14 (7) motor units per participant) could be tracked across 252 conditions at a single contraction rate (i.e., baseline, pain, isotonic and post pain at 0.25Hz-5%MVC, 253 0.25Hz-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC, independently). The number of identified motor units 254 was not affected by pain as a similar number of motor units was observed across all conditions (effect condition: p=0.39, η2=0.57). These tracked motor units (all conditions merged at a single contraction rate)
 were then considered for all subsequent analyses.

# 257 Discharge rate, recruitment threshold and neuromechanical delay

258 Results from a representative subject can be seen in Figure 2; sinusoidal contractions performed with 10% 259 MVC amplitude modulation at a frequency of 0.25Hz (A) and 1Hz (B) during the baseline (left) and painful 260 (right) conditions. Smoothed discharge rates, torque profiles and results from cross correlation between 261 the cumulative spike train and exerted torque can be seen for each of the contractions. An increase in 262 neuromechanical delay and decrease in discharge rate were observed for the painful condition at low 263 contraction speeds only (0.25Hz, A). At high speed contractions (1Hz), both the neuromechanical delay 264 and discharge rate were similar between the baseline and painful condition, and discharge rate variability 265 (CoV discharge rate) was reduced with pain. These results were confirmed for the group of participants 266 as only motor units identified at slower frequencies (0.25Hz) decreased their mean discharge rate 267 significantly during pain (frequency x condition interaction: p=0.01,  $\eta^2=0.25$ ) and motor units identified at 268 faster frequencies (1Hz) decreased their CoV discharge rate with pain (frequency x condition interaction: 269 p=0.02, n<sup>2</sup>=0.21) Figure 3. Minimum discharge rates were higher at the fastest contraction rate (1Hz-270 10%MVC, frequency x condition interaction: p=0.03,  $n^2=0.20$ ), while maximum discharge rate values 271 decreased across all conditions regardless of contraction speed and amplitude (condition effect: p=0.004, 272  $\eta^2$ =0.25) Figure 4. The recruitment thresholds of the identified motor units were significantly influenced 273 by both the amplitude (amplitude effect: p<0.001,  $\eta = 0.72$ ) and frequency of the contractions (frequency 274 effect: p=0.01,  $\eta 2=0.39$ ) but not by pain (condition effect: p=0.39,  $\eta 2=0.07$ ), Figure 5. Figure 6 shows the 275 association between the  $\Delta$  discharge rate (pain-baseline condition) and recruitment threshold for a 276 contraction at slow frequency and large torque amplitude (A) and fast frequency and large torque 277 amplitude (B). The results show that during the fastest contraction rate (1Hz-10% MVC), lower threshold

278 units decreased discharge rate similarly to the slower contraction speed condition (positive intercept of 279 0.58 Hz vs. 0.47 Hz at slow and fast contraction speeds, respectively). However, for higher threshold motor 280 units, a similar proportion of units either increased or decreased firing rate in response to pain in the 281 fastest contraction rate as the regression slope approached zero (Figure 6B). Additionally, the 282 neuromechanical delay increased during the painful condition at low frequencies (0.25Hz) but not at high 283 frequencies; frequency x condition interaction: p=0.033,  $\eta^2=0.19$ ), Figure 7. Finally, the cross-correlation 284 between the cumulative spike train and target torque increased with contraction frequency, with average 285 values of 0.79 (0.06), 0.85 (0.05), 0.88 (0.05) and 0.91 (0.02) at 0.25Hz-5% MVC, 0.25Hz-10% MVC, 1Hz-5% 286 MVC and 1Hz-10% MVC, respectively (frequency effect: p<0.0001,  $\eta$ 2=0.91). These cross-correlation 287 values did not change across conditions (condition effect: p=0.33,  $\eta 2=0.08$ ).

# 288 Interferential EMG

Both the tibialis anterior amplitude of activity and tibialis anterior-medial gastrocnemius co-activation did
 not vary between conditions, frequencies or torque amplitudes (p>0.071 in all possible comparisons).

# 291 DISCUSSION

292 This study demonstrates that both motor performance and motor unit firing adaptations in response to 293 pain are dependent on contraction speed. Specifically, we observed that torque amplitude and torque 294 variability were reduced at the fastest contraction speed during pain. These motor responses were 295 accompanied by a reduction in motor unit discharge rate variability and discharge rate modulation, and maintenance in mean discharge rate and neuromechanical delay. These results are possibly explained by 296 297 the greater proportion of higher threshold motor units observed during faster contractions, providing 298 compensation for the stronger inhibitory inputs received by lower threshold motor units (25). Taken 299 together, this study provides new evidence of motor adaptations to pain and further supports a 300 differential effect of nociception across the motor unit pool.

302 Torque amplitude and torque variability were reduced with pain at the fastest contraction speed. Several 303 studies have shown that individuals with pain display altered motor output (10, 20, 35). This mechanism is believed to be part of a compensatory strategy which would help to avoid further tissue damage (22). 304 305 However, there are very few studies that have examined the effect of pain on contraction speed. Ervilha 306 et al. (10) previously showed that elbow flexion movement amplitude and velocity was reduced during 307 fast contractions when pain was induced in the biceps bracchi muscle via injection of hypertonic saline. 308 Moreover, Thomas et al. (35) found that individuals in a period of remission of low back pain showed 309 reductions in lumbar spine movement excursion, velocity and acceleration during a forward reaching task 310 when the movement was performed at a fast pace but not at a slow pace. Although there are multiple 311 differences between these and the current study (i.e., dynamic contractions vs. isometric contractions), it 312 is apparent that pain can alter motor performance at faster contraction speeds. In our specific case we 313 did not observe differences in torque tracking accuracy nor contraction velocity (as this was kept constant), 314 but the reduction in torque modulation amplitude can be compared with the findings from these previous 315 studies since individuals might have reduced the amount of muscle fiber shortening and lengthening in 316 order to decrease contraction time and minimize the pain perceived during the contraction. It could be 317 argued that this could have been also experienced during slow contractions with high modulation 318 amplitude (0.25 Hz-10%MVC condition), nevertheless, it is important to note that individuals tended to 319 modulate torque beyond the 10% MVC requested at 1Hz-10%MVC, reaching torque ranges which were 320 significantly higher than those observed at slower contraction rates (Table 1).

A key finding in this study are the differences in motor unit firing behaviour across the different contraction rates and conditions. Motor unit mean discharge rate is commonly reduced in response to experimentally induced pain during low-force sustained contractions (32). This reduction in discharge rate 324 has been related to a number of mechanisms, such as type III-IV afferent inhibition (3), reduction of 325 corticospinal excitability (19) and decreased spinal excitability (19, 31). However, the exact mechanisms 326 responsible for this decrease in firing frequency are still debated. Despite the consistency of this response 327 across multiple studies, recent research has shown that the changes in discharge rate in response to pain 328 differ across the motor unit pool, particularly when high forces are exerted. Specifically, it was 329 demonstrated that during painful contractions of the tibialis anterior muscle at 70% MVC, lower threshold 330 motor units either reduced or maintained their discharge rate (recruitment threshold <35% MVC) while 331 higher-threshold motor units (recruitment threshold >35% MVC) increased their discharge rate with 332 respect to non-painful conditions (25). This finding suggested that inhibitory nociceptive inputs to low 333 threshold motor units can be compensated by increased excitation to higher-threshold motor units. 334 Higher-threshold motor units are recruited with increasing force, so that high forces can be reached in 335 painful conditions. It is possible that the excitation of higher-threshold motor units with pain is a specific 336 mechanism by the CNS to maintain the performance of challenging tasks, such as when the CNS is required 337 to perform high forces or high velocities. Therefore, here we hypothesised that maintenance of high speed 338 could be reached by a greater involvement of higher-threshold motor units in the presence of muscle pain. 339 Consistent with this observation, we identified a greater number of motor units as the speed of the 340 contraction increased. Moreover, the average recruitment threshold torque was greater in the higher 341 speed conditions (Figure 5). Therefore, at faster speeds, more higher threshold motor units were recruited, 342 as was expected (8). In these conditions, we confirmed a differential effect of pain on lower and higher 343 threshold units, as we had previously observed when comparing low and high force contractions. 344 Nevertheless, in this study we also identified differences in motor performance during pain across the 345 different contraction rates, which could have also influenced the mean discharge rate results presented 346 herein. Indeed, the reduction in torque modulation amplitude was accompanied by an increase in 347 minimum discharge rate and reductions in both maximum discharge rate and CoV discharge rate, meaning

348 that the maintenance in mean discharge rate could be due to these torgue and motor unit adjustments 349 instead of a differential effect on lower and higher threshold motor units. However, there are a number 350 of observations that still provide support for a differential effect of nociception among lower and higher 351 threshold motor units. First, it is known that lower threshold motor units exert the highest firing 352 frequencies while lower threshold units usually show the lowest firing frequencies. The fact that maximum 353 discharge rate was reduced and minimum discharge rate was increased, supports the possibility that a 354 greater proportion of higher threshold motor units, on average, increased their firing rate during the 355 painful condition to compensate for the reduction in firing rate among lower threshold units. This 356 compression in motor unit firing rates between higher and lower threshold motor units was also observed 357 in the experimental and simulated results of Martinez-Valdes et al. at high forces (25). Second, during the 358 fastest contraction, a similar number of higher threshold motor units either increased or decreased their 359 discharge rate (as reflected in the slope approaching zero for these units, Figure 6), while most of the 360 lower threshold units decreased their discharge rate (similar positive intercept to a low frequency 361 contraction), which shows recruitment-threshold related adjustments in motor unit discharge rate in 362 response to pain. Third, mean discharge rate was reduced at the slowest contraction velocity during pain, 363 despite observing no changes in mean torque and torque modulation. This is not a surprising finding since 364 previous studies have reported pain-related reductions in discharge rate at low forces despite observing 365 no variations in mean torque (32). Therefore, any variations in torque cannot explain variations in motor 366 unit firing properties alone. Taken together, it is plausible to assume that both the maintenance of mean 367 discharge rate and neuromechanical delay (see next sections) at fast contraction speeds is both due to 368 subtle adjustments in task performance and differential effects of nociception across the motor unit pool.

The source for a differential nociceptive response on lower and higher threshold motor units has not yet been determined, but it could be due to changes in corticospinal axon excitability and/or changes in the intrinsic properties of the motoneurons. Regarding the first possibility, Martin et al. (23) previously 372 observed an increase in corticospinal axon excitability in response to experimental muscle pain. The 373 authors specifically found non-uniform effects across the motoneuron pool, with facilitation of 374 cervicomedullary motor evoked potentials (CMEPs) at rest and during contractions at a matched level of 375 EMG, which likely reflects a preferential excitation of high-threshold motoneurons by group III and IV 376 afferents. Another proposed candidate for differences in excitability/inhibition across the motor unit pool 377 to pain are persistent inward currents (PICs) (27). PICs have a long-lasting effect in low-threshold motor 378 units and are very sensitive to inhibitory synaptic input (21). In contrast, high-threshold motor units do 379 not largely depend on PICs but on an increased excitatory synaptic input (27). Therefore, even in the case 380 of a uniform nociceptive inhibition across the motoneuron pool, the decline in PICs would mainly affect 381 low-threshold motor units, as this pool relies on PICs to sustain firing. Nevertheless, this latter observation 382 remains speculative since the effects of pain on PICs has never been tested.

# 383 Changes in the neural drive and force relationships due to pain: effect of contraction speed

384 This study is the first to assess how nociception affects the relation between motor unit firing and force 385 production at different contraction speeds. The delay between motor unit activation and force is referred 386 to as the neuromechanical delay (7) and decreases with contraction speed, since higher discharge rates 387 and faster recruitment are required to exert faster contractions. In this study, the same motor units were 388 followed across conditions so we were able to assess how the neuromechanical delay was affected by 389 nociception at different contraction speeds. Our findings showed that nociception induced a larger 390 neuromechanical delay during slow contractions only (0.25 Hz). As mentioned previously, the main neural 391 determinants for the neuromechanical delay are motor unit discharge rate and recruitment. However, 392 force tracking accuracy (correlation between force-matching target and exerted torque) and intrinsic 393 properties of the muscle-tendon unit can also influence this variable i.e. changes in muscle-fibre twitch 394 force and muscle tendon compliance. Since we followed the same motor units across all conditions, the

395 effect of recruitment of additional units on the neuromechanical delay can be discarded. Therefore, three 396 possible mechanisms for this increase in delay can be due to changes in muscle-tendon properties, 397 changes in torque modulation amplitude and adjustments in discharge rate. Farina et al. (14) previously 398 showed that experimentally induced pain increases motor unit peak twitch force during very low-force 399 contractions, which could potentially decrease the neuromechanical delay during painful conditions, 400 however, this increase in twitch force was not correlated with the pain-related decrease in discharge rate 401 and was even maintained after the pain ceased (post-pain condition). Twitch contraction velocity is 402 another potential candidate to explain differences in neuromechanical delay across conditions and 403 contraction speeds. For instance, Roatta et al. (30) previously reported that pain induced via the cold 404 pressor test reduced twitch half-relaxation time among low threshold motor units. However, these 405 changes were also accompanied by an increase in discharge rate, which was not observed in the present 406 study. When using the cold pressor test, motor unit responses are not measured in the painful area (in 407 the study by Roatta et al. (30) the authors measured the tibialis anterior muscle and induced pain at the 408 hand), therefore, it is likely that a more generalized sympathetic response to cold-induced pain could have 409 increased both the excitability to motoneurons and contraction velocity. As mentioned previously, pain 410 induced with hypertonic saline usually shows a reduction in discharge rate among low threshold units (32), 411 although this reduction in discharge rate could be partially compensated by an increase in peak twitch 412 force, this has not shown to increase action potential propagation velocity (14). In fact, twitch 413 force/velocity could have only explained changes in neuromechanical delay during pain if we would have 414 observed a reduced delay at slower contraction speeds. Therefore, the effect of twitch force/velocity on 415 the neuromechanical delay during slow and painful contractions can be discarded. Changes in torque 416 modulation amplitude is another mechanism by which we could have observed differences in 417 neuromechanical delay across conditions. We observed that torque variability and peak-to-peak 418 (minimum-maximum) torque values were lower in the faster contraction rate during pain. This decrease

419 in torque modulation reduces the amount of force required to match the torque sinusoidal target. 420 Therefore, if we consider that mean discharge rate was similar across conditions but the difference 421 between maximum and minimum discharge rate values was lower, it is possible that the neuromechanical 422 delay was specifically adjusted to these variations in torque modulation in order to be able to maintain 423 the contraction speed. Conversely, at slow contraction rates we did not observe any differences in torque 424 modulation but observed a reduction in mean firing rate, therefore, the maintenance of modulation 425 amplitude and changes in firing rate across conditions (see next section) might have induced the increase 426 in neuromechanical delay observed at 0.25Hz contractions. Finally, another mechanism explaining 427 changes in neuromechanical delay across conditions and contraction speeds can again be related to 428 differential changes in firing properties across populations of motor units. Indeed, as mentioned 429 previously, recruitment threshold was dependent on contraction frequency and a larger number of higher 430 threshold motor units were identified at faster contraction speeds. The excitation of this group of motor 431 units could have compensated for the inhibitory effect of pain on lower threshold motor units, helping to 432 maintain the neuromechanical delay at the same level of a non-painful contraction at faster contraction 433 speeds.

#### 434 Functional implications

The findings of this study have important functional implications. The adaptations observed among higher-threshold motor units during fast contractions, support previous findings showing that acute tonic pain can induce a re-organization in the activity of the motor unit pool, where the inhibitory effects of nociception on some units is compensated by greater excitation to other motor units (25, 36-38). Thus, the increased excitation, either via spinal or supraspinal inputs, to higher threshold motor units during pain allows the exertion of fast submaximal contractions, which are required to maintain function when needed (30). Nevertheless, it has been consistently shown that experimental muscle pain decreases the 442 ability to perform maximal forces (16, 17) and contractions at maximal speeds (10, 11), therefore, these 443 responses might have an upper limit where the increase in excitability to higher threshold motor units will 444 not be able to compensate for the strong inhibition received by the lower threshold motor unit pool. 445 Moreover, the over-reliance on higher threshold motor units to maintain a task in the presence of pain 446 can have adverse consequences if prolonged, since the fatigability of this motor unit pool is higher and 447 would likely induce greater stress on the muscle tissue. Studies assessing the effects of pain on fatigue 448 have shown that contractions can be sustained for significantly shorter times when nociceptive 449 substances are infused into the muscle (5, 17, 33).

450 Certain limitations need to be acknowledged. Although we identified a greater number of motor units at 451 faster contraction speeds, we could not determine the total number of recruited units at each contraction 452 speed directly. To date, it is still not possible to quantify the total number of recruited units during a 453 contraction with any motor unit decomposition technique. Therefore, it is possible that the sample of 454 identified motor units may have been biased towards those with greater action potential amplitudes 455 (which tend to have higher recruitment thresholds).

456 Conclusion

Both changes in motor performance and firing behaviour of motor units in response to muscle pain is dependent on contraction speed. The reductions in torque and firing rate modulation amplitude in conjunction with a maintenance in mean firing rate and neuromechanical delay at faster contraction speeds allows for the execution of fast submaximal tasks despite the presence of pain. These compensatory motor strategies are likely enabled by an increase in excitability of higher threshold motor units, which could potentially increase fatigability and persistence of symptoms in the long term.

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#### 464 **DISCLOSURES**

465 No conflicts of interest, financial or otherwise, are declared by the authors.

#### 466 AUTHOR CONTRIBUTIONS

- 467 E.M.-V., D. Farina and D. Falla conceived and designed research; E.M.-V. and D. Falla performed
- 468 experiments; E.M.-V., F.N. and M.A. analyzed data; E.M.-V., F.N., D. Farina and D. Falla interpreted results
- 469 of experiments; E.M.-V. prepared figures; E.M.-V. drafted manuscript; E.M.-V., F.N., D. Farina and D. Falla
- 470 edited and revised manuscript; E.M.-V., F.N., M.A., D. Farina and D. Falla approved final version of
- 471 manuscript.

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- 568 guidelines for reporting observational studies. *Lancet* 370: 1453-1457, 2007.
- 569 FIGURE CAPTIONS
- 570 **Figure 1.** Sinusoidal torque targets representing different contraction frequencies and amplitudes. Torque
- 571 targets at four different contraction rates can be seen on the top of the figure. All targets had a mean
- 572 torque of 20% MVC and were comprised by a 2s ramp-up, 40s sinusoidal contraction and 2s ramp-down.
- 573 The combination of sinusoidal contractions at 0.25Hz and 1Hz, and 5% MVC amplitude and 10% amplitude,
- 574 represented contractions with four different rates of torque development (quantified as the product of
- the amplitude and frequency of the sinusoidal torque trajectory, bottom of the figure). Contractions
- 576 modulated at 5%MVC amplitude had variations in torque from 17.5%MVC to 22.5%MVC and contractions
- 577 modulated at 10%MVC amplitude had torque variations from 15%MVC to 25%MVC.
- 578 **Figure 2.** Representative results from one participant. Sinusoidal contractions were performed at 20%
- 579 MVC with 10% amplitude modulation at a frequency of 0.25Hz (A) and 1Hz (B) during baseline (left) and

580 painful (right) conditions. Smoothed discharge rates (DR, low pass filtered at 2Hz), force profiles and 581 results from the cross correlation (CC) between the cumulative spike train and torgue can be seen for 582 each of the contractions. An increase in neuromechanical delay (NMD) and decrease in discharge rate 583 (dashed horizontal line) can be seen for the painful condition at low contraction speeds only (A). At high 584 speed contractions (B), both the NMD and DR were similar between baseline and pain, while the 585 coefficient of variation of torque (CoV torque) and coefficient of variation in discharge rate (CoV DR) were 586 lower during the pain condition. The same motor units were tracked across baseline and painful 587 conditions.

Figure 3. Mean discharge rate and coefficient of variation in discharge rate across conditions. Mean discharge rate results during contractions with 5% MVC amplitude modulation (A) and 10% amplitude modulation (B) and coefficient of variation (CoV) in discharge rate results in contractions with 5% MVC amplitude modulation (C) and 10% MVC amplitude modulation (D). All contractions had a mean force target of 20% MVC. The same motor units were tracked across all conditions at each amplitude and speed separately. \*p<0.05.

Figure 4. Minimum and maximum discharge rate results across conditions. Minimum discharge rate results during contractions with 5% MVC amplitude modulation (A) and 10% amplitude modulation (B) and maximum discharge rate results in contractions with 5% MVC amplitude modulation (C) and 10% MVC amplitude modulation (D). All contractions had a mean force target of 20% MVC. The same motor units were tracked across all conditions at each amplitude and speed separately. \*p<0.05 between conditions.</p>

**Figure 5.** Recruitment threshold across conditions. Motor unit recruitment threshold during 5% (A) and 10% (B) amplitude modulation (mean force target of 20% MVC) can be seen on the left and right side of the figure, respectively. Recruitment threshold was dependent on contraction speed (effect: p<0.01, #) and amplitude (effect: p<0.01,  $\Psi$ ). The same motor units were tracked across all conditions at each contraction speed separately.

Figure 6. Association between recruitment threshold and pain-related variations in mean discharge rate.
 The association between recruitment threshold at baseline (x-axes) and the difference of mean discharge
 rate between pain and baseline conditions from all the motor units identified during contractions at A)
 025Hz-10% amplitude and B) 1Hz-10% amplitude.

**Figure 7.** Neuromechanical delay across conditions. Neuromechanical delay results during 5% (A) and 10% (B) amplitude modulation (mean force target of 20% MVC) can be seen on the left and right side of the figure, respectively. At both amplitudes, nociception induced an increase in the neuromechanical delay at the slow contraction speed only (0.25Hz). The same motor units were tracked across all conditions at each amplitude and speed separately. \*p<0.05.

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