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

Martinez-Valdes, Eduardo; Negro, Francesco; Arvanitidis, Michail; Farina, Dario; Falla, Deborah

DOI:
10.1152/japplphysiol.01011.2020

Document Version
Peer reviewed version

Citation for published version (Harvard):
Martinez-Valdes, E, Negro, F, Arvanitidis, M, Farina, D & Falla, D 2021, 'Pain-induced changes in motor unit discharge depend on recruitment threshold and contraction speed', *Journal of Applied Physiology*, vol. 131, no. 4, pp. 1260-1271. https://doi.org/10.1152/japplphysiol.01011.2020

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

Eduardo Martinez-Valdes[1], Francesco Negro[2], Michail Arvanitidis[1], Dario Farina[3], Deborah Falla[1]

Affiliations

1. Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, UK
2. Department of Clinical and Experimental Sciences, Università degli Studi di Brescia, Brescia, Italy
3. Department of Bioengineering, Imperial College London, London, UK

Correspondence to:

Deborah Falla

Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, UK

Tel: +44 (0)121 41 47253

E-mail: d.falla@bham.ac.uk

Abbreviated title (50 characters): Differential motor unit behavior during pain

ABSTRACT

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

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

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.

INTRODUCTION

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

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

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

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

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 (calculated from one-way repeated measures ANOVA results of previous data (12, 15) and a potential 20% loss of data due to poor signal quality or participant withdrawal. The data from one participant had to be removed from the analysis due to poor signal quality (motor units could not be tracked across all conditions) and therefore the results are presented for 14 participants [age: 26 (3) years, eight males and six females].

Experimental muscle pain

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

Task

Participants were seated with their trunk flexed in 30˚ (in relation to the horizontal plane) on the chair of a Biodex System 3 dynamometer (Biodex Medical Systems). The subject's dominant leg (right for all participants) was positioned over a support with the knee flexed to 160° (with 180° representing full knee extension), and the foot fixed to a footplate (90° ankle joint angle). The lateral malleolus was aligned to the center of rotation of the dynamometer in order to measure ankle dorsiflexion torque. A computer monitor providing real-time feedback of the exerted dorsiflexion torque was positioned approximately 1.5 m away at eye level. At the beginning of the session, in the absence of pain, ankle dorsiflexion maximum voluntary contraction (MVC) torque was recorded three times, each separated by 2 min of rest. The maximal MVC defined the submaximal torque level exerted by the participants in the subsequent contractions. Following the MVC measurement, participants were given time to practice with the visual feedback of their exerted torque (as seen on the computer monitor), by performing the same sinusoidal contractions used in the main protocol (see below) in order to familiarize themselves with the task and reduce the possibility that learning effects would affect the results. After 5 min of rest, participants were asked to track sinusoidal torque trajectories at a frequency of 0.25 or 1 Hz and amplitudes of 5 or 10% 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). Participants performed four sinusoidal contractions in all conditions (baseline, isotonic, pain and post pain) with a 30s rest between contractions. The combination of sinusoidal frequencies and amplitudes were: 1) 0.25Hz and 5% amplitude, 2) 0.25Hz and 10% amplitude, 3) 1Hz and 5% amplitude and 4) 1 Hz and 10% amplitude **(Figure 1)**. These combined frequencies and amplitudes represented different contraction rates (quantified as the product of the amplitude and frequency of the sinusoidal torque trajectory) with a rate 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.

Electromyography

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

Motor unit decomposition and tracking

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

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

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

spike trains, and motor unit firing data was quantified as the inverse of the inter-spike interval (instantaneous firing rate). Mean discharge rate was analyzed on the central part of the contraction after the first sinusoid and before the last sinusoid at each contraction rate. Discharge rate variability was quantified on the same region with the coefficient of variation of discharge rate (CoV discharge rate, SD discharge rate/mean discharge rate * 100). Maximum and minimum discharge rate values were calculated as the maximum and minimum instantaneous discharge rates observed at the point which corresponded with maximum and minimum torque values. Finally, motor unit recruitment threshold was defined as the ankle dorsi-flexion torque (%MVC) at the time when the motor units began discharging action potentials. After these analyses, individual motor unit discharge timings from all tracked motor units across conditions were summed to generate a cumulative spike train as done previously (7, 28). The cumulative spike train and torque signals were filtered (4^{th} order zero-phase Butterworth, 2Hz low-pass filter) and cross correlated in order to quantify the neuromechanical delay, which is defined as the time delay (ms) between the rise time of the motor unit action potentials and the resultant torque output (7). The cumulative spike train and torque signals were divided into one-cycle time frames and the cross-correlation between the cumulative spike train and torque was computed for each time frame and then averaged across all time frames. The time lag of the peak of the cross-correlation function provided an estimate of the neuromechanical delay (7).

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).

Statistics

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

RESULTS

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.

Torque variability and tracking accuracy

Mean torque was maintained across all conditions and contraction rates ($p>0.08$ in all cases). Moreover, torque tracking accuracy did not vary across baseline, isotonic, pain and post-pain conditions (cross-

correlation condition effect: p=0.26, η^2=0.096 and MSE condition effect: p=0.102, η2=0.146). However, tracking accuracy was less at the highest frequency (1Hz, frequency effect: p<0.001) and lowest amplitude (5% MVC, amplitude effect: p<0.001). Torque variability was significantly reduced during the pain condition at the fastest contraction rates when calculated both in terms of SD torque (frequency x condition interaction: p=0.007, η^2=0.267) and CoV torque (frequency x condition interaction: p<0.001, η2=0.35). Finally, maximum torque decreased and minimum torque increased during pain at the fastest contraction speed (frequency x condition interaction: p<0.01, η2=0.26 and p=0.01, η2=0.24, respectively). Mean values for measures of torque tracking accuracy and variability can be seen in **Table 1**.

Motor unit decomposition

When merging the different contraction rates in a single condition (i.e. 0.25Hz-5%MVC, 0.25Hz-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC during baseline, pain, isotonic and post-pain conditions independently), the number of identified motor units was dependent on both the frequency and amplitude of the 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, respectively (frequency x amplitude interaction: p=0.038, η^2=0.29). Most importantly, recruitment threshold was not affected by contraction frequency nor amplitude as all motor units that were tracked across the different contraction rates maintained their recruitment threshold in each individual condition (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-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC, respectively, frequency x amplitude interaction: p=0.283, η2=0.088). A total of 229 (24) motor units (14 (7) motor units per participant) could be tracked across conditions at a single contraction rate (i.e., baseline, pain, isotonic and post pain at 0.25Hz-5%MVC, 0.25Hz-10%MVC, 1Hz-5%MVC and 1Hz-10%MVC, independently). The number of identified motor units 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.

Discharge rate, recruitment threshold and neuromechanical delay

Results from a representative subject can be seen in **Figure 2**; sinusoidal contractions performed with 10% MVC amplitude modulation at a frequency of 0.25Hz (A) and 1Hz (B) during the baseline (left) and painful (right) conditions. Smoothed discharge rates, torque profiles and results from cross correlation between the cumulative spike train and exerted torque can be seen for each of the contractions. An increase in neuromechanical delay and decrease in discharge rate were observed for the painful condition at low contraction speeds only (0.25Hz, A). At high speed contractions (1Hz), both the neuromechanical delay and discharge rate were similar between the baseline and painful condition, and discharge rate variability (CoV discharge rate) was reduced with pain. These results were confirmed for the group of participants as only motor units identified at slower frequencies (0.25Hz) decreased their mean discharge rate significantly during pain (frequency x condition interaction: p=0.01, η^2=0.25) and motor units identified at faster frequencies (1Hz) decreased their CoV discharge rate with pain (frequency x condition interaction: p=0.02, η^2=0.21) **Figure 3**. Minimum discharge rates were higher at the fastest contraction rate (1Hz-10%MVC, frequency x condition interaction: p=0.03, η^2=0.20), while maximum discharge rate values decreased across all conditions regardless of contraction speed and amplitude (condition effect: p=0.004, η^2=0.25) **Figure 4**. The recruitment thresholds of the identified motor units were significantly influenced by both the amplitude (amplitude effect: p<0.001, η2=0.72) and frequency of the contractions (frequency effect: p=0.01, η2=0.39) but not by pain (condition effect: p=0.39, η2=0.07), **Figure 5**. **Figure 6** shows the association between the Δ discharge rate (pain-baseline condition) and recruitment threshold for a contraction at slow frequency and large torque amplitude (A) and fast frequency and large torque amplitude (B). The results show that during the fastest contraction rate (1Hz-10% MVC), lower threshold

units decreased discharge rate similarly to the slower contraction speed condition (positive intercept of 0.58 Hz vs. 0.47 Hz at slow and fast contraction speeds, respectively). However, for higher threshold motor units, a similar proportion of units either increased or decreased firing rate in response to pain in the fastest contraction rate as the regression slope approached zero (**Figure 6B**). Additionally, the neuromechanical delay increased during the painful condition at low frequencies (0.25Hz) but not at high frequencies; frequency x condition interaction: $p=0.033$, $\eta^2=0.19$), **Figure 7**. Finally, the cross-correlation between the cumulative spike train and target torque increased with contraction frequency, with average 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% MVC and 1Hz-10% MVC, respectively (frequency effect: $p<0.0001$, $\eta2=0.91$). These cross-correlation values did not change across conditions (condition effect: $p=0.33$, $\eta2=0.08$).

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).

DISCUSSION

This study demonstrates that both motor performance and motor unit firing adaptations in response to pain are dependent on contraction speed. Specifically, we observed that torque amplitude and torque variability were reduced at the fastest contraction speed during pain. These motor responses were 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 the greater proportion of higher threshold motor units observed during faster contractions, providing compensation for the stronger inhibitory inputs received by lower threshold motor units (25). Taken together, this study provides new evidence of motor adaptations to pain and further supports a differential effect of nociception across the motor unit pool.

Effect of contraction speed on torque, discharge rate and recruitment threshold

Torque amplitude and torque variability were reduced with pain at the fastest contraction speed. Several 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). However, there are very few studies that have examined the effect of pain on contraction speed. Ervilha et al. (10) previously showed that elbow flexion movement amplitude and velocity was reduced during fast contractions when pain was induced in the biceps bracchi muscle via injection of hypertonic saline. Moreover, Thomas et al. (35) found that individuals in a period of remission of low back pain showed reductions in lumbar spine movement excursion, velocity and acceleration during a forward reaching task when the movement was performed at a fast pace but not at a slow pace. Although there are multiple differences between these and the current study (i.e., dynamic contractions vs. isometric contractions), it is apparent that pain can alter motor performance at faster contraction speeds. In our specific case we did not observe differences in torque tracking accuracy nor contraction velocity (as this was kept constant), but the reduction in torque modulation amplitude can be compared with the findings from these previous studies since individuals might have reduced the amount of muscle fiber shortening and lengthening in order to decrease contraction time and minimize the pain perceived during the contraction. It could be argued that this could have been also experienced during slow contractions with high modulation amplitude (0.25 Hz-10%MVC condition), nevertheless, it is important to note that individuals tended to modulate torque beyond the 10% MVC requested at 1Hz-10%MVC, reaching torque ranges which were 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

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

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

observed an increase in corticospinal axon excitability in response to experimental muscle pain. The authors specifically found non-uniform effects across the motoneuron pool, with facilitation of cervicomedullary motor evoked potentials (CMEPs) at rest and during contractions at a matched level of EMG, which likely reflects a preferential excitation of high-threshold motoneurons by group III and IV afferents. Another proposed candidate for differences in excitability/inhibition across the motor unit pool to pain are persistent inward currents (PICs) (27). PICs have a long-lasting effect in low-threshold motor units and are very sensitive to inhibitory synaptic input (21). In contrast, high-threshold motor units do not largely depend on PICs but on an increased excitatory synaptic input (27). Therefore, even in the case of a uniform nociceptive inhibition across the motoneuron pool, the decline in PICs would mainly affect low-threshold motor units, as this pool relies on PICs to sustain firing. Nevertheless, this latter observation remains speculative since the effects of pain on PICs has never been tested.

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

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

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

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

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

ability to perform maximal forces (16, 17) and contractions at maximal speeds (10, 11), therefore, these responses might have an upper limit where the increase in excitability to higher threshold motor units will not be able to compensate for the strong inhibition received by the lower threshold motor unit pool. Moreover, the over-reliance on higher threshold motor units to maintain a task in the presence of pain can have adverse consequences if prolonged, since the fatigability of this motor unit pool is higher and would likely induce greater stress on the muscle tissue. Studies assessing the effects of pain on fatigue have shown that contractions can be sustained for significantly shorter times when nociceptive substances are infused into the muscle (5, 17, 33).

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

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.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

1. **Afsharipour B, Manzur N, Duchcherer J, Fenrich KF, Thompson CK, Negro F, Quinlan KA, Bennett DJ, and Gorassini MA**. Estimation of self-sustained activity produced by persistent inward currents using firing rate profiles of multiple motor units in humans. *J Neurophysiol* 124: 63-85, 2020.
2. **Beretta Piccoli M, Rainoldi A, Heitz C, Wuthrich M, Boccia G, Tomasoni E, Spirolazzi C, Egloff M, and Barbero M**. Innervation zone locations in 43 superficial muscles: toward a standardization of electrode positioning. *Muscle Nerve* 49: 413-421, 2014.
3. **Bigland-Ritchie BR, Dawson NJ, Johansson RS, and Lippold OC**. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol* 379: 451-459, 1986.
4. **Boccia G, Martinez-Valdes E, Negro F, Rainoldi A, and Falla D**. Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher in open compared with closed kinetic chain exercise. *J Appl Physiol (1985)* 127: 950-958, 2019.
5. **Ciubotariu A, Arendt-Nielsen L, and Graven-Nielsen T**. The influence of muscle pain and fatigue on the activity of synergistic muscles of the leg. *Eur J Appl Physiol* 91: 604-614, 2004.
6. **De la Fuente C, Martinez-Valdes E, Cruz-Montecinos C, Guzman-Venegas R, Arriagada D, Pena YLR, Henriquez H, and Carpes FP**. Changes in the ankle muscles co-activation pattern after 5 years following total ankle joint replacement. *Clin Biomech (Bristol, Avon)* 59: 130-135, 2018.
7. **Del Vecchio A, Ubeda A, Sartori M, Azorin JM, Felici F, and Farina D**. Central nervous system modulates the neuromechanical delay in a broad range for the control of muscle force. *J Appl Physiol (1985)* 125: 1404-1410, 2018.
8. **Desmedt JE, and Godaux E**. Ballistic contractions in fast or slow human muscles: discharge patterns of single motor units. *J Physiol* 285: 185-196, 1978.
9. **Desmedt JE, and Godaux E**. Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. *J Physiol* 264: 673-693, 1977.
10. **Ervilha UF, Arendt-Nielsen L, Duarte M, and Graven-Nielsen T**. Effect of load level and muscle pain intensity on the motor control of elbow-flexion movements. *Eur J Appl Physiol* 92: 168-175, 2004.

11. **Ervilha UF, Farina D, Arendt-Nielsen L, and Graven-Nielsen T**. Experimental muscle pain changes motor control strategies in dynamic contractions. *Exp Brain Res* 164: 215-224, 2005.
12. **Farina D, Arendt-Nielsen L, and Graven-Nielsen T**. Experimental muscle pain reduces initial motor unit discharge rates during sustained submaximal contractions. *J Appl Physiol (1985)* 98: 999-1005, 2005.
13. **Farina D, Arendt-Nielsen L, Merletti R, and Graven-Nielsen T**. Effect of experimental muscle pain on motor unit firing rate and conduction velocity. *J Neurophysiol* 91: 1250-1259, 2004.
14. **Farina D, Arendt-Nielsen L, Roatta S, and Graven-Nielsen T**. The pain-induced decrease in low-threshold motor unit discharge rate is not associated with the amount of increase in spike-triggered average torque. *Clin Neurophysiol* 119: 43-51, 2008.
15. **Farina D, Negro F, Gizzi L, and Falla D**. Low-frequency oscillations of the neural drive to the muscle are increased with experimental muscle pain. *J Neurophysiol* 107: 958-965, 2012.
16. **Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsoe B, and Bliddal H**. Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally mediated mechanism. *Muscle Nerve* 26: 708-712, 2002.
17. **Graven-Nielsen T, Svensson P, and Arendt-Nielsen L**. Effects of experimental muscle pain on muscle activity and co-ordination during static and dynamic motor function. *Electroencephalogr Clin Neurophysiol* 105: 156-164, 1997.
18. **Hodges PW, Ervilha UF, and Graven-Nielsen T**. Changes in motor unit firing rate in synergist muscles cannot explain the maintenance of force during constant force painful contractions. *J Pain* 9: 1169-1174, 2008.
19. **Le Pera D, Graven-Nielsen T, Valeriani M, Oliviero A, Di Lazzaro V, Tonali PA, and Arendt-Nielsen L**. Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clinical Neurophysiology* 112: 1633-1641, 2001.
20. **Lee H, Nicholson LL, and Adams RD**. Cervical range of motion associations with subclinical neck pain. *Spine (Phila Pa 1976)* 29: 33-40, 2004.
21. **Lee RH, and Heckman CJ**. Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. *J Neurophysiol* 80: 583-593, 1998.
22. **Low A, Weymar M, and Hamm AO**. When Threat Is Near, Get Out of Here: Dynamics of Defensive Behavior During Freezing and Active Avoidance. *Psychol Sci* 26: 1706-1716, 2015.
23. **Martin PG, Weerakkody N, Gandevia SC, and Taylor JL**. Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J Physiol* 586: 1277-1289, 2008.
24. **Martinez-Valdes E, Negro F, Falla D, Dideriksen JL, Heckman CJ, and Farina D**. Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions. *J Neurophysiol* 124: 1110-1121, 2020.
25. **Martinez-Valdes E, Negro F, Farina D, and Falla D**. Divergent response of low- versus high-threshold motor units to experimental muscle pain. *J Physiol* 598: 2093-2108, 2020.
26. **Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, and Farina D**. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. *J Physiol* 595: 1479-1496, 2017.
27. **Mesquita RNO, Skarabot J, and Pearcey GEP**. Low-threshold motor units can be a pain during experimental muscle pain. *J Physiol* 598: 2545-2547, 2020.
28. **Negro F, Holobar A, and Farina D**. Fluctuations in isometric muscle force can be described by one linear projection of low-frequency components of motor unit discharge rates. *J Physiol* 587: 5925-5938, 2009.
29. **Negro F, Muceli S, Castronovo AM, Holobar A, and Farina D**. Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *J Neural Eng* 13: 026027, 2016.

30. **Roatta S, Arendt-Nielsen L, and Farina D**. Sympathetic-induced changes in discharge rate and spike-triggered average twitch torque of low-threshold motor units in humans. *J Physiol* 586: 5561-5574, 2008.
31. **Rossi A, Decchi B, and Ginanneschi F**. Presynaptic excitability changes of group Ia fibres to muscle nociceptive stimulation in humans. *Brain Res* 818: 12-22, 1999.
32. **Sanderson A, Wang SF, Elgueta-Cancino E, Martinez-Valdes E, Sanchis-Sanchez E, Liew B, and Falla D**. The effect of experimental and clinical musculoskeletal pain on spinal and supraspinal projections to motoneurons and motor unit properties in humans: A systematic review. *Eur J Pain* 2021.
33. **Smith SA, Micklewright D, Winter SL, and Mauger AR**. Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure. *Eur J Appl Physiol* 120: 2047-2058, 2020.
34. **Sohn MK, Graven-Nielsen T, Arendt-Nielsen L, and Svensson P**. Inhibition of motor unit firing during experimental muscle pain in humans. *Muscle Nerve* 23: 1219-1226, 2000.
35. **Thomas JS, France CR, Lavender SA, and Johnson MR**. Effects of fear of movement on spine velocity and acceleration after recovery from low back pain. *Spine (Phila Pa 1976)* 33: 564-570, 2008.
36. **Tucker K, Butler J, Graven-Nielsen T, Riek S, and Hodges P**. Motor unit recruitment strategies are altered during deep-tissue pain. *J Neurosci* 29: 10820-10826, 2009.
37. **Tucker KJ, and Hodges PW**. Changes in motor unit recruitment strategy during pain alters force direction. *Eur J Pain* 14: 932-938, 2010.
38. **Tucker KJ, and Hodges PW**. Motoneurone recruitment is altered with pain induced in non-muscular tissue. *Pain* 141: 151-155, 2009.
39. **von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, and Initiative S**. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 370: 1453-1457, 2007.

FIGURE CAPTIONS

Figure 1. Sinusoidal torque targets representing different contraction frequencies and amplitudes. Torque targets at four different contraction rates can be seen on the top of the figure. All targets had a mean torque of 20% MVC and were comprised by a 2s ramp-up, 40s sinusoidal contraction and 2s ramp-down. The combination of sinusoidal contractions at 0.25Hz and 1Hz, and 5% MVC amplitude and 10% amplitude, 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 modulated at 5%MVC amplitude had variations in torque from 17.5%MVC to 22.5%MVC and contractions modulated at 10%MVC amplitude had torque variations from 15%MVC to 25%MVC.

Figure 2. Representative results from one participant. Sinusoidal contractions were performed at 20% MVC with 10% amplitude modulation at a frequency of 0.25Hz (A) and 1Hz (B) during baseline (left) and

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

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: $p<0.01$, #)

603 and amplitude (effect: $p<0.01$, Ψ). The same motor units were tracked across all conditions at each
604 contraction speed separately.

605 **Figure 6.** Association between recruitment threshold and pain-related variations in mean discharge rate.
606 The association between recruitment threshold at baseline (x-axes) and the difference of mean discharge
607 rate between pain and baseline conditions from all the motor units identified during contractions at A)
608 025Hz-10% amplitude and B) 1Hz-10% amplitude.

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

614