EXPERIMENTAL MUSCLE PAIN INCREASES THE VARIABILITY OF THE
NEURAL DRIVE TO MUSCLE AND DECREASES MOTOR UNIT COHERENCE IN
THE TREMOR FREQUENCY BAND

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ABSTRACT

It has been observed that muscle pain influences force variability and low-frequency (<3 Hz) oscillations in the neural drive to muscle. In this study, we aimed to investigate the effect of experimental muscle pain on the neural control of muscle force at higher frequency bands, associated with afferent feedback (alpha band, 5-13Hz) and with descending cortical input (beta band, 15-30 Hz). Single motor unit activity was recorded, in two separate experimental sessions, from the abductor digiti minimi (ADM) and the tibialis anterior (TA) muscles using intramuscular wire electrodes, during isometric abductions of the fifth finger at 10% of the maximal force (MVC) and ankle dorsiflexions at 25% MVC. The contractions were repeated under three conditions: no pain (baseline), after intramuscular injection of isotonic (0.9%, control), and hypertonic (5.8%, painful) saline. The results showed an increase of the relative power of both the force signal and the neural drive at the tremor frequency band (alpha, 5-13Hz) between the baseline and the hypertonic (painful) conditions for both muscles (p<0.05), but no effect on the beta band. Additionally, the strength of motor unit coherence was lower (p<0.05) in the hypertonic condition in the alpha band for both muscles and in the beta band for the ADM. These results indicate that experimental muscle pain increases the amplitude of the tremor oscillations due to an increased variability of the neural control (common synaptic input) in the tremor band. Moreover, the concomitant decrease in coherence suggests an increase in independent input in the tremor band due to pain.

KEYWORDS: Motor unit, experimental muscle pain, tremor, motor unit coherence.
INTRODUCTION

Alpha motor neurons receive common synaptic inputs from spinal and supraspinal sources during the execution of sustained contractions (Farina et al., 2014b). For this reason, motor neuron spike trains show some level of correlation, that can be estimated using time (cross-correlation) (De Luca et al., 1982; Nordstrom et al., 1992) or frequency (coherence) domain (Farmer et al., 1993; Myers et al., 2004) methods. In addition to low frequency components (common drive) (De Luca et al., 1982), the effective control signal to the muscle is also partly reflected in the alpha band (5-13 Hz), that is commonly associated with un-voluntary oscillations, termed physiological tremor (McAuley and Marsden, 2000). Common input in this frequency band is also reflected in the variability of force since these neural oscillations are not fully filtered out by the contractile properties of muscles (Baldissera et al., 1998). Higher common frequencies (beta band) are believed to be related to the monosynaptic corticospinal projections (Halliday and Conway, 1999; Negro and Farina, 2011a), but their direct influence on force output is probably negligible because of the severe muscle low-pass filtering (Negro and Farina, 2011b; Farina and Negro, 2014).

Nociception influences motor output with effects ranging from decreased maximal force (Graven-Nielsen et al., 1997) to reduced force steadiness during submaximal tasks (Farina et al., 2012). The way in which tasks are executed is influenced by pain, but the influence depends on the demands of the task and on the constraints imposed to execute the task. For this reason, studies analyzing different tasks have observed a different change in task accuracy (e.g., force steadiness) in painful conditions (e.g., Farina et al., 2004; Smith et al. 2006; Bandholm et al., 2008; Farina et al., 2012). A large inter-subject variability of the influence of muscle pain in force control is also often reported (Hodges and Moseley, 2003; Sae-Lee et al., 2008).
Previously we observed that the impaired ability to maintain a constant force during an unaccustomed task of the fifth digit with experimental muscle pain was mediated by an increase in the amplitude of oscillations of the common low-frequency components (<3 Hz) of motor unit spike trains (Farina et al., 2012). The increased variability in these components reflects greater variability in the common synaptic input received by the motor neurons at low frequency. The steadiness of this low frequency component is controlled by the integration of supraspinal (descending drive) input and afferent synaptic input. Thus, a high variability of the low-frequency component may suggest a suboptimal compensation of afferent input by the descending drive due to nociceptive stimulation. In this study, we focus on the influence of experimental muscle pain on higher frequency bands of the neural drive to muscle. The results of the study contribute to the understanding of the influence of pain on the execution of fine motor tasks as controlling contraction of a muscle at targeted force.

MATERIALS AND METHODS

Subjects

Data from 23 healthy subjects were analyzed. All subjects were right hand dominant and none reported symptoms of neuromuscular disorders or musculoskeletal pain. Ethical approval for the study was granted by the Ethics Committee of Nordjylland, Denmark (ref. N-20090019). Informed written consent was obtained from all participants and the procedures were conducted in accordance with the Declaration of Helsinki.

Signal recording
For the present study, we re-analyzed the intramuscular electromyography (iEMG) data reported in two previous studies (Farina et al., 2004, 2012) that investigated the discharge properties of motor units during experimental pain in the abductor digiti minimi (ADM) (11 subjects, Experiment 1) and in the tibialis anterior (TA) (12 subjects, Experiment 2) muscles. In both experiments, single motor unit action potentials were recorded using sterilized Teflon-coated stainless steel intramuscular wire electrodes (diameter 0.1 mm; A-M System, Carlsborg, WA). The wires were cut to expose the cross section of the tip without insulation. Following disinfection of the skin, the wires were inserted into the muscle belly via a 25-gauge hypodermic needle, which was removed immediately after the insertion, leaving the wire electrodes inside the muscle. The bipolar iEMG signals were amplified (Counterpoint EMG, Dantec Medical, Skovlunde, Denmark) with a gain of 500, band-pass filtered between 500 Hz to 5000 Hz, and sampled at 10,000 sample/s for both experiments. A reference electrode was placed around the right wrist in Experiment 1 and the right ankle in Experiment 2.

The fifth digit abduction force (ADM) and ankle dorsiflexion force (TA) was recorded and used for online visual feedback for the subjects in both experiments. Since highly accurate decomposition of a relatively high number of motor units is required for coherence analysis, the analysis of the final results was obtained only from the most accurate decomposed data that corresponded to 9 of 11 subjects (9 men; age, 25.1 ± 2.5 yrs) for the ADM muscle, and 7 of 12 subjects (7 men; age, 24.2 ± 2.1 yrs) for the TA muscle. Decompositions with abnormal inter-spike intervals due to a high incidence of missed or wrongly matched discharges were excluded. This was judged by researchers through visual examination of the decomposition results. Decompositions with inter-spike intervals outside of physiological limits were excluded (Moritz et al., 2005).
Experimental muscle pain

In both experiments, experimental muscle pain was induced by injection (27G cannula) of 0.5 ml sterile hypertonic saline (5.8%). Isotonic saline (0.5 ml, 0.9 %) was used as a control injection. The injections into the ADM were performed manually and the bolus was delivered over 10 s. The location of the injection was ~10 mm distal and ~5 mm transverse to the intramuscular wires. Participants were asked to verbally rate their level of perceived pain intensity on an 11 point numerical rating scale (NRS) anchored with “no pain” (0) and “the worst possible pain imaginable” (10) every 30 s until pain was no longer reported. In the second experiment, a computer-controlled syringe pump (IVAC, model 770) was used to infuse the saline into the TA muscle over 40 s. The location of the needle was ~10 mm transverse to the intramuscular wires. The level of perceived pain intensity was rated by the subjects every 5 s until pain was no longer reported.

Experiment 1

Participants were seated on an adjustable chair with the right arm and the first four digits fixed on a force measuring device with Velcro straps. The fifth finger was fixed to a load cell to measure isometric abduction force. The participants performed three maximum voluntary contractions (MVC) of right fifth finger abduction with 2-min rest intervals between trials. Verbal encouragement was provided to the subject to promote higher forces in each trial. The highest value of force recorded was selected as the reference MVC. After the MVC recordings, the participants performed one sustained abduction of the fifth finger at 10% of MVC for 60 s, under three conditions: baseline, isotonic saline, and hypertonic saline. Visual feedback of finger abduction force was provided on an oscilloscope. The participants were asked to keep the force signal at the target level that was provided as visual feedback (resolution adjusted as ±10% of the
target force) as accurately as possible. The order of the baseline and the isotonic saline conditions were randomized across subjects and were followed lastly by the hypertonic saline condition. A rest of 5 min was given between each condition.

Experiment 2

The participants were seated in a comfortable chair with their ankle at 90° of flexion, knee at 120° of extension and hip at 90° of flexion, and with their foot fixed in an isometric force brace. The ankle dorsiflexion force was measured with a torque transducer which was incorporated in the isometric force plate (Aalborg University, Aalborg, Denmark). In this experiment, the same procedure was applied in the same experimental session on both legs, apart from the type of infusion (hypertonic saline in the right leg, painful condition; isotonic saline in the left leg, control condition).

Subjects performed three MVCs of ankle dorsiflexion with 2-min rest in between each contraction. Verbal encouragement was provided to the subject to promote higher forces in each trial and the highest value of force was selected as the reference MVC. After 5 min of rest, subjects performed sustained isometric dorsiflexion for 4-min at 25% MVC. Visual feedback of ankle dorsiflexion force was provided on an oscilloscope. The participants were asked to keep the force signal at the target level that was provided as visual feedback (resolution adjusted as ±10% of the target force) as accurately as possible. The subject then had a 20-min rest, after which hypertonic (right leg) or isotonic (left leg) saline was infused. Two minutes after the beginning of the infusion, the subject was asked to perform a 4-min-long contraction at 25% MVC, identical to the previous one. Thus a total of four contractions, two for each leg, were performed by the subject with and without injection of saline. The order of assessment of the two legs was
randomized. For the current study, a data segment of 60 s duration in the interval of maximum pain was decomposed and analyzed.

**Signal and data analysis**

iEMG signals were filtered with a 1000 Hz high-pass filter and decomposed using the EMG-Lab decomposition program (McGill et al., 2005). Furthermore, each motor unit spike train was manually edited using the visual interface of the program for inspecting unusually long or short inter-spike intervals (ISI). The motor units were discriminated from the segment of iEMG where the signal quality was high enough to provide a reliable decomposition and where the highest level of perceived pain intensity was approximately constant.

The discharge properties of motor units, coherence, power spectrum and statistical analysis were performed using custom designed software written in MATLAB (Matworks, Natick, Massachusetts, U.S.A.). After decomposition, the smoothed spike trains (Figure 1) were examined for each trial to eliminate the motor unit spike trains which had abnormally low or high inter-spike intervals (Semmler, 2002).

**Figure 1 here**

The force signal was preprocessed applying a low-pass filter with 20-Hz cutoff frequency after removing the offset. In order to examine the strength of force oscillations in the three conditions (baseline, isotonic, and pain), the standard deviation and power spectral density (PSD) of the force signal were computed from intervals of 5 s and 0.5s, respectively. Welch’s method (Welch, 1967) was used for PSD estimation of the force signal, windowed using Hamming function without overlap. Additionally, to investigate the distribution of power over frequency for
the neural drive to muscle, the PSD of the cumulative spike trains (CST) was calculated by a
similar method as for the force signal.

To estimate the relative proportion of common input with respect to independent input to
motor units in each condition (baseline, hypertonic, isotonic), the coherence analysis was
performed on the CST for each subject and condition individually. The magnitude of the
coherece function increases quadratically with the number of motor unit spike trains considered
in the CST resulting in better estimates (Negro and Farina, 2011a; Farina et al., 2014a). In the
present study, the number of decomposed motor units was only four in some cases. Therefore, the
CST was calculated from the possible unique combinations of motor unit pairs (Figure 2). In this
way, all conditions were analyzed with the same number of motor units, so that the coherence
values could be compared between conditions (Negro and Farina, 2012).

To compute the coherence function, the Welch’s averaged, modified periodogram method
was used:

\[ k(\omega) = \frac{|X_{ij}(\omega)|^2}{\sqrt{X_{ii}(\omega)X_{jj}(\omega)}} \] (1)

where \( X_{ij}(\omega) \) is the cross-spectrum (the Fourier transform of the cross-correlation function) of the
two CSTs and \( X_{ii}(\omega), X_{jj}(\omega) \) the respective autospectra. The discriminated spike trains were
divided into non-overlapping 0.5s windows (Hamming) with a length for the fast Fourier
transform (FFT) of 10 times sampling rate (10*Fs) (zero padding). To define the significance
threshold for coherence peaks, the confidence level was calculated as (Rosenberg et al., 1989):

\[ CL = 1 - (1 - \alpha) \frac{1}{N-1} \] (2)
where $N$ and $\alpha$ represent the number of segments used in the coherence calculation (data length / number of windows) and the confidence level (95%), respectively.

The Fisher’s $z$-transform was applied to the coherence values and confidence level in order to compare the normalized coherence values between the three conditions (Rosenberg et al., 1989; Laine et al., 2014). The integration of coherence values over the confidence level, which is an indication of synchronization of motor units, was calculated for the 2-50 Hz frequency band.

To compare the strength of coherence, the PSD of force and of the CST, relative to the total power (averaged on 2 Hz bin size), repeated measures ANOVA was used with two factors (frequency and conditions) for both muscles. The Tukey’s honestly significant difference (HSD) post-hoc analysis was used to define differences between conditions in specific frequency bands. The difference between the subjective pain scores (NRS), number of motor units, mean discharge rate, coefficient of variation (CoV) of ISI, and CoV of force in the three conditions was investigated by a one-way ANOVA for both muscles. Significant differences revealed by ANOVA were followed by post hoc Fisher’s least significant difference (LSD) pairwise comparisons. Statistical significance was set at $P < 0.05$.

**RESULTS**

*Experimental pain scores*

The injection of hypertonic saline elicited a significant painful sensation for both muscles. The peak pain intensity, as measured by the NRS score, was greater following the injection of hypertonic saline (ADM: $4.4 \pm 2.1$; TA: $3.5 \pm 1.2$) compared to the isotonic saline (ADM: $1.0 \pm 0.4$; TA: $0.3 \pm 0.1$) in both muscles ($p<0.05$) (Table 1). The NRS scores were $4.7\pm1.8$ and
4.2±1.9 for the ADM, 3.8±1.2 and 3.4±1.0 for the TA in the hypertonic saline condition at the
beginning and at the end of 60-s interval respectively. The painful sensation did not change over
time during the 60-s interval of analysis (p>0.05) for both muscles.

Motor unit properties

The discharge properties of motor units were compared under the three conditions for
both the ADM and TA muscles. A total of 165 and 134 individual motor units were identified
from the ADM and TA muscles, respectively. Motor unit discharge rates were not significantly
different between conditions for both ADM (p=0.185) and TA (p=0.061) muscles, although there
was a tendency for lower rates in the hypertonic saline condition (Table 1). No significant
differences in the number of analyzed motor units were found between conditions for the ADM
(p=0.367) and the TA muscle (p=0.257). The CoVs of ISI for the ADM and the TA muscles
were not significantly different across conditions (Table 1).

Force

The variability of the force increased after the injection of hypertonic saline. The standard
deviation of force was significantly greater for the hypertonic saline condition of both muscles
(ADM: p<0.01; TA: p< 0.01). The repeated measures ANOVA showed a significant effect for
condition (p<0.01) and an interaction between frequency and condition (p<0.01). The post-hoc
test revealed that the power of the force signal was greatest in the 4-8 Hz frequency band (tremor
oscillation) and 2-4 Hz (common drive oscillation) frequency bands for both muscles (p<0.05)
during the hypertonic saline condition with respect to the other two conditions. For the TA
muscle, the power of force was higher in the isotonic saline condition than baseline despite
higher power during hypertonic saline condition (Figure 3a-b). In line with these results, the
relative variability of the force signal (CoV of force) depended on the condition for both muscles
(ADM: p<0.05; TA: p<0.05). Post hoc analysis revealed that the CoV of force was greater in the
hypertonic saline condition compared to both baseline (ADM: p<0.05; TA: p<0.05) and isotonic
saline (ADM: p<0.01; TA: p<0.01) conditions (Table 1).

Figure 3 here

Composite Spike Train

The CST was examined in the frequency domain, as an estimate of the neural drive to the
muscle. The repeated measures ANOVA revealed a significant interaction between frequency
and condition (p < 0.01). The post-hoc test revealed that the relative power (PSD of the smoothed
CST divided by the integral of the PSD function) of the neural drive to the muscle in the
hypertonic (painful) saline condition was significantly greater than in the baseline condition in
the lower frequency band (2-4 Hz) for both muscles (p<0.05), as previously shown (Farina et al.,
2012). Moreover, the hypertonic saline condition was associated with greater power of the CST
also in the frequency interval from 8 to 12 Hz for the ADM (p < 0.01) and from 6 to 12 Hz for
the TA muscle (p < 0.01) (alpha band) (Figure 4a-b). No effect of experimental pain was
observed for higher frequencies (p>0.10).

Figure 4 here

Motor Unit Coherence

The influence of experimental muscle pain on the common input to motor units was
investigated by calculating the coherence between CST. An average of 31 CST pairs per subject
(intervals: 3-45 for baseline, 3-100 for hypertonic, and 15-100 for isotonic conditions) were
calculated for the TA and 33 CST pairs (intervals: 3-45 for baseline, 3-100 for hypertonic, and 6-100 for isotonic conditions) for the ADM.
Representative examples of coherence estimates are shown in Figure 5a, b. In these examples, the coherence functions present a low frequency component in the 2-4 Hz band, a narrow peak in the 6-10 Hz band and a broad and lower peak in the 15-25 Hz band. The repeated measures ANOVA revealed that, for both muscles, the strength of coherence between motor units was affected by condition (p<0.05) and that there was an interaction between frequency and condition (p<0.05). The strength of coherence between motor units in the hypertonic saline condition was significantly lower than in the other two conditions in the bands 6-8 Hz (ADM: p<0.05; TA: p<0.05) and 8-10 Hz (ADM: p<0.01; TA: p<0.01; Figure 5c, d). Moreover, for the ADM muscle only, a significantly lower coherence strength in the hypertonic condition was observed with respect to the baseline and the isotonic saline conditions at the frequencies 14-18 Hz (beta band) (p<0.05; Figure 5c). The coherence strength was significantly lower in the hypertonic saline condition compared to the baseline condition for the frequencies 18-22 Hz for ADM and 14-22 Hz for TA muscles, however the hypertonic saline condition was not different with respect to isotonic saline condition at these frequencies. No significant difference was found in the low frequency band (2-4 Hz) between conditions for both muscles (p>0.05).

Figure 5 here

To confirm that the present results were not influenced by the average discharge rates of the analyzed motor units, the correlation between discharge rate of motor units and their strength of coherence at the analyzed frequency bands in the three conditions was calculated and no significant correlation between the two variables was found for both the ADM (p=0.30) or TA (p=0.06) muscles.

The integral of the coherence function in the full bandwidth (2-50 Hz), which is associated with the strength of short-term synchronization (Semmler et al., 2004), was lower in
the hypertonic saline condition compared to both baseline and isotonic conditions for both
muscles (ADM: p<0.05; TA: p<0.05) (Table 1).

**Table 1 here**

**DISCUSSION**

Experimental muscle pain increased the physiological tremor component in the neural
drive to muscle but did not influence the components in the beta band.

Consistent with previous observations in experimental (Bandholm et al., 2008; Farina et
al., 2012) and clinical pain conditions (Falla et al., 2010; Muceli et al., 2011), we observed a
higher variability in the force oscillations (increased standard deviation of the force signal) in the
hypertonic (painful) saline condition for both muscles. The high variability in the force signal
indicates an impairment of the central nervous system to accurately control force when a
nociceptive stimulus is present (Farina et al., 2012). Previous work has shown that the low
frequency oscillations (<3 Hz) of the motor unit spike trains increase during experimental muscle
pain, impairing force steadiness (Farina et al., 2012). This frequency band represents the largest
common input variation and is highly correlated with force fluctuations (Negro et al., 2009). In
the present study, we showed that the delta band (2-4Hz) of the neural drive to muscle had the
highest power during pain, which confirms these earlier observations, and we extended the
analysis to the full frequency bandwidth of the neural drive. The most consistent finding was an
increased power (i.e., variability) of the neural drive to muscle in the alpha (tremor) frequency
band (5-13Hz) and a decreased motor unit coherence in the same band for both muscles. These
changes in the neural drive to muscle corresponded to an increase in the force oscillations in the
tremor frequency band.
Force oscillations in the alpha band (physiological tremor) increased during the hypertonic saline condition in both muscles. This observation is consistent with earlier work (Jaberzadeh et al., 2003) which documented a similar increase in physiological tremor (force signal) with experimental muscle pain induced in the extensor digitorum longus muscle. Previous studies demonstrated that tremor oscillations in force (6-10 Hz) are highly correlated with the activity of muscle spindle afferents (Halliday and Redfearn, 1958; Lippold, 1970; Laine et al., 2013, 2014). For this reason, an increase in the amplitude of the oscillations of the control signal (combination of cortical and afferent projections) would likely increase also the oscillations of the neural drive that are associated with the tremor frequency of the force spectrum. Indeed, the results of the present study showed that the relative power of the CST signal in the tremor band was greatest during the hypertonic saline condition.

Motor unit spike trains recorded during sustained isometric contractions show common oscillations in the delta, alpha and beta frequency bands (Grosse et al., 2002). In these frequency bands, the shared synaptic projections over the motor neuron pool generate a significant level of coherence between multiple spike trains (Lowery et al., 2007; Negro and Farina, 2011a). The coherent oscillations in the delta and alpha bands are transmitted to the force signal, whereas those at higher frequencies are filtered by the contractile properties of the muscle (Baldissera et al., 1998; Halliday et al., 1999).

The magnitude of the coherence between motor unit spike trains in the alpha band (6-10 Hz) significantly decreased in the presence of experimental muscle pain. This result was consistent in both the ADM and TA muscles and suggests that nociceptive afferent input may influence the relative strength of the common input with respect to the independent synaptic input to motor neurons in the tremor band. Oscillations in the alpha band are known to be influenced
by the modulation of muscle spindle activity, e.g. under ischemic conditions (Erimaki and Christakos, 2008) and muscle stretching (Semmler, 2002; Negro et al., 2012). Specifically, results from previous studies have shown that the coherence between motor units increases with increasing muscle spindle activity. It is relevant to note that increased force signal power in the alpha band is not in contradiction with a decrease in motor unit coherence in the same bandwidth since coherence expresses the relative strength of the correlation in the synaptic input, but not its power (Halliday et al., 1995; Mima et al., 1999; Farina and Negro, 2014). Additionally, it has been shown that, while experimental muscle pain increases the stretch reflex amplitude, the H-reflex is not influenced (Matre et al., 1998), which suggests that experimental muscle pain directly provokes muscle spindle activity rather than modulating muscle spindle afferent input presynaptically. Together with the results from the PSD analysis, one possible interpretation can be that pain increased both the power of the common oscillatory inputs to motor neurons at the tremor band (increased PSD of neural drive) that caused increased force oscillations at the same frequencies, as well as the relative power of independent input with respect to common input (decreased coherence). The increased activity of monosynaptic muscle spindles during pain would increase the common synaptic input to motor neurons in the tremor band as discussed above. Despite this, the increase in strength of the independent input (synaptic noise) relative to the common synaptic input to motor neurons in the alpha band may be due to the fact that nociceptive afferents have polysynaptic innervation on motor units (Andersen et al., 2000). Therefore this polysynaptic input may have increased the strength of independent input due to the non-uniform synaptic distribution (Jankowska and Roberts, 1972; Powers and Binder, 1985) that may have divergent projections on the motor neuron pool.

In addition to the alpha band, the coherence values in the 14-22 Hz band (beta band) were lower in the hypertonic saline condition with respect to the baseline condition but only for the
ADM muscle. Coherence peaks in the beta band are thought to be mostly associated with the firing oscillations generated by the motor and somatosensory cortex (Farmer et al., 1993; Conway et al., 1995; Halliday et al., 1999). Thus, a decrease in coherence in the beta band can be interpreted as a reduction of the common input from the motor and somatosensory cortex to the motor neuron pool due to pain. The observation that hand muscles have broader corticospinal projections to their motor neuron pools compared with leg muscles (Brouwer and Ashby, 1990) may explain the lack of this effect in the TA muscle.

The average motor unit discharge rate influences the estimation of coherence magnitude (Christou et al., 2007). In this study, motor unit discharge rates decreased with pain (Farina et al., 2004, 2012), although not significantly. Moreover, the mean motor unit discharge rate was not correlated with the magnitude of the estimated coherence, in all three conditions and for both muscles, which excludes an influence of discharge rate on the conclusions drawn.

In conclusion, we observed a higher variability of force and greater oscillations of the neural drive to the muscles in the tremor band during hypertonic saline condition (painful) with respect to isotonic and baseline conditions. At the same time, the coherence in the same frequency band decreased, suggesting a relatively greater increase in independent inputs (synaptic noise) to motor neurons with respect to the increase in common input oscillations. Therefore, one interpretation of present results can be that muscle pain increases the power of both common inputs to motor neurons, which are translated into greater force oscillations, and of independent input.

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FIGURE LEGENDS

Figure 1: Smoothed spike trains from the abductor digiti minimi (ADM) muscle and force recorded from one representative subject during the baseline condition and following the injection of hypertonic and isotonic saline. The top trace in each condition denotes the force signal. Each trace in gray tone below the force trace denotes different motor units.

Figure 2: Estimation of coherence. a) The raw intramuscular signal was decomposed into single motor units (2-s interval of intramuscular signal). b) Discharge times of the discriminated single motor units. c) Composite spike trains (CST) were calculated from all possible unique combinations of two motor units. d) The normalized coherence was estimated from the CST (dashed horizontal line indicates the 95% confidence level).

Figure 3: Averaged power spectrum density (PSD) estimates for the force signal of the abductor digiti minimi (ADM) (A) and tibialis anterior (TA) (B) muscles. Baseline, Isotonic and Hypertonic conditions are indicated by solid black line, solid gray and dashed black lines, respectively. The shaded (light gray) area shows the frequency interval where significantly higher power was observed for the hypertonic saline condition compared to the other conditions (p<0.05).

Figure 4: Averaged (over subjects) power spectrum density (PSD) estimations of smoothed composite spike train (CST) in the time interval analyzed for the abductor digiti minimi (ADM) (A) and tibialis anterior (TA) muscles. Baseline, Isotonic and Hypertonic conditions are illustrated by solid black, solid gray and dashed black lines respectively. The shaded (light gray) area shows the frequency interval where significantly higher power was observed for the
hypertonic saline condition compared to the baseline condition. The PSD is shown only for the
delta and alpha bands for clarity. No differences were detected among conditions for higher
frequencies.

**Figure 5:** Coherence estimates for a representative subject and for the pooled data. A) Coherence
for the abductor digiti minimi (ADM) muscle of Subject 2. B) Coherence for the tibialis anterior
(TA) muscle of Subject 2. C) Pooled coherence for the ADM muscle. D) Pooled coherence for
the TA muscle. Baseline, Isotonic and Hypertonic conditions are illustrated by solid, dotted and
dashed lines, respectively. *: Coherence estimates significantly lower in the hypertonic condition
compared to the other conditions in the corresponding frequency band (p<0.05). Horizontal lines
(solid, dotted and dashed) show the 95% confidence interval for all conditions. Coherence values
are Z-transformed.

**TABLE LEGEND**

**Table 1:** Average values ± standard deviation of the motor unit (MU) discharge rate (DR),
coefficient of variation (CoV) of the interspike interval (ISI) and force, and integral value of the
coherence function between 2 Hz and 50 Hz (CohInt) for the abductor digiti minimi (ADM) and
tibialis anterior (TA) muscles recorded during a baseline condition and following the injection of
hypertonic and isotonic saline. *: significantly different in the hypertonic saline condition with
respect to the baseline and isotonic saline conditions for the same muscle (p<0.05).
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<th>ADM</th>
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<td>Isotonic</td>
<td>Baseline</td>
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<td>Pain (NRS)</td>
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<td>4.4 ± 2.1*</td>
<td>1.0 ± 0.4</td>
<td>-</td>
<td>3.5 ± 1.2*</td>
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<td>51</td>
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<td>DR (spike/s)</td>
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<td>7.9 ± 1.9</td>
<td>8.2 ± 2.2</td>
<td>8.3 ± 0.7</td>
<td>7.4 ± 1.4</td>
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<tr>
<td>CoV of ISI (%)</td>
<td>16 ± 4</td>
<td>16 ± 5</td>
<td>16 ± 5</td>
<td>11 ± 3</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>CoV of Force (%)</td>
<td>3.5 ± 1.6</td>
<td>3.8±1.5*</td>
<td>3.5 ± 1.6</td>
<td>1.5 ± 0.6</td>
<td>2.0 ± 1.0*</td>
</tr>
<tr>
<td>CohInt (Hz)</td>
<td>304 ± 58</td>
<td>284 ± 61*</td>
<td>298 ± 58</td>
<td>290 ± 78</td>
<td>265 ± 64*</td>
</tr>
</tbody>
</table>