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Divergent response of low- *versus* high-threshold motor units to experimental muscle pain

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Key points

- The neural strategies behind the control of force during muscle pain are not well understood as previous research has been limited in assessing pain responses only during low-force contractions.
- Here we compared, for the first time, the behaviour of motor units recruited at low and high forces in response to pain.
- The results showed that motor units activated at low forces were inhibited while those recruited at higher forces increased their activity in response to pain.
- When analysing lower- and higher-threshold motor unit behaviour at high forces we observed differential changes in discharge rate and recruitment threshold across the motor unit pool.
- These adjustments allow the exertion of high forces in acutely painful conditions but could eventually lead to greater fatigue and stress of the muscle tissue.

Abstract During low-force contractions, motor unit discharge rates decrease when muscle pain is induced by injecting nociceptive substances into the muscle. Despite this consistent observation, it is currently unknown how the central nervous system regulates motor unit behaviour in the presence of muscle pain at high forces. For this reason, we analysed the tibialis anterior motor unit behaviour at low and high forces. Surface EMG signals were recorded from 15 healthy participants (mean age (SD) 26 (3) years, six females) using a 64-electrode grid while performing isometric ankle dorsiflexion contractions at 20% and 70% of the maximum voluntary force (MVC). Signals were decomposed and the same motor units were tracked across painful (intramuscular hypertonic saline injection) and non-painful (baseline, isotonic saline, post-pain) contractions. At 20% MVC, discharge rates decreased significantly in the painful condition (baseline *vs.* pain: 12.7 (1.1) Hz to 11.5 (0.9) Hz, $P < 0.001$). Conversely, at 70% MVC, discharge rates increased significantly during pain (baseline *vs.* pain: 19.7 (2.8) Hz to 21.3 (3.5) Hz, $p = 0.029$) and recruitment thresholds

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decreased (baseline *vs.* pain: 59.0 (3.9) %MVC to 55.9 (3.2) %MVC, $p = 0.02$). These results show that there is a differential adjustment between low- and high-threshold motor units during painful conditions. An increase in excitatory drive to high-threshold motor units is likely required to compensate for the inhibitory influence of nociceptive afferent inputs on low-threshold motor units. These differential mechanisms allow the force output to be maintained during acute pain but this strategy could lead to increased muscle fatigue and symptom aggravation in the long term.

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Introduction

Despite extensive research over the past decades, there is still no consensus on the exact nature of pain-induced adaptations in muscle behaviour. Various experimental pain models have been used to investigate the mechanisms underlying adaptations in the neural control of muscle, most commonly with the use of intramuscular injection of hypertonic saline solution (Graven-Nielsen *et al.* 1997*a*). This is one of the most applied pain models since it induces moderate levels of pain, is self-limited and typically lasts up to 10 min with the quality of the pain comparable to clinical muscle pain (Graven-Nielsen *et al.* 2000). However, unlike clinical pain conditions, it is not confounded by psychosocial factors (Falla & Farina, 2008). Some studies have supported a model for which pain increases muscle activation, leading to a vicious cycle of continued pain (Roland, 1986), while other studies have shown inhibition of the painful muscle and facilitation of the antagonist muscle (pain adaptation model (Lund *et al.* 1991)). More recent research has challenged these stereotypical views by proposing that the central nervous system adapts to painful conditions in a task-dependent and meaningful way by activating muscle regions that are not affected by the noxious stimuli, in order to protect and avoid further damage to the painful tissues (moving differently in pain theory (Hodges & Tucker, 2011)). Thus, the central nervous system could potentially inhibit and facilitate different regions of a painful muscle, which has been partly confirmed by motor unit recordings during the infusion of noxious substances into muscles (Tucker *et al.* 2009). According to this explanation, the decrease in discharge rate commonly observed in low-threshold motor units in response to pain (Farina *et al.* 2004) may be compensated by the recruitment of additional motor units, possibly not affected by the noxious stimuli, allowing the motor task to be performed despite the presence of pain (Tucker & Hodges, 2009; Tucker *et al.* 2009). Nevertheless, studies examining motor unit adjustments to painful stimuli have been limited to very low contraction forces (Farina *et al.* 2004, 2005; Tucker *et al.* 2009) and it remains unknown how pain affects the behaviour of high-threshold motor units.

Previous studies have shown that pain impairs the ability to produce maximal forces, suggesting that nociceptive afferent input determines a uniform inhibition of the whole motor unit pool (Graven-Nielsen *et al.* 1997*b*, 2002). Nonetheless, it has been extensively shown that submaximal contractions at high force levels (but not maximal forces) can be produced in painful conditions (Graven-Nielsen *et al.* 1997*b*; Wang *et al.* 2000; Shimada *et al.* 2015). In these conditions, it is unknown whether low- and high-threshold motor units respond in the same way or differently to muscle pain. To address this question, here we compared changes in low- and high-threshold tibialis anterior motor unit firing properties during experimentally induced pain via injection of hypertonic saline. It was hypothesized that at high forces, the central nervous system will increase the synaptic input received by high-threshold motor units, in order to compensate for the inhibition of low-threshold motor units. To further test this hypothesis, in addition to the experimental findings, a computational model was used to simulate different levels of inhibition/excitation across the motor unit pool during a high-force contraction.

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). All procedures were approved by the University of Birmingham's ethical committee (approval number: 16-0934) and were conducted in accordance with the *Declaration of Helsinki*, except for registration in a public database. The report of this study is in accordance with STROBE guidelines (von Elm *et al.* 2007). 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. Fifteen volunteers participated in the experiments (age: mean (SD) 26 (3) years, nine males and six females). Inclusion criteria: healthy males and females between 18 and 35 years old. Exclusion criteria: current or previous history of lower limb pain, past history of orthopaedic disorders

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affecting the leg, history of neurological disorders, known bleeding disorders and taking anticoagulant medication. The sample size was calculated based on a 95% confidence interval, a power of 0.8, an effect size of (f) 0.36 (calculated from the one-way repeated measures ANOVA results of Farina *et al.* (2005; 2012) and a potential 20% loss of data due to signal quality or participant withdrawal.

Experimental muscle pain

Experimental muscle pain was induced by injection (27-gauge cannula) of 0.5 ml of sterile hypertonic saline (5.8%) into the tibialis anterior muscle. Isotonic saline (0.5 ml, 0.9%) was used as a control injection at a similar location. The location of the injection was within the proximal portion of the muscle, 10 mm distal to the third column of the EMG electrode grid (see below). The bolus was injected manually over a 10 s period.

Participants performed isometric ankle dorsiflexion under four conditions: baseline, isotonic saline, hypertonic saline (pain condition) and post-pain. Baseline and isotonic saline conditions were randomized across participants and were always followed by hypertonic saline and post-pain conditions. Therefore, for each participant the isotonic saline injection was administered before the hypertonic injection; however, participants were advised that both injections may or may not be painful. A rest of 5 min was allowed between the first three conditions. The set of contractions in the post-pain condition was performed 15 min after pain was no longer reported.

The 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 injections and every 30 s until pain was no longer reported. By the end of the experiment, participants also drew the region where they felt pain.

Task

Participants were seated in a reclined position on the chair of a Biodex System 3 (Biodex Medical Systems), with the back flexed at 30°. The right leg (dominant for all subjects) was positioned over a support, the knee was flexed to 160° (with 180° representing full knee extension), and the foot was fixed to a footplate (90° ankle joint angle). The centre of rotation of the ankle joint (lateral malleoli) was aligned to the centre of rotation of the device in order to accurately quantify the ankle dorsiflexion torque. At the beginning of the session, the ankle dorsiflexion maximum voluntary contraction (MVC) torque was recorded three times, separated by 2 min of rest. The maximal MVC defined the submaximal torque levels. Following the MVC measurement, participants were allowed to practise with the visual feedback of their exerted torque (displayed on a computer monitor), by performing brief ramp-hold contractions at low torque levels (20% MVC) in order to familiarize themselves with the task. Then, after 5 min of rest, participants performed ramp-hold contractions at 20 or 70% MVC. Participants were asked to increase their torque at a rate of 10% MVC/s and then to hold the contraction at the target level for 10 s, therefore reaching the 20% MVC target in 2 s and the 70% MVC in 7 s. Two 20% MVC and two 70% MVC contractions were performed on each condition (four contractions per condition). 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 using high-density, 64-channel surface EMG electrode grids (OT Bioelettronica, Torino, Italy). Each grid consists of 5 × 13 electrodes (1 mm diameter, 8 mm interelectrode distance in both directions), with one electrode absent from the upper right corner. The array was located centrally between the proximal and distal tendons of the muscle, with the columns oriented parallel to the tibia bone (Laine *et al.* 2014). Signals from the gastrocnemius medialis were recorded in bipolar mode with Ag–AgCl electrodes (Ambu Neuroline 720, Ballerup, Denmark; conductive area 28 mm^2), which were mounted according to guidelines (Beretta Piccoli *et al.* 2014). Signals were amplified and recorded (2048 Hz sampling rate) using an OT Bioelettronica Quattrocento amplifier (16-bit analogue–digital converter). The EMG data were processed and analysed offline using MATLAB 2017b (MathWorks). Before further processing, the 64 monopolar EMG channels (referenced at the lateral malleoli) were re-referenced offline to form 59 bipolar channels (i.e. obtaining the differential signals between adjacent electrodes in the direction of the muscle fibres).

Motor unit decomposition and tracking

The HDEMG signals were decomposed into motor unit spike trains with a state-of-the-art algorithm based on blind source separation, which provides automatic identification of motor unit activity (Negro *et al.* 2016a). Since HDEMG allows the tracking of the same motor units within and across sessions (Martinez-Valdes *et al.* 2017), we merged all contractions performed at the same torque level across conditions (i.e. first repetition at 20% MVC during baseline was merged with the first repetitions of the 20% MVC contractions during the isotonic, pain and post-pain conditions). This merged file was then

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decomposed offline to identify motor units that could be identified in 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 (Negro *et al.* 2016*a*). Only motor units with an accuracy >90% were included in the analysis. Moreover, further examination of each spike train was performed and all firings separated from the next by <33.3 ms or >200 ms (Martinez-Valdes *et al.* 2016) were re-checked manually by an experienced operator (Boccia *et al.* 2019).

Torque and motor unit analysis

The torque signal was low-pass filtered (15 Hz) and then used to quantify torque steadiness (coefficient of variation of torque) from the stable part of the contractions. Discharge times of the identified motor units were converted into binary spike trains. Mean discharge rate and discharge rate variability (coefficient of variation of the interspike interval, CoVisi) were calculated from the stable plateau torque region. Motor unit recruitment and de-recruitment thresholds were defined as the ankle dorsiflexion torques (%MVC) at the times when the motor units began and stopped discharging action potentials, respectively. Discharge rates at recruitment and de-recruitment were calculated using the first and last six discharges of the motor units. Motor units were analysed in four different ways. First, we compared the average behaviour of the motor units identified during 20% MVC contractions with those identified at 70% MVC contractions with recruitment thresholds >50% MVC from each subject individually. In this way, we compared the behaviour of motor units with thresholds in the ranges (0–20%) MVC and (50–70%) MVC in separate contractions (low and high forces). Second, the activity of all the motor units identified across all subjects and conditions at these two force levels (0–20% and 50–70% MVC) was analysed by linear regression. Across conditions, the regression slopes for the increase in discharge rate from recruitment to target torque (mean discharge rate minus discharge rate at recruitment, Δ discharge rate) relative to the increase in torque from the recruitment threshold (target torque %MVC minus recruitment threshold torque, Δ torque), were computed as an indirect estimate of the input–output gain of the tibialis anterior motoneurons (Martinez-Valdes *et al.* 2018*a*; Boccia *et al.* 2019; Del Vecchio *et al.* 2019). This estimate represents the changes in discharge rate with force as determined by all sources of input to motoneurons, such as descending drive from supraspinal centres (Olivier *et al.* 1995), as well as afferent Ia input (Miles *et al.* 1989), among others. Modification in the Δ discharge rate – Δ torque relationship implies a change in the gain of motoneurons when the system requires force generation. Third, for the contractions at 70% MVC we compared the behaviour of the lower-threshold motor units (0–35%) MVC with the higher-threshold motor units (35–70%) MVC. These two groups of units were compared to check the relative contribution of low- and high-threshold motor units concurrently recruited at high forces per each individual participant. Finally, all motor units identified during the 70% MVC contractions from all participants (range 0–70% MVC) were analysed together to check differences in the behaviour of the motor unit pool at high forces.

Finally, in order to account for differences in perceived pain intensity across participants, we examined the correlation between the differences in discharge rate between baseline and painful conditions at 20% MVC (baseline discharge rate–pain discharge rate, 20% MVC Δ discharge rate), and pain intensity (NRS). Additionally, for 70% MVC contractions we examined the correlation between the differences in discharge rate between painful and baseline conditions (pain discharge rate–baseline discharge rate, 70% MVC Δ discharge rate) and pain intensity.

Interferential EMG

The EMG average rectified value (ARV) obtained from the submaximal contractions was averaged across all channels of the electrode grid and computed from the same torque region where mean discharge rate and CoVisi were calculated. Co-activation was quantified as tibialis anterior ARV divided by gastrocnemius medialis ARV.

Simulations

The motoneuron model was a Hodgkin–Huxley type model, modified from Cisi and Kohn (Cisi & Kohn, 2008) and used in several previous studies (Negro & Farina, 2011; Dideriksen *et al.* 2012; Negro *et al.* 2015, 2016*b*) to confirm a number of theoretical predictions of experimental observations. The combination of the parameters used in the simulations was selected based on previous experimental results on the behaviour of motor units in healthy individuals (e.g. Fuglevand *et al.* 1993). Thus, the model was used to simulate the firing behaviour of motor units recruited from 0 to 70% MVC during non-painful (baseline) and painful conditions. The time course of the synaptic input was simulated in order to obtain a force profile similar to the experimental recordings. The first simulation mimicked a ‘no pain’ scenario, where 450 motoneurons received a mean current (voluntary drive) of 13.6 nA without afferent inhibition. In the second simulation, during the painful condition, the motor unit pool received an inhibitory current with an

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exponential distribution which was highest for the earliest recruited units (low threshold) and lowest to the latest recruited unit (high threshold) and a larger voluntary drive (mean current of 14.2 nA) to reach the same level of force of the baseline condition. In this simulation, the first recruited unit (i.e. at 0% MVC) received an inhibitory current of 8 nA while the latest recruited unit (i.e. at 70% MVC) received an inhibitory current close to 0.1 nA. The total variance of the synaptic input was selected as a percentage of the mean current in order to obtain a coefficient of variation for the interspike intervals of approximately 15% (Negro & Farina, 2012). This simulated distribution of synaptic inputs is in line with previous experimental studies (i.e. Lee & Heckman 2000) and simulations (i.e. Powers & Heckman, 2015) that demonstrated a non-uniform distribution of (supraspinal and spinal) excitatory/inhibitory synaptic inputs to the motoneuron pool. The stability of the model in terms of this main conclusion was tested empirically with a number of simulation tests that are not reported in the manuscript for the sake of brevity.

Statistics

Normality of the data was checked with the Shapiro–Wilk test and sphericity was tested with the Mauchly test. Statistical significance was set at $P < 0.05$. Results are expressed as means (SD) unless stated otherwise. Torque (mean torque, CoV torque), interferential EMG (ARV, co-activation) and motor unit variables (mean discharge rate, CoVisi, recruitment/de-recruitment threshold, discharge rate at recruitment/de-recruitment) were averaged for each participant and assessed with a two-way repeated measures analysis of variance (ANOVA) with factors time (repetitions 1, 2) and condition (baseline, isotonic, pain and post-pain) for motor units recruited at (0–20%) MVC (during 20% MVC contractions) and (50–70%) MVC (during 70% MVC contractions). ANOVA with factors recruitment (low- and high-threshold, 0–35% and 35–70% MVC, respectively) and condition, was used to compare mean discharge rate and recruitment threshold between 'low' (0–35% MVC) and 'high' (35–70% MVC) threshold motor units, for units identified during contractions with a target torque of 70% MVC. These analyses were followed by pairwise comparisons with a Student–Newman–Keuls *post hoc* test when ANOVA was significant. Linear regression was used to characterize the association between the differences in discharge rate at target torque (mean discharge rate at 20 and 70% MVC) and recruitment (calculated from the first six motor unit discharges) and between the target torque (20 and 70% MVC) and motor unit recruitment threshold (0–20 and 50–70% MVC). This analysis was used to estimate the input–output gain of the tibialis anterior motoneurons (see torque and motor unit analysis section). The slopes of these linear regressions were compared between conditions: baseline *vs.* pain, isotonic *vs.* pain and post-pain *vs.* pain by analysis of covariance (Martinez-Valdes *et al.* 2018*a*, *b*; Boccia *et al.* 2019). This analysis was applied at each contraction level independently (20 and 70% MVC) to the full pool of motor units identified at 0–20 and 50–70% MVC across all participants. Finally, linear regression analysis was applied to all motor units identified during the contractions at 70% MVC (recruitment thresholds from 0 to 70% MVC) 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). The relationship between the difference in pain and baseline recruitment threshold and baseline recruitment threshold (Δ pain/baseline recruitment threshold *vs.* baseline recruitment threshold) was also assessed. These analyses were required to evaluate the relative contribution of high- and low-threshold motor units identified at high forces (70% MVC). As an additional analysis, we also assessed the relationships between 20% MVC Δ discharge rate and 70% MVC Δ discharge rate with pain intensity by calculating the Pearson correlation coefficient (r).

Results

Pain sensation

The painful sensation lasted for the full set of contractions, reaching its peak 1 min after the injection (Fig. 1*A*) and ceasing completely within 500 s. Within-subject

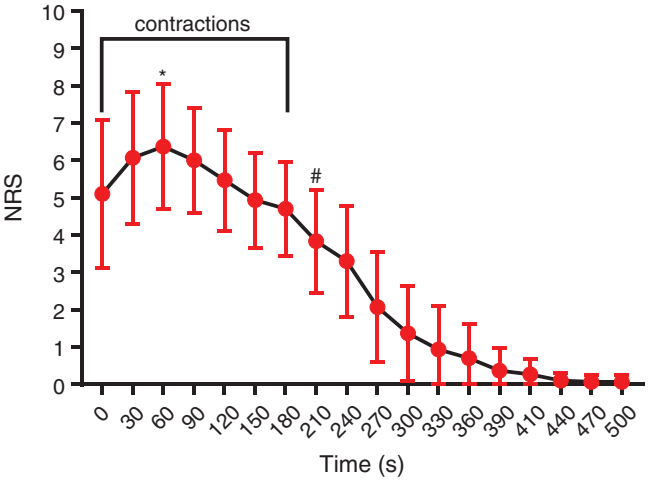

Figure 1. Pain profile. Pain sensation after the injection of hypertonic saline
Contractions were performed until 180 s. Significant increase from baseline *$P < 0.01$, significant decrease from baseline #$P < 0.01$. NRS, numerical rating scale [Colour figure can be viewed at wileyonlinelibrary.com]

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differences in pain sensation after the injection of hypertonic saline did not affect any of the motor unit variables (time effect $P > 0.21$ for all variables: discharge rate (at target torque, at recruitment and de-recruitment), recruitment de-recruitment thresholds and CoVisi), at both torque levels (0–20% and 50–70% MVC). Therefore, results correspond to the average of the two repetitions. Pain was consistently felt under the electrode grid by all participants (mainly under the 2nd, 3rd and 4th columns of the grid); however, three participants also experienced referred pain at the lateral malleoli.

Motor unit decomposition

A total of 568 and 494 motor units could be tracked across conditions (baseline, isotonic, pain, post-pain) and repetitions in all participants during contractions at 20 and 70% MVC, respectively. The average number of tracked motor units per participant at 20% MVC was 14 (8) and 11 (5) for contractions at 70% MVC. In a limited number of trials, we could observe some units that were active in some but not all conditions. However, this was unusual and the total number of identified units (considering both tracked motor units and those that were only present in certain conditions) was not significantly different across conditions (20% MVC, $p = 0.7$ and 70% MVC $p = 0.5$).

Mean target torque, torque steadiness and discharge rate variability at low and high forces

Mean target torque levels during the hold-phase of the contractions were consistent across conditions (average value across conditions: 20.0 (0.8) % MVC and 68.0 (0.04) % MVC, $P = 0.31$ and $P = 0.92$, respectively). Moreover, there were no differences in torque steadiness (average value across conditions: 2.2 (0.2) % at 20% MVC and 2.4 (0.2) % at 70% MVC, $P = 0.76$ and $P = 0.20$, respectively) and discharge rate variability (average value across conditions: 12.3 (1.0) % at 20% MVC and 21.8 (1.0) % at 70% MVC, $P = 0.29$ and $P = 0.6$, respectively).

Mean discharge rate and discharge rate at recruitment/de-recruitment in low and high forces

Figure 2 depicts a representative example from one participant showing adjustments in low- and high-threshold motor units between baseline and painful conditions. The same motor units were followed longitudinally. A reduction and increase in firing rate can be observed for this individual at 20 and 70% MVC, respectively. More specifically, at 70% MVC this individual showed differences in firing rate among low- and high-threshold motor units during the 70% MVC contraction, with a low/mid-threshold motor unit maintaining discharge rate across conditions and a high-threshold motor unit increasing discharge rate during pain (see low- and high-threshold motor units identified at 70% MVC, experiments and simulations sections). These results were also confirmed for the group of participants as mean discharge rate (stable torque part) decreased after the injection of hypertonic saline in relation to baseline and isotonic conditions and recovered during post-pain for motor units identified at 0–20% MVC ($P = 0.0003$, $\eta 2 = 0.36$, Fig. 3*A*). This contrasted with the response observed for motor units with recruitment thresholds between 50% and 70% MVC which significantly increased their discharge rate during pain in relation to baseline and isotonic conditions and did not fully recover post-pain ($P = 0.02$, $\eta 2 = 0.2$, Fig. 3*B*). Discharge rate at recruitment was not affected by pain for any of the conditions during low- and high-torque contractions (20 and 70% MVC, $P = 0.18$ and $P = 0.22$, respectively, Fig. 4*A*). However, the discharge rate at de-recruitment was significantly lower during the painful condition than at baseline and isotonic at 50–70% MVC ($P = 0.02$, $\eta 2 = 0.21$, Fig. 4*B*).

Relationship between perceived pain intensity and changes in discharge rate. Differences in discharge rate between the baseline and painful conditions at 20% MVC and 70% MVC were significantly correlated with pain intensity ($r = 0.74$, $P = 0.0018$ and $r = 0.59$, $P = 0.02$, respectively). Therefore, participants who had the largest reduction in discharge rate at 20% MVC and the largest increase in discharge rate at 70% MVC during the painful condition also showed the highest ratings of perceived pain intensity (Figs 5*A* and 5*B*).

Recruitment and de-recruitment thresholds at low and high forces

Recruitment and de-recruitment thresholds did not vary between conditions at 0–20% MVC ($P = 0.59$ and $P = 0.08$, for recruitment and de-recruitment thresholds respectively, Fig. 6*A*). However, at 50–70% MVC, both recruitment and de-recruitment thresholds decreased significantly during pain ($P = 0.02$, $\eta 2 = 0.2$ and $P = 0.008$, $\eta 2 = 0.24$, for recruitment and de-recruitment thresholds, respectively, Fig. 6*B*).

Estimated input–output gain to tibialis anterior motoneurons at low and high forces

At 0–20% MVC, the slopes of the regression lines representing change in discharge rate (mean discharge rate at target torque minus discharge rate at recruitment) *vs.* change in torque (target torque minus recruitment

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threshold) for baseline, isotonic and post-pain conditions were significantly greater than the slopes obtained during the painful condition ($P < 0.001$ in all comparisons, Fig 7*A*). Moreover, regressions deviated significantly from zero in baseline, isotonic and post-pain ($P < 0.001$), but not during the painful condition ($P = 0.7$) as the mean discharge rate became closer to the values of discharge rate at recruitment, showing less sensitivity to increases in torque during the 'ramp-up' phase of the contraction. Conversely, at 50–70% MVC, the slopes of the regression lines representing change in discharge rate *vs.* change in torque were significantly greater for the painful condition than for the baseline ($P < 0.0001$) and isotonic ($P = 0.02$), but not post-pain ($P = 0.06$), Fig. 7*B*. These results were mainly due to the decrease in recruitment threshold and lack of changes in discharge rate at recruitment during the painful condition.

Low- and high-threshold motor units identified at 70% MVC: experiments

Low-threshold motor units (0–35% MVC) identified at a target torque of 70% MVC showed a different behaviour compared with high-threshold motor units (35–70% MVC) identified from the same contractions, Fig. 8. Low-threshold motor units maintained both their recruitment thresholds and discharge rates during the painful condition while the high-threshold motor units decreased their recruitment threshold and increased their firing rates during pain (interaction effect: force ×

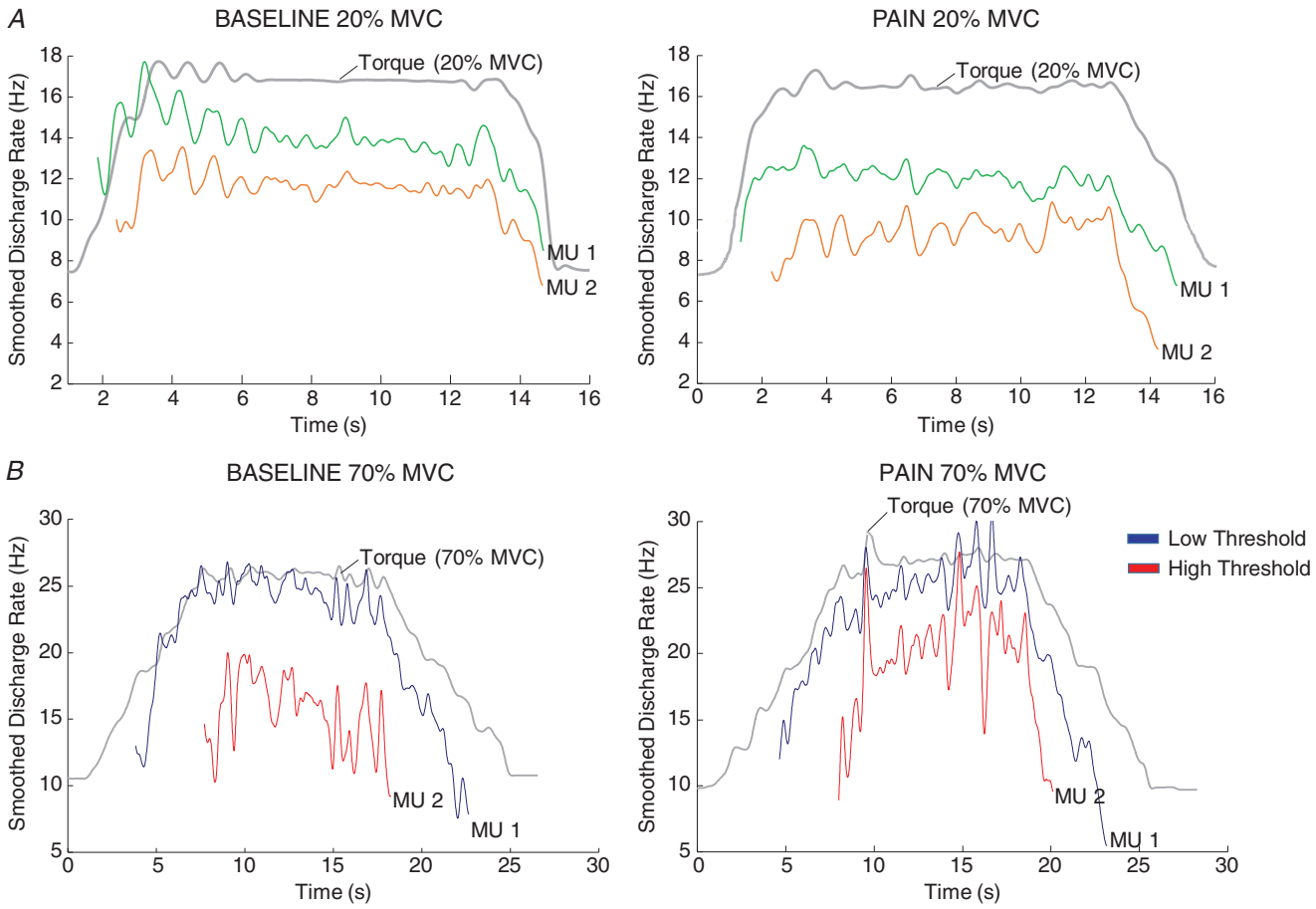

Figure 2. Representative example of motor unit behaviour during baseline and pain conditions at low and high forces
A, smoothed discharge rate from two low-threshold motor units identified at a low-force level (20% MVC). Regardless of their recruitment threshold, these two motor units' mean discharge rate (at plateau) decreased from 13.9 Hz and 11.8 Hz during baseline to 11.7 Hz and 9.1 Hz during the pain condition, respectively. *B*, smoothed discharge rate from one low-threshold (20.0% MVC recruitment threshold) and one high-threshold (57.8% MVC recruitment threshold) motor unit identified at a high force level (70% MVC). As opposed to the behaviour observed during low forces, discharge rate from these two motor units was adjusted differently according to the torque level where these units were recruited. Thus, the low-threshold motor unit slightly decreased its mean firing rate between baseline and pain conditions (from 24.8 Hz to 24.5 Hz) while the high-threshold motor unit increased its firing rate across conditions (from 15.1 Hz to 20.5 Hz) [Colour figure can be viewed at wileyonlinelibrary.com]

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condition, $p = 0.001$ and $p = 0.015$, for recruitment threshold and discharge rate, respectively). This divergent behaviour was also observed for the full pool of identified motor units at 0–70% MVC across all subjects (Fig. 9). Figure 9*A* shows the relationship between the difference in discharge rate during pain and baseline conditions and recruitment threshold obtained at baseline from the full pool of identified units between 0 and 70% MVC. Regression analysis showed that the largest increases in discharge rate between conditions were observed for high-threshold motor units ($P < 0.001$). These results were also observed for the relationship between the difference in recruitment threshold during pain and baseline conditions and recruitment threshold during baseline (Fig. 9*B*), where high-threshold motor units showed the largest decrease in recruitment threshold ($P < 0.001$).

Low- and high-threshold motor units identified at 70% MVC: simulations. Simulation results from two contractions at 70% MVC are depicted in Fig. 10. The baseline condition without afferent inhibition is shown on the left while the painful condition with increased voluntary drive and exponentially distributed inhibitory currents (i.e. high inhibition for low-threshold motor units and low inhibition for high-threshold motor units) is presented on the right. The representative firing behaviour of 20 motor units is shown in these two simulations. When considering all simulated motor units, the average discharge rate between conditions was similar (Baseline: 21.5 Hz *vs.* Pain: 21.2 Hz). However, differences could be observed when analysing motor units of different recruitment thresholds. In this figure, a low-threshold (red), mid-threshold (green) and high-threshold (red)

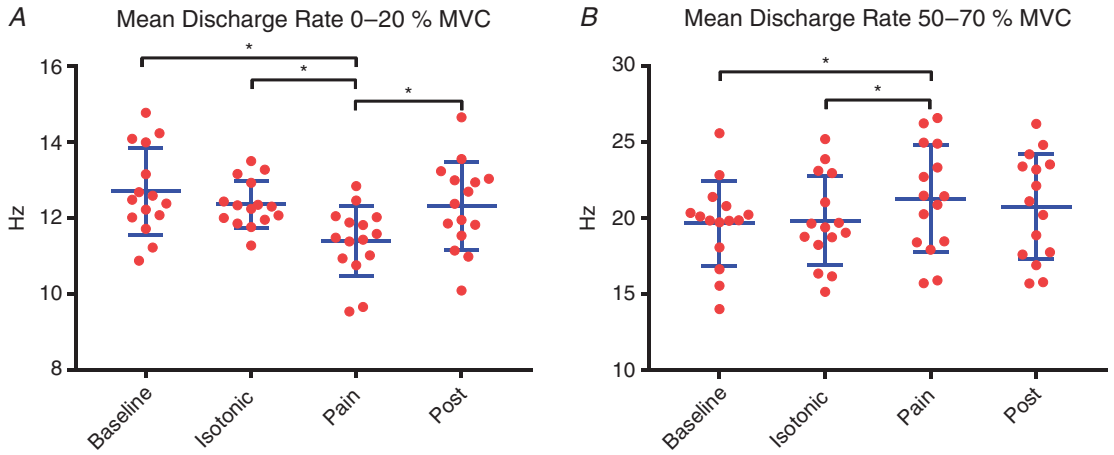

Figure 3. Changes in mean discharge rate in low- and high-threshold motor units at different forces
Changes in mean discharge rate (attained during stable torque part) across conditions can be seen for motor units recruited at low (low-threshold, 0–20% MVC, A) and high forces (high-threshold, 50–70% MVC, B). *$P < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

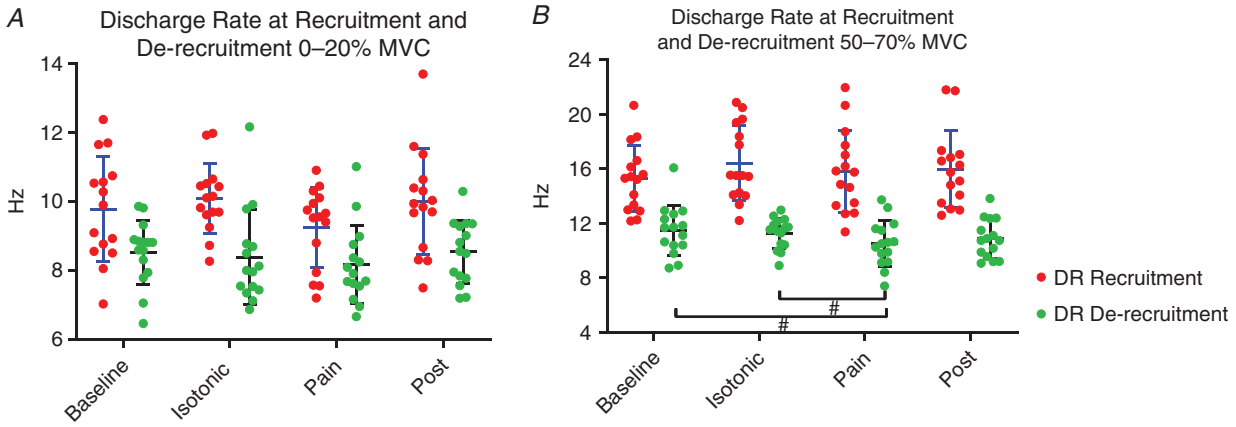

Figure 4. Changes in discharge rate at recruitment and de-recruitment for low- and high-threshold motor units at different forces
Changes in discharge rate (DR) at recruitment (first 6 firings, red) and decruitment (last 6 firings, green) can be seen for motor units recruited at low (low-threshold, 0–20% MVC, *A*) and high forces (high-threshold 50–70% MVC, *B*). #$P < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

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motor unit was followed across conditions. The motor unit firing rates for these units were 26.0 Hz, 17.9 Hz and 7.9 Hz during baseline condition while the discharge rates during the painful condition were 24.0 Hz, 18.5 Hz and 9.9 Hz, for red, green and black motor units, respectively. The results show that in order to maintain a high-force contraction (70% MVC), the reduction in firing rate of low-threshold motor units needs to be compensated by an increase in high-threshold motor unit discharge rate. This can only be possible by reduced inhibition to high-threshold motor units as increases in voluntary drive allow an increase of the excitability of these units.

Interferential EMG

ARV amplitude at 20% was not different between conditions (baseline 32.0 (16.4) μV, isotonic 30.5 (13.6) μV, pain 31.2 (14.5) μV and post-pain 29.6 (15.5) μV, $P = 0.28$). The same was observed at 70% MVC as amplitude did not differ across conditions (baseline 130.6 (42.6) μV, isotonic 134.9 (43.0) μV, pain 138.4 (48.7) μV and post-pain 135.6 (44.2) μV, $P = 0.35$). Finally, co-activation also did not change between conditions at 20% MVC (baseline 27.2 (15.0) %, isotonic 27.4 (13.1) %, pain 25.3 (12.4) % and post-pain 28.3 (16.9) %, $P = 0.9$) and 70% MVC, $P = 0.72$) and at 70% MVC (baseline

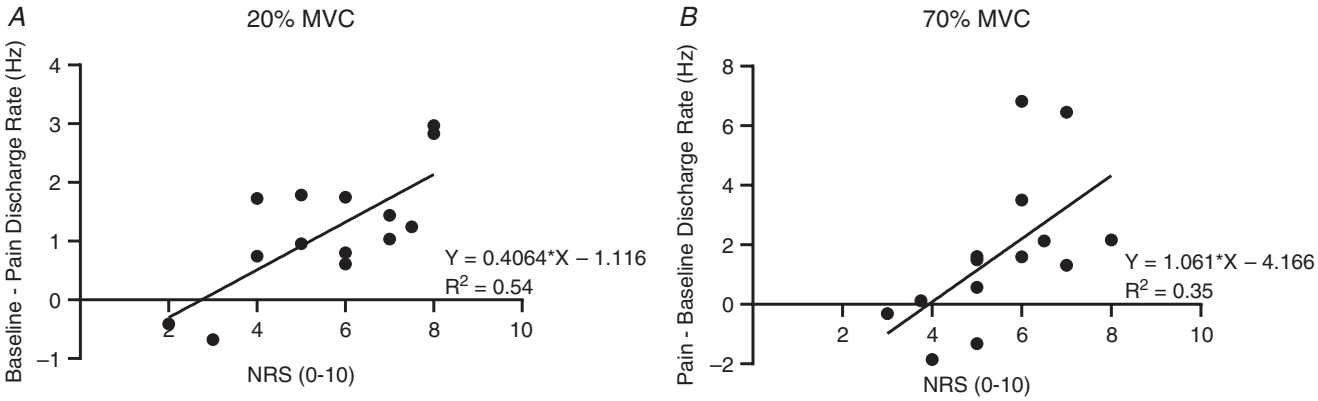

Figure 5. Relationships between perceived pain intensity and changes in discharge rate at 20% and 70% MVC
The differences between discharge rate during the baseline and pain conditions were correlated with pain intensity (numerical rating scale (NRS)) at 20% MVC (*A*) and 70% MVC (*B*). Linear regression equations and coefficients of determination (R^2) are reported for each plot

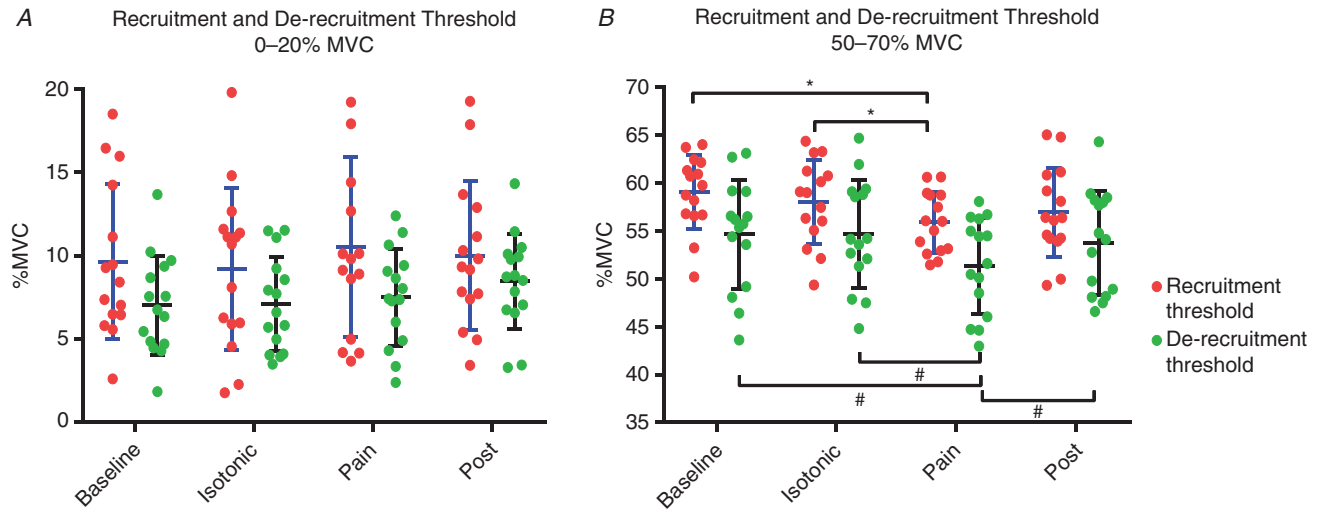

Figure 6. Changes in recruitment and de-recruitment threshold for low- and high-threshold motor units at different forces
Changes in recruitment threshold (red) and de-recruitment threshold (green) can be seen for motor units recruited at low (low-threshold, 0–20% MVC, *A*) and high forces (high-threshold, 50–70% MVC, *B*). Significant decrease in recruitment threshold $*P < 0.05$. Significant decrease in de-recruitment threshold $\#P < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

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22.4 (7.1) %, isotonic 22.9 (6.5) %, pain 25.4 (7.1) % and post-pain 22.8 (6.8) %, $P = 0.3$).

Discussion

We have shown, for the first time, that the firing frequency and recruitment strategies of low- and high-threshold motor units are adjusted differently in response to acute noxious stimuli. Thus, afferent inhibitory inputs are presumably not uniformly distributed across all motor units since otherwise differential adjustments would not be observed, as also confirmed by the results of our computational model.

Previous studies focusing on low-force sustained contractions have commonly reported a reduction in discharge rate during experimental muscle pain in low-threshold motor units (Sohn *et al.* 2000; Farina *et al.* 2004, 2005). Reflex-mediated inhibition of motoneurons via excitation of type III and IV nociceptive afferents has been described as the likely mechanism (Farina

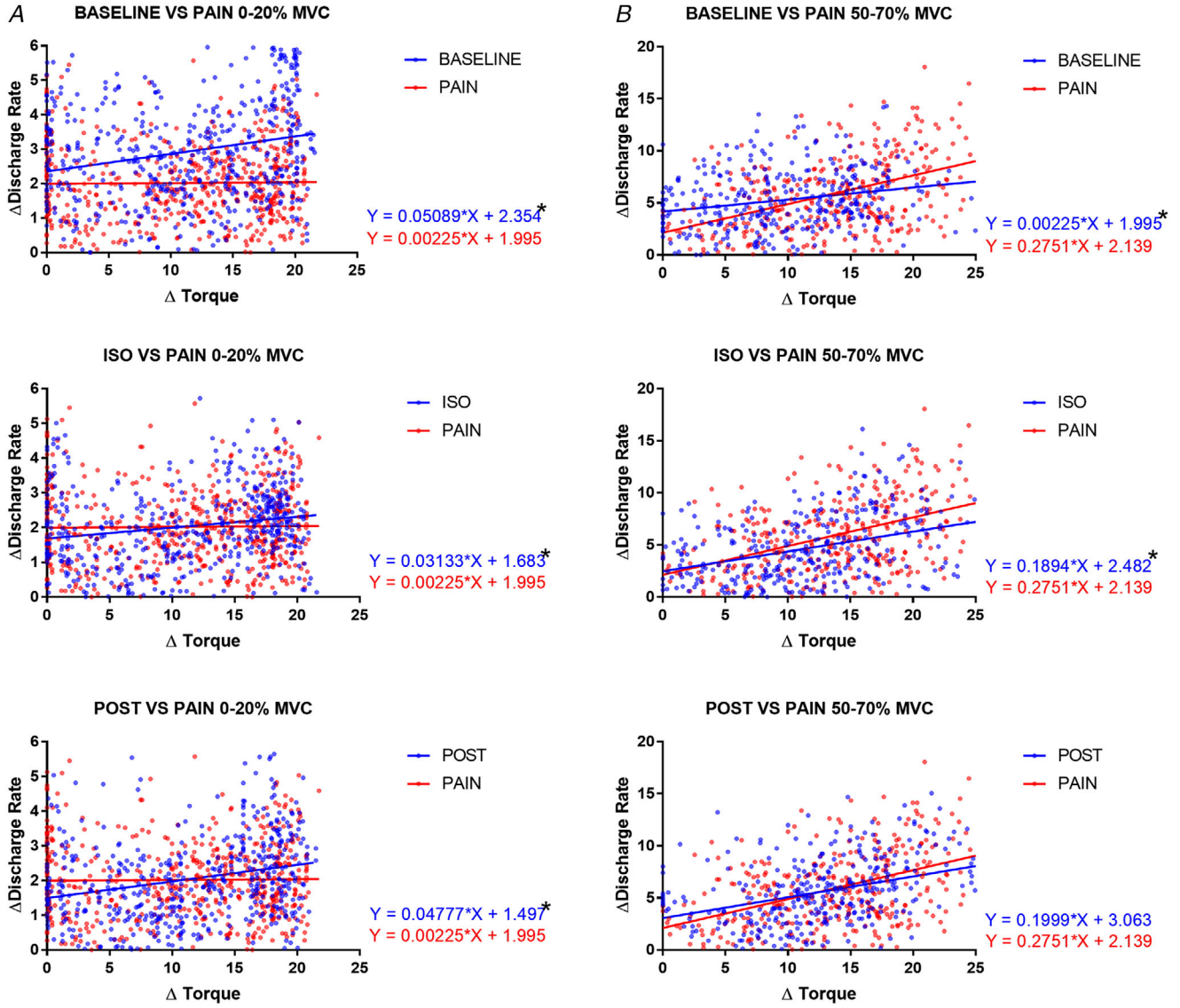

Figure 7. Estimated input–output relationship for low- and high-threshold motor units at different forces
Input–output relationship (estimated net synaptic input received by motoneurons) was estimated by linear regression analysis of the difference between mean discharge rate at target torque and discharge rate at recruitment (y-axis) and the difference between target torque (20 or 70% MVC) and recruitment threshold (x-axis), for motor units identified at low (low-threshold, 0–20% MVC, $n = 568$ motor units, *A*) and high forces (high-threshold, 50–70% MVC, $n = 361$ motor units, *B*). The comparison of the estimated synaptic input between conditions was made by comparing slopes and intercepts by analysis of covariance. *, Significant difference in slope between conditions, $P < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

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et al. 2005). Our results confirmed this decline in discharge rate and additionally showed that pain alters the input–output gain (estimated synaptic input) of the motor unit pool (Martinez-Valdes *et al.* 2018*b*; Boccia *et al.* 2019; Del Vecchio *et al.* 2019). This general decrease in discharge rate and synaptic input provides some support for the pain adaptation model (uniform inhibition to the painful muscle). Nevertheless, this inhibitory behaviour on low-threshold motor units cannot explain the maintenance of muscle force for low intensity contractions during pain. If the discharge rate is reduced and the estimated synaptic input received by the motor unit pool declines, force has to be maintained by other peripheral and/or central mechanisms. The influence of peripheral motor unit properties on the maintenance of muscle force has been discarded (Farina *et al.* 2004, 2008). Moreover, the influence of synergists compensating for reductions in discharge rate of the painful muscle has also been discarded (Hodges *et al.* 2008). More recent studies provided evidence of a centrally mediated change in recruitment strategies (Tucker & Hodges, 2009; Tucker *et al.* 2009). Indeed, additional motor units presumably not affected by the painful stimuli can be recruited to maintain force output. Although these previous results provide a plausible explanation for the maintenance of force in painful conditions at low forces, we did not observe

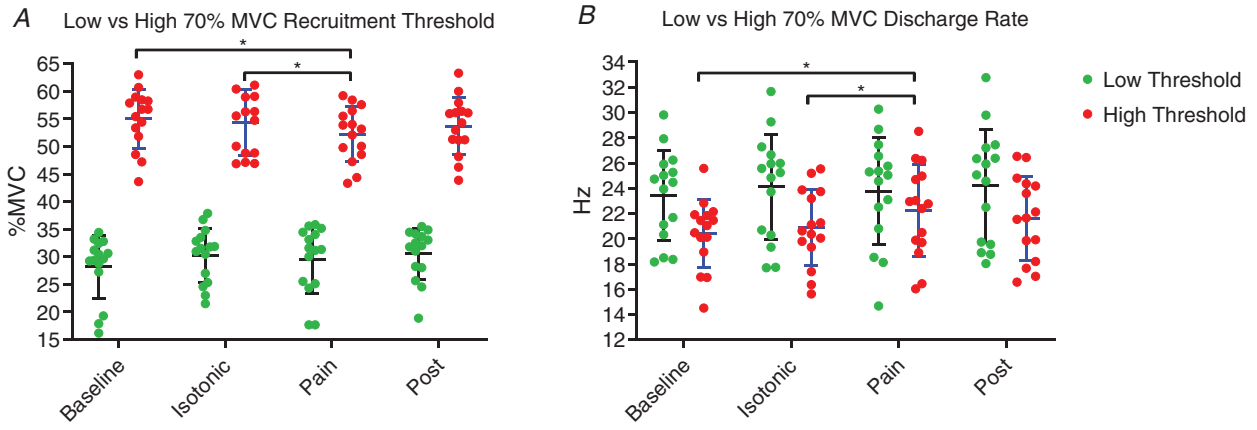

Figure 8. Recruitment threshold and mean discharge rate for low- and high-threshold motor units identified at 70% MVC
Changes in recruitment threshold (RT, *A*) and mean discharge rate (DR, *B*) across conditions was compared between lower (recruitment threshold between 0 and 35% MVC) and higher-threshold (recruitment threshold between 35 and 70% MVC) motor units identified during a contraction with a target torque of 70% MVC [Colour figure can be viewed at wileyonlinelibrary.com]

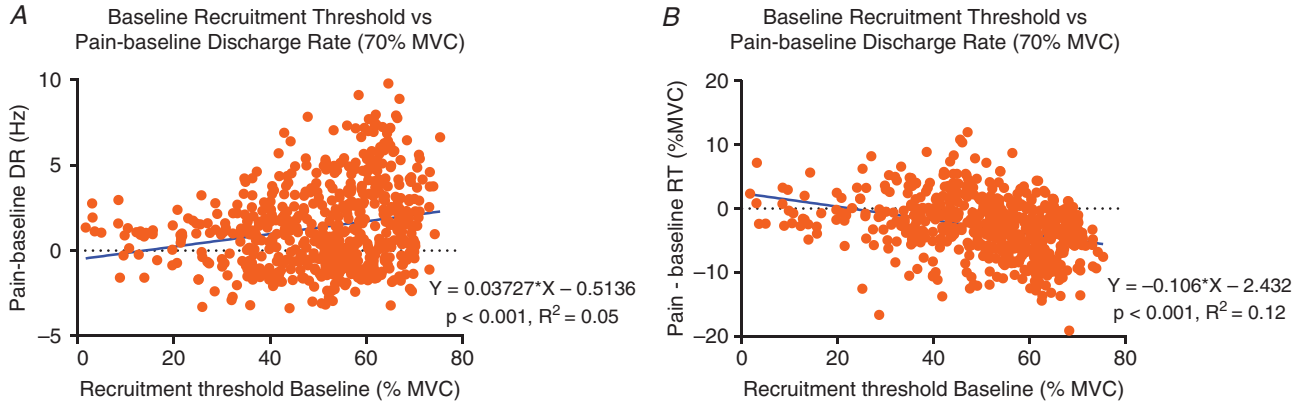

Figure 9. Difference in discharge rate and recruitment threshold between pain and baseline conditions and its relation with recruitment threshold at baseline
The association between recruitment threshold at baseline (x-axes) and the difference of mean discharge rate (*A*) and recruitment threshold (*B*, y-axes) between pain and baseline conditions from all the units identified at 70% MVC ($n = 494$) was used to analyse the relative contribution of low- and high-threshold motor units to changes in both firing and recruitment properties. Regression lines show that at 70% MVC, changes in mean discharge rate and recruitment threshold during pain, are more pronounced for high-threshold motor units [Colour figure can be viewed at wileyonlinelibrary.com]

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significant differences in the number of units identified across conditions.

Our study is the first to analyse high-threshold motor unit behaviour during experimental muscle pain. During this condition, discharge rate and the input–output gain to the high-threshold motor unit pool increased, and second, both recruitment and de-recruitment thresholds were reduced, meaning that high-threshold motor units were active for a longer period of time during the contraction. Previous studies analysing muscle responses to pain at high forces are scarce and predominantly focused on examining global EMG measures. Graven-Nielsen *et al.* observed a decline in maximal knee extension (Graven-Nielsen *et al.* 2002) and dorsiflexion force (Graven-Nielsen *et al.* 1997*b*) following the infusion of hypertonic saline into the rectus femoris and tibialis anterior, respectively. In the latter study, the authors reported that reduced maximal strength was accompanied by a decrease in tibialis anterior activity and an increase in antagonist activity during walking; findings which reportedly supported the pain adaptation model. Nevertheless, in the same study (Graven-Nielsen *et al.* 1997*b*), subjects were able to perform submaximal voluntary contractions up to 80% MVC during the painful condition with no significant changes in EMG with respect to the non-painful condition. This observation agrees with our findings. It is therefore apparent that central adjustments to high-threshold motor units are required to reach high force levels in the presence of pain. However, such adjustments will not be sufficient to reach maximum force due to the overall afferent-mediated inhibition to motoneurons during pain. High submaximal forces during pain can only be reached to the point that the voluntary drive can be increased, as this will allow the firing rate of high-threshold motor units to be increased. Accordingly, when we compared the behaviour of lower- (0–35% MVC) and higher- (35–70% MVC) threshold motor units identified at 70% MVC, we observed that the lower-threshold units maintained their discharge rate and did not change their recruitment threshold as opposed to the increase in discharge rate and decrease in recruitment threshold found for higher-threshold units (Fig. 8). These results were also confirmed across the full population of identified motor units (0–70% MVC) as the difference in discharge rate and recruitment threshold between baseline and painful conditions showed that the largest differences were found for motor units that were recruited at the highest force levels (Fig. 9). These findings, in addition to our simulation results, provide unique support for a differential inhibition of different populations of motor units. The increase in voluntary drive and the concomitant

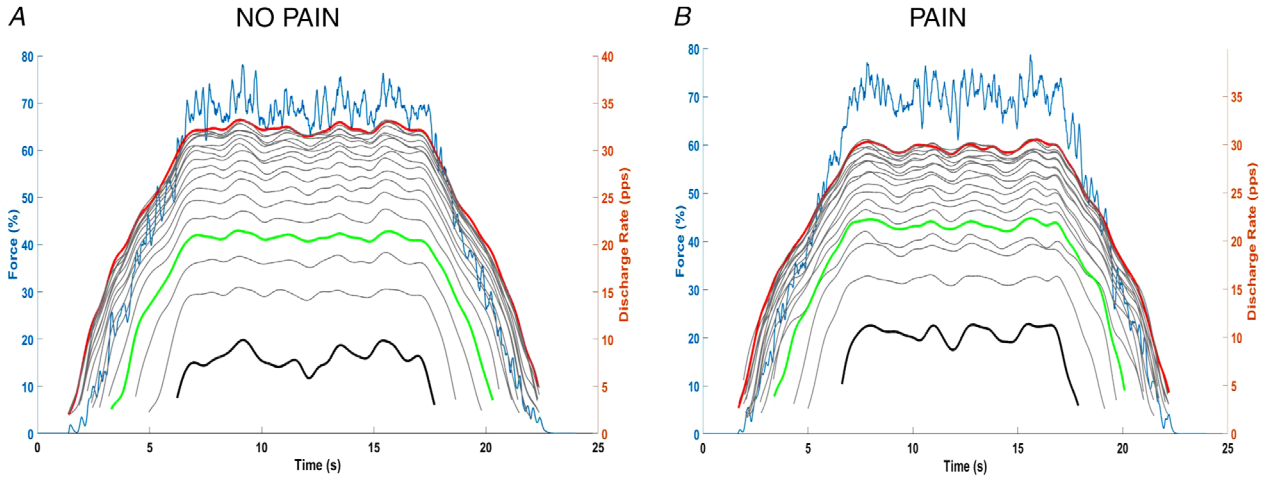

Figure 10. Simulation results
Firing behaviour of 450 motor units recruited from 0 to 70% MVC was simulated for baseline (*A*, mean current to the motor unit pool of 13.6 nA without afferent inhibition) and pain conditions (*B*, mean current to the motor unit pool of 14.2 nA, with exponentially distributed inhibitions across the pool). The smoothed firing behaviour of 20 motor units is shown for clarity (grey and coloured lines). Three representative motor units with different recruitment thresholds (low-threshold (red), low–mid-threshold (green) and high-threshold (black)) were followed across conditions. In the synthetic painful contraction (*B*), the red motor unit received an inhibitory current of 8 nA, the green motor unit received an inhibitory current of 0.2 nA, while the black unit received an inhibitory current of 0.1 nA. These different inhibitory currents in addition to the increased voluntary drive during the pain condition (increase in mean current) induced divergent responses in motor unit behaviour for these three representative units and across the motor unit pool. Thus, during no pain (*A*) the motor unit firing rate for the red, green and black motor units was 26.0 hz, 17.9 hz and 7.9 hz, respectively, which changed during the painful condition to 24.0 Hz, 18.5 hz and 9.9 hz. These results show that an increase in firing rate for high-threshold motor units is necessary to compensate for the decrease in firing rate/inhibition for low-threshold motor units. These adjustments are likely required to maintain high forces (i.e. 70% MVC) during pain. [Colour figure can be viewed at wileyonlinelibrary.com]

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increase in discharge rate and estimated input–output gain to high-threshold motor units during pain is likely determined by supraspinal (Olivier *et al.* 1995) and not spinal sources, as it has been previously reported that pain increases Ia afferent pre-synaptic inhibition (Rossi *et al.* 1999). In support of this statement, Martin *et al.* (2008) previously observed an increase in corticospinal axon activity during experimental pain. The authors concluded that the increase in motoneuron excitability was driven by high-threshold motor units, which induced compression of the range of recruitment thresholds of the motoneuron pool, which is in agreement with our findings. Such mechanism allows high-threshold motor units to be activated earlier during the contraction and highlights the increase in excitability (or decreased inhibition) to this pool during pain. Reduction of antagonist muscle activity (Patten & Kamen, 2000), decrease in twitch force (Farina *et al.* 2009) and increase in the rate of force development (Desmedt & Godaux, 1978) have been identified as potential candidates for a reduction in recruitment thresholds. However, our results and previous literature shows that, first, co-activation remained constant across conditions; second, twitch force is not related to the maintenance/reduction in muscle force during sub-maximal and maximal painful contractions (Farina *et al.* 2008); and finally, the rate of force development did not change across conditions as participants performed a fixed trajectory (ramp-up contraction at a rate of 10% MVC per second). Another interesting finding was the reduction of de-recruitment thresholds (Fig. 3*B*). This observation shows that high-threshold motor units compensate for the reduction in force of low-threshold motor units by being activated for a longer time during the contraction. Since these units typically fatigue faster (Burke, 1980), their early recruitment in addition to their prolonged activity, likely imposed an additional cost to the nervous system.

The findings from this study provide a possible explanation for the progression from acute to chronic pain as the constant activation of high-threshold motor units compensating for the inhibition of low-threshold units will eventually lead to increased muscle fatigue which will require redistribution of activity and changes of the resultant force vector in order to unload the painful tissue and satisfy the demands of the task (van Dieen *et al.* 2017). This observation is consistent with findings from studies that have analysed motor unit behaviour in painful clinical conditions, which have reported an increase in discharge rate compared with asymptomatic individuals, despite observing an overall decrease in maximum voluntary force (Kallenberg & Hermens, 2006; Falla *et al.* 2010; Yang *et al.* 2016). Nevertheless, this has to be confirmed in longitudinal studies monitoring motor unit behaviour from acute to chronic pain as an increase in firing rate can be observed in both low- and high-threshold motor units, possibly due to chronic changes across the whole motor unit pool.

Another interesting observation was the lack of recovery in discharge rate and recruitment threshold for high-threshold motor units during the post-pain condition. Previous studies have also observed long-lasting central changes following nociceptive stimulation (Le Pera *et al.* 2001; Svensson *et al.* 2003; Martin *et al.* 2008). These changes are not due to changes in afferent terminal or motoneuron excitability (Cook *et al.* 1986) but probably due to changes in interneurons (Wall & Woolf, 1984), possibly through long-term synaptic potentiation (Sandkuhler, 2000). It is possible that these effects are more pronounced for high-threshold motoneurons (Martin *et al.* 2008) and might also contribute to altered motor unit activity in chronic pain.

The present study provided a number of new findings but is not exempt from limitations. First, the estimation of the number of recruited units is limited by the small proportion of motor units identified with respect to the total number of active motor units during the contractions. Secondly, a relatively small number of low-threshold motor units were detected at 70% MVC. Decomposition algorithms rely on the energy of the EMG signal where the separation of multiple sources is biased towards the units that have the largest action potentials (Holobar & Zazula, 2007). Therefore, when strong contractions are decomposed, very low-threshold motor units (i.e. 0–20% MVC) are usually not detected. Being able to identify the activity of these units is essential to confirm their inhibition at high forces. Nevertheless, the fact that we could show that the behaviour between high-threshold and moderate- to low-threshold units (∼30% MVC) diverged, confirms the presence of differential behaviour across the motor unit pool at high forces. These two limitations might be solved in future studies by combining HDEMG and thin-film multichannel intramuscular electrodes (Muceli *et al.* 2015), allowing identification of a larger population of motor units. Finally, it is possible that the study design might have induced certain levels of fatigue, which could have potentially interfered with our findings. Nevertheless, we aimed to minimize such effects by providing long resting periods between contractions and conditions. The subjects only performed a few repetitions and the time that the contraction was maintained at target torque was relatively short (10 s). We observed that torque steadiness and discharge rate variability remained similar across conditions, indicating the absence of fatigue (Castronovo *et al.* 2015). Moreover, the pain intensity was significantly correlated with the changes in discharge rate between baseline and pain conditions during both 20% and 70% MVC contractions. Therefore, it is unlikely that the present findings reflect the effects of fatigue.

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In conclusion, we have provided novel evidence of non-uniform changes in motor unit properties at low and high forces in response to muscle pain. Specifically, at high force levels, the inhibition of low-threshold motor units can be compensated by an increase in voluntary drive which specifically increases the activity of high-threshold motor units, as these units are presumably less inhibited in response to nociceptive afferent input.

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Additional information

Author contributions

Experiments were performed at the Centre for Precision Rehabilitation (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom. E.M.-V., D. Far and D. Fal designed the research; E.M.-V and D. Fal performed the experiments; E.M.-V., F.N. and D. Far. analysed the data; E.M.-V., F.N., D. Far and D. Fal interpreted the data and contributed to the drafting of the article. All authors have read and approved the final submission. All authors agree to be accountable for all aspects of the work, ensuring that questions related to the

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accuracy or integrity of any part are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing interests

All authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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