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

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

Interestingly, the adjustments to noxious stimulation of the upper trapezius was confirmed to be independent of the location of the painful stimulus(Falla et al., 2009). In this way, the greatest reduction of EMG amplitude occurred in the cranial region, even when nociceptive afferents in the caudal region were stimulated. Assuming that adjustments to pain aim to reduce the activity of the painful region (Lund et al., 1991; Hodges and Tucker, 2011), this strategy appears suboptimal since

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

METHODS

Subjects

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

Procedure

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

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

Experimental Muscle Pain

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

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

Measures of Perceived Pain Intensity and Area

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 following the injection and every 30 s until pain was no longer reported. For each trial, the peak pain intensity and the duration of the pain were calculated. Participants documented the area of pain on a body chart. Pain drawings were subsequently digitized (ACECADD9000 + Taiwan) and pain areas were measured.

Multi-channel surface EMG

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

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

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

[FIGURE 1 AROUND HERE]

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

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

Surface EMG analysis

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

High-density surface EMG decomposition

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

Motor unit analysis

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

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

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

[FIGURE 2 AROUND HERE]

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

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

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

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

Statistical Analysis

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

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

RESULTS

Sensory characteristics

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

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

[FIGURE 3 AROUND HERE]

Surface EMG variables

The average amplitude of the surface EMG across all channels was significantly lower after the injection of hypertonic saline (58.1 ± 26.8 μV) compared to the control condition (74.9 ± 38.7 μV; p=0.011) and following the injection of isotonic saline (67.6 ± 29.9 μV; p=0.008). The difference in EMG amplitude from the control to the isotonic condition was not statistically significant (p=0.07). The reduction in mean EMG amplitude from the control to hypertonic condition (22%) was similar to those previously observed in similar conditions (approximately 20-25%

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

Motor unit behavior

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

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

[FIGURE 4 AROUND HERE]

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

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

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

Coherence between cumulative spike trains

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

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

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

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

[FIGURE 5 AROUND HERE]

DISCUSSION

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

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

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

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

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

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

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

496 average synaptic input to these motor units did not change, there was a large
497 variability in the effect of nociception on the single motor neuron (it may be inhibited
498 to a degree where it is no longer active or excited to a degree where it becomes active
499 or increases its discharge rate). The mechanism(s) underlying this adjustment are not
500 clear, but type III/IV afferents (the nerve fiber types carrying nociceptive feedback)
501 have been shown to excite as well as to inhibit motor neurons (Kniffki et al., 1981).
502 With regards to the second aim (dependence of the adjustment on pain
503 location), there was no difference between the motor unit discharge rates within the
504 individual muscle region when hypertonic saline was injected in the same or in the
505 other region (Figure 4C). This indicates that the underlying neural mechanisms
506 reflected in the relative decrease of the amplitude of the EMG signal in the cranial
507 region are similar in the two conditions. Furthermore, this observation implies that
508 when pain was induced in the caudal region of the muscle, the net activity of the
509 trapezius was redistributed to be concentrated in that area. According to a recent
510 theory of motor adaptation to pain (Hodges and Tucker, 2011), muscle activity is
511 redistributed (within or across muscles) to minimize activity of the painful region with
512 the aim of "protecting" the painful area. As described above, however, the
513 adjustments to experimentally induced muscle pain in the caudal region of the upper
514 trapezius did not optimally protect the caudal region. This suggests that, when the
515 upper trapezius muscle is painful, the adjustment aims always to preferentially
516 minimize activation of the cranial region. The functional advantage underlying this
517 strategy, however, cannot readily be identified based on the current results. The
518 fascicles of cranial region attach to the lateral part of the clavicle, while fascicles in
519 the caudal region attach along the superior border of the scapula (from the acromion
520 along the scapula ridge) (Johnson et al., 1994). In this way, during 90 degree shoulder

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

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

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

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

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

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Figure Legends

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

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

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

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

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