Reorganized trunk muscle activity during multidirectional floor perturbations after experimental low back pain

*a comparison of bilateral versus unilateral pain*

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REORGANIZED TRUNK MUSCLE ACTIVITY DURING MULTI-DIRECTIONAL FLOOR PERTURBATIONS AFTER EXPERIMENTAL LOW BACK PAIN: A COMPARISON OF BILATERAL VERSUS UNILATERAL PAIN

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ABSTRACT

Low back pain (LBP) changes the trunk muscle activity after external perturbations but the relationships between pain intensities and distributions and their impact on the trunk muscle activity remains unclear. The effects of unilateral and bilateral experimental LBP on trunk muscle activity were compared during unpredictable multi-directional surface perturbations in 19 healthy participants. Pain intensity and distribution were assessed on a visual analogue scale (VAS) and by pain drawings. Root-mean-square (RMS) of the electromyographic (EMG) signals from 6 trunk muscles bilaterally after each perturbation was extracted and averaged across perturbations. The difference (ΔRMS-EMG) and absolute difference (absolute ΔRMS-EMG) RMS from baseline conditions were extracted for each muscle during pain conditions and averaged bilaterally for back and abdominal muscle groups. Bilateral compared with unilateral pain induced higher VAS scores (P<0.005) and larger pain areas (P<0.001). Significant correlation was present between VAS scores and muscle activity during unilateral (P<0.001) and bilateral pain (p>0.001), respectively.

Compared with control injections ΔRMS-EMG increased in the back (P<0.03) and abdominal (P<0.05) muscles during bilateral and decreased in the back (P<0.01) and abdominal (P<0.01) muscles during unilateral pain. Bilateral pain caused higher absolute ΔRMS-EMG changes in the back (P<0.01) and abdominal (P<0.01) muscle groups than unilateral pain.

Perspectives

This study provides novel observations of differential trunk muscle activity in response to perturbations dependent on pain intensity and/or pain distribution. Due to complex and variable changes the relevance of clinical examination of muscle activity during postural tasks is challenged.
INTRODUCTION

The life-time prevalence of low back pain (LBP) is up to 38.9%\textsuperscript{40} and the evidence on causality is poor\textsuperscript{35, 37}. Nonetheless, genetic\textsuperscript{50} and psycho-social factors\textsuperscript{45, 58, 61} have been proposed as risk-factors in LBP, and movement strategies and muscle activation patterns may be potential factors\textsuperscript{1, 35}. Muscle function and coordination are usually altered in LBP patients\textsuperscript{21, 35} and impaired trunk muscle activation and activity gained much attention as an explanatory model for LBP\textsuperscript{34}. Although the underlying mechanisms in trunk motor control and pain are sparsely linked\textsuperscript{58}, trunk muscle training is widely implemented clinically and in sports\textsuperscript{70} with underlying assumptions on trunk muscles as spinal stabilizers during functional tasks\textsuperscript{69, 70}. Although the nature of possible changes are inconsistent complex muscle pain adaptation is evident\textsuperscript{37, 52}. Additionally, stabilization exercises have no long-term effect\textsuperscript{18} or is not superior to other treatments\textsuperscript{10, 13, 68}.

Experimental pain models therefore have been used extensively to explore the effects of LBP, aiming to mimic pain and yet exclude confounding factors in LBP patients\textsuperscript{5, 21}. In previous studies lumbar pain was induced unilateral, but differences in pain characteristics between subacute LBP patients with greater prevalence of unilateral pain and persistent LBP patients predominantly indicating bilateral pain\textsuperscript{11} highlight the importance of understanding if pain related mechanisms during motor tasks differs between unilateral and bilateral pain conditions.

Gait is the primary human locomotion function and based on gait studies in LBP it is evident that complex muscle control is related to specific, and individual, temporal and spatial demands\textsuperscript{35}. LBP patients showed inconsistent muscle activity with e.g. increased back muscle activity during the swing phase\textsuperscript{3} and increased co-activation of erector spinae and rectus abdominis muscles\textsuperscript{76}, while increased lumbar and decreased abdominal muscle activity was present in patients older than 50 years\textsuperscript{22}. Van den Hoorn et al.\textsuperscript{75} additionally found individualized synergistic muscle-strategies during treadmill walking and the trunk control synergies were affected by back and leg pain in some subjects.
The nature of the gait task is complex and involves motor planning as well as motor adaptation and the effect of pain on the underlying mechanisms in motor control during gait is challenged. Contrarily, surface perturbation is a highly standardized and still complex motor task since unpredictable surface perturbation is challenging due to non-predictable, high-velocity changes from the external perturbation. Multi-directional floor perturbations resulted in increased co-contraction of the trunk muscles in persistent LBP patients compared with a control group, indicating a trunk stiffening strategy. In contrast, Boudreau et al. found decreased trunk muscle activity after anterior and posterior perturbations after pain induction in healthy participants. It remains unknown if these observed alterations are related to the differences in the surface perturbation protocol or if the underlying musculoskeletal impairments are important. Although studies showed no changes in proprioception in LBP patients, postural repositioning is generally challenged and decreased variability in postural adjustments to perturbations after acute and persistent LBP furthermore may indicate complex trunk muscle timing and activity. Various motor adaptations in functional tasks are generally accepted, but although experimental unilateral pain affects the trunk muscle activity bilaterally and pain-related reorganization of the trunk muscle strategies during LBP is evident between and within muscles, the underlying interactions between muscles are not well understood and the effect of unilateral and bilateral pain on the trunk muscle response is unknown.

The aim of the study was to compare the effects of unilateral and bilateral experimental LBP on trunk muscle activity during unpredictable multi-directional surface perturbations in healthy participants. It was hypothesized that (1) unilateral LBP will decrease, and (2) bilateral LBP will increase trunk muscle activity during multi-directional unpredictable surface perturbations.

**METHODS**
Participants

Nineteen healthy participants [4 females; mean age 26 years (range 19-39 years); mean height 180 cm (range 160-200 cm), mean body mass index 23.7 kg/m$^2$ (range 20.4-29.2 kg/m$^2$)] without lower extremity or back related pain or dysfunction participated in the study. The study was conducted in accordance with the Helsinki Declaration, approved by the local ethics committee (N-20090053) and informed consent was obtained from each participant.

Protocol

The subjects participated in one baseline perturbation session and three successive experimental perturbations sessions on the same day with minimum 15 min break in between conditions: (1) bilateral experimental saline-induced LBP, (2) bilateral control condition, and (3) unilateral experimental saline-induced LBP. In each session, the subject was standing on a marked position on a moveable platform during a series of 20 randomized multi-directional surface perturbations delivered after an auditory warning signal. Between sessions the subjects were allowed to sit on a chair.

Experimental low back pain

The injection procedure was performed with the subject prone lying. The Th12 segment was located and L2 was down counted and verified by palpation of L4 at the line between the iliac crest bilaterally where L2 was estimated$^{15}$. At the L2 level the most bulky part of m. longissimus was palpated (typically 3-5cm from the midline) and marked as injection site. Sterile isotonic (1.0 ml, 0.9%) or hypertonic (1.0 ml, 5.8%) saline was injected perpendicular to the skin surface with a 25G × 19 mm needle, after cleaning the injection site with alcohol. Hypertonic and isotonic saline was injected bilaterally (experimental condition 1 and 2, respectively) and in experimental condition 3 one hypertonic saline injection was given in the right side immediately followed by an
injection of isotonic saline in the left side. The participants were informed about receiving injections, but were blinded to the type of saline injected. In the bilateral conditions the right injection was performed before the left and the time between injections was 30-60 s. Immediately after completion of both injections, the participant was assisted to the standing position on the platform for perturbations and started scoring the pain intensity.

During the perturbations the pain intensity was assessed on a 10-cm electronic visual analogue scale (VAS) with an external handheld slider. The VAS was anchored with ‘no pain’ and ‘maximum pain’ at 0 cm and 10 cm, respectively. The signal from the VAS was recorded after each injection until the pain vanished (sample frequency of 20 Hz). During the complete period including perturbations the mean VAS score was extracted in the time window from onset to the subsequent perturbation and the maximum VAS and average VAS scores were extracted among the 20 perturbations. The subjects were asked to recover their balance as fast as possible after the perturbation, and only then, they were allowed to update the VAS. After each condition the subjects were asked to indicate the pain distribution on a body chart. The pain area was extracted from the drawings (ImageJ 1.47V, Rasband, NIH, USA) and mean areas were extracted.

**Perturbations**

Surface perturbations were performed by a computer-controlled moveable platform. The participant stood on the platform in a relaxed position with the feet in approximately shoulder-width distance, the arms along the body, and instructed to look straight forward on a marker on the wall (4 m distance, 5 cm diameter). The foot position was marked on the platform to ensure that the position from the baseline condition was used during all 3 conditions. Ten perturbations in different directions were conducted as acclimatization before the data collection started. The perturbation protocol aimed to challenge the postural demands substantially in the standing position, but still allowing the participants to maintain the limits of stability of the standing
position without stepping. A series of perturbations consisted of 20 randomized multidirectional surface perturbations (1: sagittal anterior and posterior 3° tilt, velocity 30°/s and peak acceleration 200°/s²; 2: frontal left and right 10° tilt, velocity 40°/s and peak acceleration 140°/s²; 3: frontal left and right 100 mm displacement, velocity 0.4 m/s and peak acceleration 140 m/s²) with randomly 4-8 s intervals between, and minimum 3 repetitions of each perturbation types in each series. Each perturbation was initiated by an auditory cue and the perturbation was conducted after 0.2 – 5.0 s at random and trials including stepping strategies after perturbation (in all 7 trials in different directions) were excluded.

**Electromyography recordings**

The skin was shaved and cleaned with alcohol. The ground electrode (Blue sensor P 34mm, Ambu Neuroline, Denmark) was mounted on the skin over the most prominent spinal process at C6, C7 or Th1. Surface electrode pairs (Ambu Neuroline 720, Denmark) were mounted bilaterally on back muscles according with previous recommendations: M. iliocostalis (one finger width medial from a line from posterior superior iliac spine (PSIS) to lowest point of lower rib at L2 level, longissimus (2 fingers width lateral from L1 spinal process), m. multifidus (line from caudal tip of PSIS to L1-L2 interspace at L5 process. Likewise electrodes were mounted bilaterally on abdominal muscles: M. obliquus internus (along horizontal line between left and right anterior superior iliac spine, medial from inguinal ligament), m. rectus abdominis (3 to 4 cm lateral to the navel), and m. obliquus externus (upper electrode directly below most inferior point of costal margin of PSIS).

The electromyography (EMG) signals were band pass filtered (10–500 Hz), sampled at 2048 Hz with a gain of 2000 using a 16-channel surface EMG-USB amplifier (LiSiN-OT Bioelettronica, Torino, Italy) and converted to digital form by a 12-bit analog-to-digital converter. The EMG signals were synchronized with the onset of perturbation.
Data analysis

Root-mean-square (RMS) values were derived from the EMG signals in 10 non-overlapping signal epochs of 50 ms from the perturbation onset and the average value across epochs was extracted for each perturbation\textsuperscript{66,67} (hereafter defined as RMS-EMG). The RMS-EMG during experimental pain and control sessions was expressed as a percentage of the baseline RMS-EMG ($\Delta$RMS-EMG; baseline is 100%) value for each muscle and perturbation type individually and furthermore averaged across all perturbation types. Calculation of $\Delta$RMS-EMG was used to account for the large inter-individual variability in pain-related muscle activity changes\textsuperscript{28}. Since changes expressed by $\Delta$RMS-EMG could cover increased and decreased values the absolute values of the $\Delta$RMS-EMG was calculated to indicate absolute changes from the baseline condition\textsuperscript{28}. Finally, $\Delta$RMS-EMG and absolute $\Delta$RMS-EMG, respectively, were averaged across unilateral and bilateral back (m. iliocostalis, m. longissimus, and m. multifidus) and abdominal (m. obliquus internus, m. rectus abdominis, and m. obliquus externus) muscle groups. The data were initially analyzed for main effects in the individual muscles between the perturbations and since this was not the case, further analyzes were conducted across the perturbations.

Statistics

Data are presented as mean and standard error of the mean (SEM). All statistical analyses were performed in SPSS\textsuperscript®22.0 (IBM). The data was tested for normality by the Shapiro-Wilk test and was generally normally distributed ($P > 0.05$). Data analyses were conducted by repeated measures analysis of variance with a Greenhouse-Geisser correction (RM-ANOVA) and when significant, Bonferroni adjusted post-hoc t-tests were used to conduct pairwise comparisons between conditions. The experimental pain areas were analyzed by one-way RM-ANOVA with condition (bilateral control, unilateral pain and bilateral pain) as main factor. Mean and peak VAS scores
were analyzed by two-way RM-ANOVAs with factors condition and time (20 perturbations). Additionally, the mean VAS scores were analyzed by two-way RM-ANOVAs with factors condition and time across perturbation 1-10 and 11-20, respectively. For the relative and absolute ∆RMS-EMG averaged across the six perturbation types RM-ANOVAs were conducted by respectively (i) 2-way RM-ANOVA with factors muscle group (abdominal and back) and condition, (ii) 3-way RM-ANOVA with factors side (left and right), muscle group, and condition, (iii) 3-way RM-ANOVA with factors side, muscle (m. iliocostalis, m. longissimus, m. multifidus, m. obliquus internus, m. rectus abdominis, and m. obliquus externus), and condition, and additionally, comparison between perturbations were conducted by a 3-way RM-ANOVA with factors muscle (left and right m. iliocostalis, m. longissimus, m. multifidus, m. obliquus internus, m. rectus abdominis, and m. obliquus externus), perturbation type (anterior tilt, posterior tilt, left displacement, left tilt, right displacement and right tilt), and condition.

To examine correlation between RMS-EMG and pain intensity during unilateral and bilateral pain, these parameters were examined by a Pearson’s correlation between VAS scores and RMS-EMG across all perturbation types and left and right side muscle groups. Moreover, correlation between pain spreading area and mean RMS-EMG across the 20 perturbations in each session were examined by a Pearson’s correlation and when significant the values were Bonferroni corrected. Statistical significance was accepted at P < 0.05.

RESULTS

Experimental low back pain

The mean VAS scores were significantly higher after bilateral compared with unilateral hypertonic saline injections during all 20 perturbations (Fig. 1A; ANOVA: F (2,720) = 85.2; P<0.001; Bonferroni: P<0.005). The maximal VAS score after control injections was 1.1 ± 0.3 cm and significantly lower than after unilateral (5.0 ± 1.0 cm, Bonferroni: P<0.005) and bilateral injection of hypertonic saline.
(6.5 ± 1.1 cm; ANOVA: F (2,720) = 851.6; P<0.001; Bonferroni: P<0.005). Moreover, the maximal VAS score was higher after bilateral compared with unilateral hypertonic saline injections (Bonferroni: P<0.001).

The mean VAS score across the 10 first perturbations was higher compared with the mean VAS score across the 10 last perturbations (ANOVA: F (1,189) = 154.4; P<0.001; Bonferroni: P<0.001) and compared with the last time window post-hoc tests showed higher mean VAS scores in the first time window during unilateral (Bonferroni: P<0.001) and bilateral (Bonferroni: P<0.001) pain.

Following unilateral and bilateral injection of hypertonic saline, pain was primarily perceived in the low back area and injection of isotonic saline only resulted in pain around the injection site (Fig. 1B). The mean perceived area of pain was 2.8 ± 2.3 in arbitrary units (a.u.) after bilateral control injections, 25.5 ± 9.6 a.u. after unilateral and 62.4 ± 22.7 a.u. after bilateral hypertonic saline injection. The mean perceived area of pain after bilateral hypertonic saline injection was bilateral and 245% larger than during unilateral pain (ANOVA: F (2,36) = 93.6, P<0.001; Bonferroni: P<0.005).

**Motor response following surface perturbation**

The initial motor responses following a perturbation occurred typically around 100 ms after the perturbation onset with peak muscle activity between 150 and 300 ms (Fig. 2). The differences from baseline recordings (ΔRMS-EMG) are illustrated in Fig. 3 for the six muscles, left and right sides, six perturbations and three experimental conditions. A 3-way RM-ANOVA showed a significant interaction between muscles, perturbation type, and conditions (ANOVA: F (110,1980) = 6.1, P<0.001) with no main effect on perturbations (ANOVA: F (5,90) = 2.4, P=0.07) and muscles (ANOVA: F (11,198) = 0.4, P=0.80) but main effect on conditions (ANOVA: F (2,36) = 6.1, P<0.01) where post-hoc analyses showed significant changes between the two pain conditions in i) right m. longissimus (Bonferroni: P<0.001) and m. multifidus (Bonferroni: P<0.03) after right
displacement, ii) left (Bonferroni: P<0.01) and right (Bonferroni: P<0.01) m. multifidus and left m. obliquus externus (Bonferroni: P<0.03) after left displacement, and iii) left m. obliquus internus after right tilt (Bonferroni: P<0.05). Compared with control injections, unilateral pain resulted in significantly decreased ∆RMS-EMG in the three back muscles after more perturbations, particularly after left and right displacement and left tilt and primarily in the right-sided muscles (Bonferroni: P<0.05), and bilateral pain resulted in significantly lower muscle activity in right m. iliocostalis after right tilt (Bonferroni: P<0.01) and higher muscle activity in right m. multifidus after respectively anterior tilt (Bonferroni: P<0.02) and left displacement (Bonferroni: P<0.04), left m. obliquus internus after right tilt (Bonferroni: P<0.01) and right m. obliquus internus (Bonferroni: P<0.01)

**Perturbation evoked muscle activity across all perturbations**

A 3-way RM-ANOVA of ∆RMS-EMG in the individual muscles averaged across all perturbations (Fig. 4A) demonstrated a significant interaction between muscles, sides and conditions (ANOVA: F (10, 1130)= 3.1, P<0.001) with no main effect on muscles (ANOVA: F (5,565) = 0.8, P=0.50) but main effect on sides (ANOVA: F (1,113) = 5.1, P<0.03) conditions (ANOVA: F (2,226) = 15.5, P<0.001). Post-hoc analyses showed significant decreased ∆RMS-EMG in right m. iliocostalis (Bonferroni: P<0.02), m. longissimus (Bonferroni: P<0.001) and m. multifidus (Bonferroni: P<0.005) after unilateral pain compared with control injections and increased ∆RMS-EMG in left m. obliquus internus (Bonferroni: P<0.02) and right m. longissimus (Bonferroni: P<0.005), m. multifidus (Bonferroni: P<0.001), m. obliquus internus (Bonferroni: P<0.001) and m. rectus abdominis (Bonferroni: P<0.03) after bilateral pain compared with unilateral pain. In addition, ∆RMS-EMG were significantly increased in right m. rectus abdominis during bilateral pain compared with control injections (Bonferroni: P<0.01).
Perturbation evoked muscle activity across muscle groups and perturbations

A 3-way RM-ANOVA of ∆RMS-EMG averaged across all perturbations and muscle groups resulted in a significant interaction between muscle groups, sides, and conditions (Fig. 4B; ANOVA: F (2,682) = 3.21, P<0.04) with no main effect on muscle group (ANOVA: F (5,565) = 1.7, P=0.16) and sides (ANOVA: F (1,113) = 0.7, P=0.42) but main effect on conditions (ANOVA: F (2,226) = 15.7, P<0.001). Post-hoc analyses showed increased ∆RMS-EMG during bilateral and decreased ∆RMS-EMG during unilateral pain in the left back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) and right back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) muscle groups. Compared with the control condition, significantly decreased ∆RMS-EMG was observed during unilateral pain in the right back muscle group (Bonferroni: P<0.001) and in the left abdominal muscle group (Bonferroni: P<0.02) and increased ∆RMS-EMG was found during bilateral pain in the right abdominal muscle group (Bonferroni: P<0.005).

Across left and right abdominal and back muscles, a 2-way ANOVA of ∆RMS-EMG demonstrated a significant interaction between muscle groups and conditions (Fig. 4C; ANOVA: F (2,1366) = 3.8, P<0.03) with main effect on muscle groups (ANOVA: F (2,1366) = 6.1, P<0.02) and conditions (ANOVA: F (2,1366) = 23.5, P<0.001). Post-hoc analyses showed significantly decreased ∆RMS-EMG during unilateral pain and increased ∆RMS-EMG during bilateral pain in the back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) muscle groups. Compared with the control condition significantly decreased ∆RMS-EMG during unilateral pain was observed in the back (Bonferroni: P<0.001) and the abdominal muscle group (Bonferroni: P<0.02) and during bilateral pain significant increased ∆RMS-EMG was observed in the abdominal muscle group (Bonferroni: P<0.005).

Absolute muscle activity changes across all perturbations
The 3-way RM-ANOVA of the absolute ∆RMS-EMG in the left and right sided muscles averaged across all perturbations (Fig. 5A) demonstrated a 3-way interaction between sides, muscles and conditions (ANOVA: F (10,1130)= 6.6, P<0.001) with no main effect on side (ANOVA: F (1,114) = 0.2, P=0.64) and muscles (ANOVA: F (5,565) = 2.3, P=0.08) but main effect on conditions (ANOVA: F (2,226) = 10.8, P<0.001). Post-hoc analyses showed significant higher absolute ∆RMS-EMG during bilateral pain compared with unilateral pain in right m. iliocostalis (Bonferroni: P<0.001), m. longissimus (Bonferroni: P<0.001), m. rectus abdominis (Bonferroni: P<0.001) and m. obliquus externus (Bonferroni: P<0.02) and during bilateral pain compared with control injections in right m. iliocostalis (Bonferroni: P<0.001), m. longissimus (Bonferroni: P<0.02), m. rectus abdominis (Bonferroni: P<0.03) and m. obliquus externus (Bonferroni: P<0.001).

**Absolute muscle activity changes in muscle groups across sides**

The 3-way RM-ANOVA of the absolute ∆RMS-EMG across left and right sided muscles groups averaged across all perturbations (Fig. 5B ) demonstrated a significant interaction between sides, muscle groups, and conditions (ANOVA: F (2, 682)=3.2, P<0.02) with no main effect on side (ANOVA: F (1,341) = 0.2, P=0.56) and muscle groups (ANOVA: F (1,341) = 0.2, P=0.64) but main effect on conditions (ANOVA: F (2,682) = 14.6, P<0.001). Post-hoc analyses showed significant higher absolute ∆RMS-EMG during bilateral pain compared with unilateral pain in the left (Bonferroni: P<0.04) and right (Bonferroni: P<0.001) back and left (Bonferroni: P<0.001) abdominal muscle groups. Compared with control conditions the absolute ∆RMS-EMG was significant higher in the right back (Bonferroni: P<0.001) and abdominal muscle groups (Bonferroni: P<0.001).

Across all perturbations and left and right abdominal and back muscles respectively (Fig. 5C) a 2-way ANOVA of absolute ∆RMS-EMG demonstrated a significant interactions between muscle groups and conditions (ANOVA: F (1.81, 1235.57) = 16.93 P<0.01) with no main effect on muscle
groups (ANOVA: F (1, 683) = 0.2; P=0.64) but main effect on conditions (ANOVA: F (2, 1366) = 16.4, P<0.001). Post-hoc analyses showed significant higher absolute ΔRMS-EMG during bilateral compared with unilateral pain in the back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) muscle groups and during bilateral compared to control conditions in the back (Bonferroni: P<0.01) and abdominal (Bonferroni: P<0.01) muscle groups.

**Correlation between experimental pain and perturbation evoked muscle activity**

Across all perturbations there were negative correlation between VAS scores and RMS-EMG during unilateral pain in the abdominal muscle group in the left (r² =0.52; P<0.001; Bonferroni: P<0.001) and right (r² =0.55; P<0.001; Bonferroni: P<0.001) side and in the back muscle group in the left (r² =0.60; P<0.001; Bonferroni: P<0.001) and right (r² =0.79; P<0.001; Bonferroni: P<0.001) side. During bilateral pain there were positive correlation between VAS scores and RMS-EMG in the abdominal muscle group in the left (r² =0.53; P<0.001; Bonferroni: P<0.001) and right (r² =0.57; P<0.001; Bonferroni: P<0.001) side and in the back muscle group in the left (r² =0.60; P<0.001; Bonferroni: P<0.001) and right (r² =0.54; P<0.001; Bonferroni: P<0.001) side.

There were no significant correlation between size of pain area and muscle activity in the left and right abdominal and back muscle groups during unilateral pain (P>0.29) or bilateral pain (P>0.14).

**DISCUSSION**

This is the first study to compare the impact of unilateral versus bilateral experimental LBP and control conditions on the motor response following surface perturbations in healthy participants. The impact of bilateral pain was generally increased trunk muscle activity while unilateral pain resulted in decreased trunk muscle activity in line with the hypotheses. The individual pain-related
changes in muscle activity across muscle groups were higher after bilateral compared with unilateral experimental pain and control injections and during the first time window after pain induction, bilateral pain resulted in higher muscle activity, compared with the last. It has recently been argued that pain-driven muscle activity changes are important protective mechanisms of the spine\textsuperscript{36, 37, 44, 77}, but the results of the present study supported a more complex reorganization of the muscle activity related to the pain intensity but not the pain spreading.

**Subjective characteristics of experimental low back pain**

Control injections of isotonic saline in the m. longissimus resulted in low pain intensity around the injection site with minor spreading in few participants and most participants reported no pain. Injection of hypertonic saline resulted in muscle pain of moderate intensity, consistent with previous reports in relation to average pain intensity\textsuperscript{7, 12, 29, 49, 51, 73, 75} and peak pain\textsuperscript{32} intensity. Higher intensity was previously found after bilateral compared with unilateral injection of hypertonic saline into the trapezius muscle\textsuperscript{19} and temporal muscle\textsuperscript{42}, but the present study is the first demonstrating such spatial summation effects\textsuperscript{20} in the lower back muscles. These mechanisms may also explain the significant increased spreading of the pain areas during bilateral injections. In line with previous studies\textsuperscript{4}, bilateral pain resulted in referred pain to the groin and lateral femoral areas in some participants and it was obvious that of the pain intensity remained relative high and unchanged during the 10 first perturbations after bilateral pain induction and then gradually decreased throughout the session.

**Perturbation of the motor system to explore the motor adaption**

Several approaches have been used to induce sudden disruptions in balance to explore the role of the trunk muscles, including self-initiated perturbations by shoulder flexion\textsuperscript{30}, sudden release of mechanical loads\textsuperscript{60} and surface perturbation\textsuperscript{7}. The motor control related to perturbation includes
preprogrammed anticipatory postural mechanisms in the time window immediately before and around 150 ms after perturbation onset and subsequent corrections of posture. This study investigated the reactive strategies and the effect of pain on the muscle control in a randomized non-predictable multi-directional floor perturbation set-up. This approach was established to support studies of postural reactions and implemented in LBP research to explore the role of the trunk muscles, an important component of the motor output after external perturbations. Sequential exposure of participants to perturbations may reduce the initial responses that monosynaptic and polysynaptic reflexes represent to postural corrections and influence the following motor strategy in the studied time window. Learning effects in healthy has been observed after both motor imagery and standing reaching training. These observations were related to anticipatory muscle activity, but in our study the possible learning effect was challenged by randomized 0.2-5.0 second latency between the auditory signal in combination with a randomized multi-directional approach that challenge the postural adaptation. Decreased risk of learning has previously been challenged by e.g. unexpected perturbation and randomized pre-perturbation feedback protocols. The postural demands in the present study were extensive due to the selected force and velocity of the perturbations and auditory cues were utilized to avoid unintentionally pre-tension in trunk muscles or risk of falls.

The latency phase after perturbation was generally 50-150 ms after perturbation onset, in line with findings of the voluntary response phase after self-initiated perturbation and unexpected surface perturbation during acute experimental pain. However, the observed variable motor adaptation to postural challenges is in line with previous results and the variability after control injections (Fig. 5A,B,C) supported that the surface perturbation approach resulted in extensive challenge of the reactive postural control and confirmed that the motor response to pain is flexible as suggested by Hodges et al. Decreased ∆RMS-EMG after unilateral pain is in line with a recent study from Boudreau et. al.
ΔRMS-EMG increased during bilateral pain (Fig. 4A and 5A) which is in line with the trunk muscle activity in pain-free recurrent LBP patients\(^{44}\). The larger changes in the assessed muscle, by means of higher absolute ΔRMS EMG (Fig. 5.A,B,C), indicated that pain intensity is playing a major role. However, the absolute ΔRMS-EMG changes from baseline during unilateral pain generally equal the absolute changes after control injections and variability in muscle responses between trials are thereby considerable, but high pain intensity will increase this further, particularly after bilateral pain induction.

**The impact of unilateral and bilateral pain conditions on trunk muscle activity**

Previous findings illustrated that pain influenced the trunk muscle activity in variable and individual manners\(^{21, 35, 36, 78}\). Protective stiffening of the trunk\(^{8}\) although has been suggested as the primary role of the trunk muscles after sudden postural constraints\(^{33, 43}\). These assumptions are based on biomechanical considerations\(^{38, 72}\) to avoid threatening of the tissue in the stabilizing system\(^{7}\) after sudden surface perturbation. Although the underlying mechanisms remain unclear increased co-contraction of the trunk muscles in pain-free non-specific LBP patients\(^{43}\) and decreased trunk muscle activity after pain induction in healthy participants\(^{7}\) have been observed and therefore the overall muscle activity was hypothesized to decrease during unilateral and increase during bilateral pain. No sex differences were present in back muscle reflex responses in persistent LBP patients\(^{46}\) and in line with previous perturbation studies\(^{7, 44}\) males and females were included in the present study.

During bilateral experimental pain, the trunk muscle activity increased in most muscles (Fig. 3) and across the muscle groups (Fig. 4A) compared with baseline values, while the effect of unilateral pain was more widespread and resulted in significantly decreased overall muscle activity across the muscle groups (Fig. 4C). The different impact from bilateral pain induction in the early time window after bilateral injections of hypertonic saline and the higher impact of the painful stimuli
on the pain intensity and spreading during bilateral pain could be a better proxy of clinical LBP\textsuperscript{5,21}. However, Farina et al.\textsuperscript{17} found that decreased motor unit firing rates correlated to the pain intensity and the observed different correlations between pain intensity and muscle activity during experimental pain conditions in the present study therefore is suggested to be a result of other protective mechanisms controlled by the central nervous system\textsuperscript{30} and adapted to the motor task dependent on the pain intensity. In LBP patients, Falla et al.\textsuperscript{16} recently showed reduced variability of back extensor muscle activity during repetitive lifting tasks in comparison with matched healthy participants and Jones et al.\textsuperscript{43} furthermore found increased co-activation of the trunk and lower extremity muscles during multidirectional surface perturbations in recurrent LBP patients during pain-free periods. The observations from the present study supported a non-stereotypical effect of pain on the activity in the individual muscles, although the results across the muscles makes it probable that the motor responses to maintain stability can be established by reorganization of the motor system in healthy participants during acute pain.

In motor tasks requiring high accuracy of the lower and upper limbs in healthy participants Salomoni and Graven-Nielsen\textsuperscript{65} showed that the force variability was influenced by experimental pain without affecting the muscle activity significantly. In the present study the participants were challenged during a series of complex motor tasks and in line with previous observations\textsuperscript{36} the effect of pain on the trunk muscle activity was not stereotypical. The individual variability in the motor output has been observed in more studies\textsuperscript{21,35,43,74} and the absolute differences in RMS-EMG\textsuperscript{27} reflects the sum of changes indicating that bilateral pain generally had a stronger impact on the muscle activity in the trunk muscles. Although a trend towards minor decreased muscle activity was observed in all trunk muscles in the non-affected side during unilateral pain (Fig. 4A and 4B), these changes were not significantly decreased compared with control injections. Such changes may illustrate compensatory strategies by reorganization of the muscle activity to the non-affected side and thereby allowing the larger decreases in the affected side. In line with this,
Hirata et al\textsuperscript{26} previously suggested that the area of pain could influence postural control to a greater extent than could pain intensity. In the present study there were no significant correlation between the pain distribution and muscle activity. Since pain distribution only was collected after each series of 20 perturbations it is unknown if the time factor after pain induction is playing a role likewise the analysis of pain intensity when comparing the first and last time windows. The absolute changes in $\Delta$RMS-EMG (Fig. 5A and 5B) after unilateral pain in the present study however showed identical changes to control injections whereas bilateral pain resulted in generally more changes. Compared with the correlation between pain intensity and muscle activity this may indicate a stronger relations between the pain intensity and the functional aspects of the trunk muscles in a potential stiffening of the trunk\textsuperscript{6}, although these mechanisms during functional motor tasks remains unclear\textsuperscript{48}.

**Limitations**

The non-randomized order of injections limited the possibility to discover if the impact of unilateral pain induction was influenced by preceding induction of bilateral pain. However, this might not be the case given that the level of pain intensity during unilateral pain was equivalent to the pain intensity level in studies based on similar pain induction methods\textsuperscript{7,29}.

Normalization of surface EMG measurements is important when comparing muscle activity between muscles and participants\textsuperscript{62}. The most widely used method is normalization to maximal voluntary contraction that is generally accepted as reliable\textsuperscript{14}, but encumbered with constraints related to the validity and participants’ ability to develop maximal exertion. Given the high variability in the motor strategy during pain\textsuperscript{35} the individual differences in the muscle activity from a pain-free baseline condition were studied\textsuperscript{9} and additionally this method allowed calculating the absolute differences in the muscle activity\textsuperscript{28}.
Clinical implications

Increased trunk muscle activity after acute bilateral LBP with high intensity and large pain distribution was present in the present study where muscle activity correlated with the pain intensity. This is a reasonable protective reaction during postural tasks as observed in pain-free recurrent LBP patients. Subsequent decreased muscle activity was present during unilateral pain in a similar series of postural tasks as reported in recent studies. It may therefore be suggested that it is clinically important to support intervention strategies aiming to reduce both the pain intensity and area. The results of the present study furthermore challenge the relevance of clinical examination of muscle activity during functional motor tasks since it would be difficult to know what constitutes impaired muscle function, due to the present complex and variable changes.

CONCLUSION

Pain intensity dependent reorganization of trunk muscle activity in healthy participants after experimental pain induction was observed after multi-directional surface perturbations in stance with generally increased muscle activity after bilateral and decreased activity after unilateral pain across the perturbations and functional muscle groups.
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FIGURE LEGENDS

Figure 1. Experimental induction of bilateral pain (black bars), unilateral pain (grey bars) and bilateral control (white bars) by injections of hypertonic saline and isotonic saline, respectively, into the longissimus muscle. (A) Average visual analogue scale (VAS) scores (+SEM, N=19) during the individual perturbations. Significantly higher VAS scores after bilateral pain than control injections (*, Bonferroni: P<0.01) and unilateral hypertonic saline injections (#, Bonferroni: P<0.05). (B) Superimposed perceived areas (N=19) of experimental pain following bilateral control (B1), unilateral pain and control (B2), and bilateral pain (B3) induction in the longissimus muscle. Significantly increased pain areas following bilateral compared with unilateral and pain.

Figure 2. Mean baseline (N=19) root-mean-square electromyographic (RMS-EMG) responses 500 ms following perturbation onset in the left side trunk muscles after an anterior perturbation. The muscle activity varied generally after the perturbation onset and peak values were reached between 150 and 300 ms after perturbation.

Figure 3. Mean (+ SEM, N=19) ΔRMS-EMG expressed as a percentage of the baseline RMS-EMG and averaged across the 10 post-perturbation epochs for 3 back (A, B, C) and abdominal muscles (D, E, F). Each muscle is illustrated separately for left and right muscles (X-axes, left and right) and the six different perturbations (Y-axes, 1 = anterior tilt, 2 = posterior tilt, 3 = left displacement, 4 = left tilt, 5 = right displacement, 6 = right tilt) showing ΔRMS-EMG values following bilateral control (white), unilateral pain (grey), and bilateral pain (black). Significant differences between conditions is illustrated (*, Bonferroni: P<0.05).

Figure 4. Mean ΔRMS-EMG after the 3 different injection trials. (A) Mean (± SEM, N=19) percentage change of ΔRMS-EMG across all perturbation in individual muscles. (B) Mean (± SEM, N=19) percentage change of ΔRMS-EMG across all perturbation in left and right back and abdominal muscles. (C) Mean (± SEM, N=19) percentage changes of ΔRMS-EMG across all perturbation in back and abdominal muscles. Significant differences (*, Bonferroni: P<0.05) with increased muscle activity during bilateral pain and decreased muscle activity during unilateral pain in muscles, across muscle groups and across sides and muscle groups.

Figure 5. Absolute changes in muscle activity across all 6 perturbations (Absolute ΔRMS-EMG) after the 3 different injection trials. (A) Mean (+ SEM, N=19) absolute changes of ΔRMS-EMG
across all perturbation in individual muscles. (B) Mean (+ SEM, N=19) absolute changes of ΔRMS-EMG across right and left back and abdominal muscles and (C) mean (+ SEM, N=19) absolute changes of ΔRMS-EMG across back and abdominal muscles bilaterally. Significant differences (*, Bonferroni: P<0.05) with higher absolute changes in the muscle activity in muscles, across muscle groups and across sides and muscle groups during bilateral pain compared with unilateral pain and control injections.
FIGURES

A

B

Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
REORGANIZED TRUNK MUSCLE ACTIVITY DURING MULTI-DIRECTIONAL FLOOR PERTURBATIONS AFTER EXPERIMENTAL LOW BACK PAIN: A COMPARISON OF BILATERAL VERSUS UNILATERAL PAIN

REORGANIZED TRUNK MUSCLE ACTIVITY AFTER BILATERAL COMPARED WITH UNILATERAL LOW BACK PAIN DURING MULTI-DIRECTIONAL FLOOR PERTURBATIONS

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Trunk muscle activity, floor perturbations, and experimental low back pain

ABSTRACT

Low back pain (LBP) changes the trunk muscle activity after external perturbations but the relationships mechanisms between different LBP pain intensities and distributions and their effects impact on the trunk muscle activity remains unclear. In this study the effects of unilateral and bilateral experimental LBP on trunk muscle activity were compared during unpredictable multi-directional surface perturbations in 19 healthy participants. The pain intensity and distribution were assessed on a visual analogue scale (VAS) and by pain drawings.

Electromyography (EMG) was recorded bilaterally from 6 trunk muscles and the root-mean-square (RMS) of the electromyographic (EMG) signals from 6 trunk muscles bilaterally after each perturbation was extracted and averaged across perturbations. The difference (∆RMS-EMG) and absolute difference (absolute ∆RMS-EMG) RMS from baseline conditions were extracted for each muscle during the pain conditions and averaged bilaterally for the back and abdominal muscle groups. Bilateral compared with unilateral pain induced higher VAS scores (P<0.005) and larger pain areas (P<0.001). Significant correlation was present between VAS scores and muscle activity during unilateral (P<0.001) and bilateral pain (p>0.001), respectively.

Compared with control injections ∆RMS-EMG increased in the back (P<0.03) and abdominal (P<0.05) muscles during bilateral and decreased in the back (P<0.01) and abdominal (P<0.01) muscles during unilateral pain. Bilateral pain furthermore caused higher absolute ∆RMS-EMG changes in the back (P<0.01) and abdominal (P<0.01) muscle groups than unilateral pain.

Perspectives

This study provides novel observations of differential trunk muscle activity in response to perturbations that appears dependent on the distribution (unilateral versus bilateral) and pain intensity and/or pain distribution of pain. Due to complex and variable changes the relevance of clinical examination of muscle activity during postural tasks is challenged.
INTRODUCTION

The life-time prevalence of low back pain (LBP) is up to 38.9%\textsuperscript{40} and the evidence on causality is poor\textsuperscript{35,37}. Nonetheless, genetic\textsuperscript{50} and psycho-social factors\textsuperscript{45,58,61} have been proposed as risk-factors in LBP, and movement strategies and muscle activation patterns are potentially important factors\textsuperscript{1,35}. Muscle function and coordination are usually altered in LBP patients\textsuperscript{21,35} and impaired trunk muscle activation\textsuperscript{activation and activity} gained much attention as an explanatory model for LBP\textsuperscript{34}. Although the underlying mechanisms in trunk motor control and pain are sparsely linked\textsuperscript{58}, trunk muscle training is widely implemented clinically and in sports\textsuperscript{70} with underlying assumptions on ing trunk muscles as spinal stabilizers during functional tasks\textsuperscript{69,70}. Although the nature of possible changes are inconsistent complex muscle pain adaptation is evident\textsuperscript{37,52}. Additionally, and-stabilization exercises have no long-term effect\textsuperscript{18} or is not superior to other treatments\textsuperscript{10,13,68}. Experimental pain models therefore have been used extensively order to explore understand the effects of LBP pain in the lower back, aiming to mimic pain and yet exclude confounding factors in LBP patients\textsuperscript{5,21}. In previous studies lumbar pain was induced unilateral, but differences in pain characteristics between subacute LBP patients with greater prevalence of unilateral pain and persistent LBP patients predominantly indicating bilateral pain\textsuperscript{11} highlight the importance of understanding if pain related mechanisms during motor tasks differs between in both unilateral and bilateral pain conditions.

Gait is the primary human locomotion function and based on gait studies in LBP it is evident that complex muscle control is related to specific, and individual, temporal and spatial demands\textsuperscript{35}. LBP patients showed inconsistent muscle activity with e.g. increased back muscle activity during the swing phase\textsuperscript{3} and increased co-activation of erector spinae and rectus abdominis muscles\textsuperscript{76}, while increased lumbar and decreased abdominal muscle activity was present in patients older...
than 50 years. Van den Hoorn et al. additionally found individualized synergistic muscle-strategies during treadmill walking and the trunk control synergies were affected by back and leg pain in some subjects.

The nature of the gait task is complex and involves motor planning as well as motor adaptation and the effect of pain on the underlying mechanisms in motor control during gait is challenged. Contrarily, surface perturbation is a highly standardized and still complex motor task since and unpredictable surface perturbation is challenging due to non-predictable, high-velocity changes from the external perturbation. Multi-directional floor perturbations resulted in increased co-contraction of the trunk muscles in persistent LBP patients compared with a control group, indicating a trunk stiffening strategy. In contrast, Boudreau et al. found decreased trunk muscle activity after anterior and posterior displacement and tilt perturbations after pain induction in healthy participants. It remains unknown if these observed alterations are related to the differences in the surface perturbation protocol or if the underlying musculoskeletal impairments are important. Postural repositioning is generally challenged in LBP patients although studies showed no changes in proprioception in LBP patients, postural repositioning is generally challenged and decreased variability in postural adjustments to perturbations after acute and persistent LBP furthermore may indicate complex trunk muscle timing and activity. Various motor adaptations in functional tasks are generally accepted, but although experimental unilateral pain additionally affects the trunk muscle activity bilaterally and pain-related reorganization of the trunk muscle strategies during LBP is evident between and within muscles. These results suggest that in painful conditions the underlying interactions between muscles are not well understood and the effect of unilateral and bilateral pain on the trunk muscle response is unknown. Various motor adaptations in functional tasks is generally accepted, but the effect of unilateral and bilateral pain on the trunk muscle response after surface perturbation is unknown.
During gait LBP patients showed inconsistent muscle activity with e.g. increased back muscle activity during the swing phase and increased co-activation of erector spinae and rectus abdominis muscles, while increased lumbar and decreased abdominal muscle activity was present in patients older than 50 years. Van den Hoorn et al. found individualized synergistic muscle strategies during treadmill walking where especially the trunk control synergies were affected by back and leg pain in some subjects, indicating complex muscle control related to specific temporal and spatial individual demands.

The aim of the study was to compare the effects of unilateral and bilateral experimental LBP on trunk muscle activity during unpredictable multi-directional surface perturbations in healthy participants. It was hypothesized that (1) unilateral LBP will decrease, and (2) bilateral LBP will increase trunk muscle activity during multi-directional unpredictable surface perturbations.

METHODS

Participants

Nineteen healthy participants [4 females; mean age 26 years (range 19-39 years); mean height 180 cm (range 160-200 cm), mean body mass index 23.7 kg/m$^2$ (range 20.4-29.2 kg/m$^2$)] without lower extremity or back related pain or dysfunction participated in the study. The study was conducted in accordance with the Helsinki Declaration, approved by the local ethics committee (N-20090053) and informed consent was obtained from each participant.

Protocol

The subjects participated in one baseline perturbation session and three successive experimental perturbations sessions on the same day with minimum 15 min break in between conditions: (1) bilateral experimental saline-induced LBP, (2) bilateral control condition, and (3) unilateral
Experimental saline-induced LBP. In each session, the subject was standing on a marked position on a moveable platform during a series of 20 randomized multi-directional surface perturbations delivered after an auditory warning signal. Between sessions the subjects were allowed to sit on a chair.

Experimental low back pain

The injection procedure was performed with the subject prone lying. The Th12 segment was located and L2 was down counted and verified by palpation of L4 at the line between the iliac crest bilaterally where L2 was estimated\textsuperscript{15}. At the L2 level the most bulky part of m. longissimus was palpated (typically 3-5cm from the midline) and marked as injection site. Sterile isotonic (1.0 ml, 0.9\%) or hypertonic (1.0 ml, 5.8\%) saline was injected perpendicular to the skin surface with a 25G x 19 mm needle, after cleaning the injection site with alcohol. Hypertonic and isotonic saline was injected bilaterally (experimental condition 1 and 2, respectively) and in experimental condition 3 one hypertonic saline injection was given in the right side immediately followed by an injection of isotonic saline in the left side. The participants were informed about receiving injections, but were blinded to the type of saline injected. In the bilateral conditions the right injection was performed before the left and the time between injections was 30-60 s. Immediately after completion of both injections, the participant was assisted to the standing position on the platform for perturbations and started scoring the pain intensity.

During the period including perturbations the pain intensity was assessed on a 10-cm electronic visual analogue scale (VAS) with an external handheld slider. The VAS was anchored with ‘no pain’ and ‘maximum pain’ at 0 cm and 10 cm, respectively. The signal from the VAS was recorded after each injection until the pain vanished (sample frequency of 20 Hz). During the complete period including perturbations the mean VAS score was extracted in the time window from onset to the subsequent perturbation and the maximum VAS and average VAS scores were
extracted among the 20 perturbations. The subjects were asked to recover their balance as fast as 
possible after the perturbation, and only then, they were allowed to update the VAS. After each 
condition the subjects were asked to indicate the pain distribution on a body chart. The pain area 
was extracted from the drawings (ImageJ 1.47V, Rasband, NIH, USA) and mean areas were 
extracted.

**Perturbations**

Surface perturbations were performed by a computer-controlled moveable platform. The 
participant stood on the platform in a relaxed position with the feet in approximately shoulder-
width distance, the arms along the body, and instructed to look straight forward on a marker on 
the wall (4 m distance, 5 cm diameter). The foot position was marked on the platform to ensure 
that the position from the baseline condition was used during all 3 conditions. Ten perturbations 
in different directions were conducted as acclimatization before the data collection started. 
The perturbation protocol aimed to challenge the postural demands substantially in the standing 
position, but still allowing the participants to maintain the limits of stability of the standing 
position without stepping. A series of perturbations consisted of 20 randomized multi-
directional surface perturbations (1: sagittal anterior and posterior 3° tilt, velocity 30°/s and peak 
acceleration 200°/s²; 2: frontal left and right 10° tilt, velocity 40°/s and peak acceleration 140°/s²; 
3: frontal left and right 100 mm displacement, velocity 0.4 m/s and peak acceleration 140 m/s²) 
with randomly 4-8 s intervals between, and minimum 3 repetitions of each perturbation types in 
each series. Each perturbation was initiated by an auditory cue and the perturbation was 
conducted after 0.2 – 5.0 s at random and trials including stepping strategies after perturbation (in 
all 7 trials in different directions) were excluded.

**Electromyography recordings**
The skin was shaved and cleaned with alcohol. The ground electrode (Blue sensor P 34mm, Ambu Neuroline, Denmark) was mounted on the skin over the most prominent spinal process at C6, C7 or Th1. Surface electrode pairs (Ambu Neuroline 720, Denmark) were mounted bilaterally on back muscles according with previous recommendations\textsuperscript{25}: M. iliocostalis (one finger width medial from a line from posterior superior iliac spine (PSIS) to lowest point of lower rib at L2 level, longissimus (2 fingers width lateral from L1 spinal process), m. multifidus (line from caudal tip of PSIS to L1-L2 interspace at L5 process. Likewise electrodes were mounted bilaterally on abdominal muscles: M. \textit{obliquus internus} (along horizontal line between left and right anterior superior iliac spine, medial from inguinal ligament\textsuperscript{1}, m. rectus abdominis (3 to 4 cm lateral to the navel\textsuperscript{57}), and m. \textit{obliquus externus} (upper electrode directly below most inferior point of costal margin of PSIS \textsuperscript{2}).

The electromyography (EMG) signals were band pass filtered (10–500 Hz), sampled at 2048 Hz with a gain of 2000 using a 16-channel surface EMG-USB amplifier (LISiN-OT Bioelettronica, Torino, Italy) and converted to digital form by a 12-bit analog-to-digital converter. The EMG signals were synchronized with the onset of perturbation.

**Data analysis**

Root-mean-square (RMS) values were derived from the EMG signals in 10 non-overlapping signal epochs of 50 ms from the perturbation onset and the average value across epochs was extracted for each perturbation\textsuperscript{66,67} (hereafter defined as RMS-EMG). The RMS-EMG during experimental pain and control sessions was expressed as a percentage of the baseline RMS-EMG (ΔRMS-EMG; baseline is 100%) value for each muscle and perturbation type individually and furthermore averaged across all perturbation types. Calculation of ΔRMS-EMG was used to account for the large inter-individual variability in pain-related muscle activity changes\textsuperscript{28}. Since changes expressed by ΔRMS-EMG could cover increased and decreased values the absolute values of the ΔRMS-EMG was calculated to indicate absolute changes from the baseline condition\textsuperscript{28}. Finally, ΔRMS-EMG and
absolute ΔRMS-EMG, respectively, were averaged across unilateral and bilateral back (m. iliocostalis, m. longissimus, and m. multifidus) and abdominal (m. obliquis internus, m. rectus abdominis, and m. obliquis externus) muscle groups. The data were initially analyzed for main effects in the individual muscles between the perturbations and since this was not the case, further analyzes were conducted across the perturbations.

Statistics

Data are presented as mean and standard error of the mean (SEM). All statistical analyses were performed in SPSS®22.0 (IBM). The data was tested for normality by the Shapiro-Wilk test and was generally normally distributed (P > 0.05). Data analyses were conducted by repeated measures analysis of variance with a Greenhouse-Geisser correction (RM-ANOVA) and when significant, Bonferroni adjusted post-hoc t-tests were used to conduct pairwise comparisons between conditions. The experimental pain areas were analyzed by one-way RM-ANOVA with condition (bilateral control, unilateral pain and bilateral pain) as main factor. Mean and peak VAS scores were analyzed by two-way RM-ANOVAs with factors condition and time (20 perturbations).

Additionally, the mean VAS scores were analyzed by two-way RM-ANOVAs with factors condition and time across perturbation 1-10 and 11-20, respectively. For the relative and absolute ΔRMS-EMG averaged across the six perturbation types RM-ANOVAs were conducted by respectively (i) 2-way RM-ANOVA with factors muscle group (abdominal and back) and condition, (ii) 3-way RM-ANOVA with factors side (left and right), muscle group, and condition, (iii) 3-way RM-ANOVA with factors side, muscle (m. iliocostalis, m. longissimus, m. multifidus, m. obliquis internus, m. rectus abdominis, and m. obliquis externus), and condition, and additionally, comparison between perturbations were conducted by a 3-way RM-ANOVA with factors muscle (left and right m. iliocostalis, m. longissimus, m. multifidus, m. obliquis internus, m. rectus abdominis, and m. multifidus).
To examine correlation between RMS-EMG and pain intensity during unilateral and bilateral pain, these parameters were examined by a Pearson’s correlation between VAS scores and RMS-EMG across across all perturbation types and left and right side muscle groups. Moreover, correlation between pain spreading area and mean RMS-EMG across the 20 perturbations in each session were examined by a Pearson’s correlation and when significant the values were Bonferroni corrected. Statistical significance was accepted at P < 0.05.

RESULTS

Experimental low back pain

The mean VAS scores were significantly higher after bilateral compared with unilateral hypertonic saline injections during all 20 perturbations (Fig. 1A; ANOVA: F (2,720) = 85.2; P<0.001; Bonferroni: P<0.005). The maximal VAS score after control injections was 1.1 ± 0.3 cm and significantly lower than after unilateral (5.0 ± 1.0 cm, Bonferroni: P<0.005) and bilateral injection of hypertonic saline (6.5 ± 1.1 cm; ANOVA: F (2,720) = 851.6; P<0.001; Bonferroni: P<0.005). Moreover, the maximal VAS score was higher after bilateral compared with unilateral hypertonic saline injections (Bonferroni: P<0.001).

The mean VAS score across the 10 first perturbations was higher compared with the mean VAS score across the 10 last perturbations (ANOVA: F (1,189) = 154.4; P<0.001; Bonferroni: P<0.001) and compared with the last time window post-hoc tests showed higher mean VAS scores in the first time window during unilateral (Bonferroni: P<0.001) and bilateral (Bonferroni: P<0.001) pain.

Following unilateral and bilateral injection of hypertonic saline, pain was primarily perceived in the low back area and injection of isotonic saline only resulted in pain around the injection site (Fig. 1B). The mean perceived area of pain was 2.8 ± 2.3 in arbitrary units (a.u.) after bilateral
control injections, 25.5 ± 9.6 a.u. after unilateral and 62.4 ± 22.7 a.u. after bilateral hypertonic saline injection. The mean perceived area of pain after bilateral hypertonic saline injection was bilateral and 245% larger than during unilateral pain (ANOVA: F (2,36) = 93.6, P<0.001; Bonferroni: P<0.005).

**Motor response following surface perturbation**

The initial motor responses following a perturbation occurred typically around 100 ms after the perturbation onset with peak muscle activity between 150 and 300 ms (Fig. 2). The differences from baseline recordings (ΔRMS-EMG) are illustrated in Fig. 3 for the six muscles, left and right sides, six perturbations and three experimental conditions. A 3-way RM-ANOVA showed a significant interaction between muscles, perturbation type, and conditions (ANOVA: F (110,1980) = 6.1, P<0.001) with no main effect on perturbations (ANOVA: F (5,90) = 2.4, P=0.07) and muscles (ANOVA: F (11,198) = 0.4, P=0.80) but main effect on conditions (ANOVA: F (2,36) = 6.1, P<0.01) where post-hoc analyses showed significant changes between the two pain conditions in i) right m. longissimus (Bonferroni: P<0.001) and m. multifidus (Bonferroni: P<0.03) after right displacement, ii) left (Bonferroni: P<0.01) and right (Bonferroni: P<0.01) m. multifidus and left m. obliquus externus (Bonferroni: P<0.03) after left displacement, and iii) left m. obliquus internus after right tilt (Bonferroni: P<0.05). Compared with control injections, unilateral pain resulted in significantly decreased ΔRMS-EMG in the three back muscles after more perturbations, particularly after left and right displacement and left tilt and primarily in the right-sided muscles (Bonferroni: P<0.05), and bilateral pain resulted in significantly lower muscle activity in right m. iliocostalis after right tilt (Bonferroni: P<0.01) and higher muscle activity in right m. multifidus after respectively anterior tilt (Bonferroni: P<0.02) and left displacement (Bonferroni: P<0.04), left m. obliquus internus after right tilt (Bonferroni: P<0.01) and right m. obliquus internus (Bonferroni:
P<0.01)

**Perturbation evoked muscle activity across all perturbations**

A 3-way RM-ANOVA of ∆RMS-EMG in the individual muscles averaged across all perturbations (Fig. 4A) demonstrated a significant interaction between muscles, sides and conditions (ANOVA: F (10, 1130)= 3.1, P<0.001) with no main effect on muscles (ANOVA: F (5,565) = 0.8, P=0.50) but main effect on sides (ANOVA: F (1,113) = 5.1, P<0.03) conditions (ANOVA: F (2,226) = 15.5, P<0.001). Post-hoc analyses showed significant decreased ∆RMS-EMG in right m. iliocostalis (Bonferroni: P<0.02), m. longissimus (Bonferroni: P<0.001) and m. multifidus (Bonferroni: P<0.005) after unilateral pain compared with control injections and increased ∆RMS-EMG in left m. obliquus internus (Bonferroni: P<0.02) and right m. longissimus (Bonferroni: P<0.005), m. multifidus (Bonferroni: P<0.001), m. obliquus internus (Bonferroni: P<0.001) and m. rectus abdominis (Bonferroni: P<0.03) after bilateral pain compared with unilateral pain. In addition, ∆RMS-EMG were significantly increased in right m. rectus abdominis during bilateral pain compared with control injections (Bonferroni: P<0.01).

**Perturbation evoked muscle activity across muscle groups and perturbations**

A 3-way RM-ANOVA of ∆RMS-EMG averaged across all perturbations and muscle groups resulted in a significant interaction between muscle groups, sides, and conditions (Fig. 4B; ANOVA: F (2,682) = 3.21, P<0.04) with no main effect on muscle group (ANOVA: F (5,565) = 1.7, P=0.16) and sides (ANOVA: F (1,113) = 0.7, P=0.42) but main effect on conditions (ANOVA: F (2,226) = 15.7, P<0.001). Post-hoc analyses showed increased ∆RMS-EMG during bilateral and decreased ∆RMS-EMG during unilateral pain in the left back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) and right back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) muscle groups. Compared with the control condition, significantly decreased ∆RMS-EMG was observed.
during unilateral pain in the right back muscle group (Bonferroni: P<0.001) and in the left abdominal muscle group (Bonferroni: P<0.02) and increased ∆RMS-EMG was found during bilateral pain in the right abdominal muscle group (Bonferroni: P<0.005).

Across left and right abdominal and back muscles, a 2-way ANOVA of ∆RMS-EMG demonstrated a significant interaction between muscle groups and conditions (Fig. 4C; ANOVA: F (2,1366) = 3.8, P<0.03) with main effect on muscle groups (ANOVA: F (2,1366) = 6.1, P<0.02) and conditions (ANOVA: F (2,1366) = 23.5, P<0.001). Post-hoc analyses showed significantly decreased ∆RMS-EMG during unilateral pain and increased ∆RMS-EMG during bilateral pain in the back (Bonferroni: P<0.001) and abdominal (Bonferroni: P<0.001) muscle groups. Compared with the control condition significantly decreased ∆RMS-EMG during unilateral pain was observed in the back (Bonferroni: P<0.001) and the abdominal muscle group (Bonferroni: P<0.02) and during bilateral pain significant increased ∆RMS-EMG was observed in the abdominal muscle group (Bonferroni: P<0.005).

Absolute muscle activity changes across all perturbations

The 3-way RM-ANOVA of the absolute ∆RMS-EMG in the left and right sided muscles averaged across all perturbations (Fig. 5A) demonstrated a 3-way interaction between sides, muscles and conditions (ANOVA: F (10,1130)= 6.6, P<0.001) with no main effect on side (ANOVA: F (1,114) = 0.2, P=0.64) and muscles (ANOVA: F (5,565) = 2.3, P=0.08) but main effect on conditions (ANOVA: F (2,226) = 10.8, P<0.001). Post-hoc analyses showed significant higher absolute ∆RMS-EMG during bilateral pain compared with unilateral pain in right m. iliocostalis (Bonferroni: P<0.001), m. longissimus (Bonferroni: P<0.001), m. rectus abdominis (Bonferroni: P<0.001) and m. obliquus externus (Bonferroni: P<0.02) and during bilateral pain compared with control injections in right m. iliocostalis (Bonferroni: P<0.001), m. longissimus (Bonferroni: P<0.02), m. rectus abdominis (Bonferroni: P<0.03) and m. obliquus externus (Bonferroni: P<0.001).
**Absolute muscle activity changes in muscle groups across sides**

The 3-way RM-ANOVA of the absolute $\Delta$RMS-EMG across left and right sided muscles groups averaged across all perturbations (Fig. 5B) demonstrated a significant interaction between sides, muscle groups, and conditions (ANOVA: $F(2, 682) = 3.2, P<0.02$) with no main effect on side (ANOVA: $F(1,341) = 0.2, P=0.56$) and muscle groups (ANOVA: $F(1,341) = 0.2, P=0.64$) but main effect on conditions (ANOVA: $F(2,682) = 14.6, P<0.001$). Post-hoc analyses showed significant higher absolute $\Delta$RMS-EMG during bilateral pain compared with unilateral pain in the left (Bonferroni: $P<0.04$) and right (Bonferroni: $P<0.001$) back and left (Bonferroni: $P<0.001$) abdominal muscle groups. Compared with control conditions the absolute $\Delta$RMS-EMG was significant higher in the right back (Bonferroni: $P<0.001$) and abdominal muscle groups (Bonferroni: $P<0.001$).

Across all perturbations and left and right abdominal and back muscles respectively (Fig. 5C) a 2-way ANOVA of absolute $\Delta$RMS-EMG demonstrated a significant interactions between muscle groups and conditions (ANOVA: $F(1.81, 1235.57) = 16.93 P<0.01$) with no main effect on muscle groups (ANOVA: $F(1,683) = 0.2, P=0.64$) but main effect on conditions (ANOVA: $F(2,1366) = 16.4, P<0.001$). Post-hoc analyses showed significant higher absolute $\Delta$RMS-EMG during bilateral compared with unilateral pain in the back (Bonferroni: $P<0.001$) and abdominal (Bonferroni: $P<0.001$) muscle groups and during bilateral compared to control conditions in the back (Bonferroni: $P<0.01$) and abdominal (Bonferroni: $P<0.01$) muscle groups.

**Correlation between experimental pain and perturbation evoked muscle activity**

Across all perturbations there were negative correlation between VAS scores and RMS-EMG during unilateral pain in the abdominal muscle group in the left ($r^2 =0.52; P<0.001$; Bonferroni: $P<0.001$) and right ($r^2 =0.55; P<0.001$; Bonferroni: $P<0.001$) side and in the back muscle group in
the left ($r^2 =0.60$; P<0.001; Bonferroni: P<0.001) and right ($r^2 =0.79$; P<0.001; Bonferroni: P<0.001) side. During bilateral pain there were positive correlation between VAS scores and RMS-EMG in the abdominal muscle group in the left ($r^2 =0.53$; P<0.001; Bonferroni: P<0.001) and right ($r^2 =0.57$; P<0.001; Bonferroni: P<0.001) side and in the back muscle group in the left ($r^2 =0.60$; P<0.001; Bonferroni: P<0.001) and right ($r^2 =0.54$; P<0.001; Bonferroni: P<0.001) side.

There were no significant correlation between size of pain area and muscle activity in the left and right abdominal and back muscle groups during unilateral pain (P>0.29) or bilateral pain (P>0.14).

**DISCUSSION**

This is the first study to compare the impact of unilateral versus bilateral experimental LBP and control conditions on the motor response following surface perturbations in healthy participants. The impact of bilateral pain was generally increased trunk muscle activity while unilateral pain resulted in decreased trunk muscle activity in line with the hypotheses. The individual pain-related changes in muscle activity across muscle groups were higher after bilateral compared with unilateral experimental pain and control injections and during the first time window after pain induction, bilateral pain resulted in higher muscle activity, compared with the last- like reorganization across abdominal and back muscles respectively was evident. It has recently been argued that pain-driven muscle activity changes are important protective mechanisms of the spine\textsuperscript{36, 37, 44, 77}, but the results of the present study supported a more complex and reorganization of the muscle activity related to the pain spreading and intensity but not the pain spreading.

**Subjective characteristics of experimental low back pain**
Control injections of isotonic saline in the m. longissimus resulted in low pain intensity around the injection site with minor spreading in few participants and most participants reported no pain. Injection of hypertonic saline resulted in muscle pain of moderate intensity, consistent with previous reports in relation to average pain intensity and peak pain intensity.

Higher intensity was previously found after bilateral compared with unilateral injection of hypertonic saline into the trapezius muscle and temporal muscle, but the present study is the first demonstrating such spatial summation effects in the lower back muscles. These mechanisms may also explain the significant increased spreading of the pain areas during bilateral injections. In line with previous studies, bilateral pain resulted in referred pain to the groin and lateral femoral areas in some participants and it was obvious that of the pain intensity remained relative high and unchanged during the 10 first perturbations after bilateral pain induction and then gradually decreased throughout the session.

**Perturbation of the motor system to explore the motor adaption**

Several approaches have been used to induce sudden disruptions in balance to explore the role of the trunk muscles, including self-initiated perturbations by shoulder flexion, sudden release of mechanical loads and surface perturbation. The motor control related to perturbation includes preprogrammed anticipatory postural mechanisms in the time window immediately before and around 150 ms after perturbation onset and subsequent corrections of posture. This study investigated the reactive strategies and the effect of pain on the muscle control in a randomized non-predictable multi-directional floor perturbation set-up. This approach was established to support studies of postural reactions and implemented in LBP research to explore the role of the trunk muscles, an important component of the motor output after external perturbations. Sequential exposure of participants to perturbations may reduce the initial responses that monosynaptic and polysynaptic reflexes represent to postural corrections and
influence the following motor strategy in the studied time window. Learning effects in healthy has been observed after both motor imagery\textsuperscript{71} and standing reaching training\textsuperscript{64}. These observations were related to anticipatory muscle activity, but in our study the possible learning effect was challenged by randomized 0.2-5.0 second latency between the auditory signal in combination with a randomized multi-directional approach that challenge the postural adaptation\textsuperscript{23}. Decreased risk of learning has previously been challenged by e.g. unexpected perturbation\textsuperscript{41} and randomized pre-perturbation feedback protocols\textsuperscript{44}. The postural demands in the present study were extensive due to the selected force and velocity of the perturbations and auditory cues were utilized to avoid unintentionally pre-tension in trunk muscles or risk of falls.

The latency phase after perturbation was generally 50-150 ms after perturbation onset, in line with findings of the voluntary response phase after self-initiated perturbation\textsuperscript{53} and unexpected surface perturbation during acute experimental pain\textsuperscript{27,41}. However, the observed variable motor adaptation to postural challenges is in line with previous results\textsuperscript{27,37,47} and the variability after control injections (Fig. 5A,B,C) supported that the surface perturbation approach resulted in major-extensive challenge of the reactive postural control and confirmed that the motor response to pain is flexible as suggested by Hodges et al\textsuperscript{36}. Decreased ΔRMS-EMG after unilateral pain is in line with a recent study from Boudreau et. al\textsuperscript{7}. However, during bilateral pain the increased ΔRMS-EMG compared with all other conditions, ΔRMS-EMG increased during bilateral pain-(Fig. 4A and 5A) which is in line with the trunk muscle activity in pain-free recurrent LBP patients\textsuperscript{44}. The larger changes in the muscles assessed muscle, by means of seen as-higher absolute ΔRMS EMG (Fig. 5.A,B,C) indicated that pain intensity is playing a major role. However, the absolute ΔRMS-EMG changes from baseline during unilateral pain generally equal the absolute changes after control conditions injections and variability in muscle responses between trials are thereby considerable, but and high pain intensity and widespread pain will increase this further, particularly after bilateral pain induction.
The impact of unilateral and bilateral pain conditions on trunk muscle activity

Previous findings illustrated that pain influenced the trunk muscle activity in variable and individual manners\textsuperscript{21, 35, 36, 78}. Protective stiffening of the trunk\textsuperscript{8} although has been suggested as the primary role of the trunk muscles after sudden postural constraints\textsuperscript{33, 43}. These assumptions are based on biomechanical considerations\textsuperscript{38, 72} to avoid threatening of the tissue in the stabilizing system\textsuperscript{7} after sudden surface perturbation. Although the underlying mechanisms remain unclear increased co-contraction of the trunk muscles in pain-free non-specific LBP patients\textsuperscript{43} and decreased trunk muscle activity after pain induction in healthy participants\textsuperscript{7} have been observed and therefore the overall muscle activity was hypothesized to decrease during unilateral and increase during bilateral pain. No sex differences were present in back muscle reflex responses in persistent LBP patients\textsuperscript{46} and in line with previous perturbation studies\textsuperscript{7, 44} males and females were included in the present study.

During bilateral experimental pain, the trunk muscle activity increased in most muscles (Fig. 3) and across the muscle groups (Fig. 4A) compared with baseline values, while the effect of unilateral pain was more widespread and resulted in significantly decreased overall muscle activity across the muscle groups (Fig. 4C). The different impact from bilateral pain induction in the early time window after bilateral injections of hypertonic saline and\textsuperscript{17} the higher impact of the painful stimuli on the pain intensity and spreading during bilateral pain could be a better proxy of clinical LBP\textsuperscript{5, 21}. However, Farina et al.\textsuperscript{17} found that decreased motor unit firing rates correlated to the pain intensity and the observed different correlations between pain intensity and distribution and increased muscle activity during bilateral experimental pain conditions in the present study therefore is suggested to be a result of other protective mechanisms controlled by the central nervous system\textsuperscript{30} and adapted to the motor task dependent on the pain intensity. In LBP patients, Falla et al.\textsuperscript{16} recently showed reduced variability of back extensor muscle activity during repetitive
lifting tasks in comparison with matched healthy participants and Jones et al.\textsuperscript{43} furthermore found increased co-activation of the trunk and lower extremity muscles during multidirectional surface perturbations in recurrent LBP patients during pain-free periods. The observations from the present study supported a non-stereotypical effect of pain on the activity in the individual muscles, although the results across the muscles makes it probable that the motor responses to maintain stability can be established by reorganization of the motor system in healthy participants during acute pain.

In motor tasks requiring high accuracy of the lower and upper limbs in healthy participants Salomoni and Graven-Nielsen\textsuperscript{65} showed that the force variability was influenced by experimental pain without affecting the muscle activity significantly. In the present study the participants were challenged during a series of complex motor tasks and in line with previous observations\textsuperscript{36} the effect of pain on the trunk muscle activity was not stereotypical. The individual variability in the motor output has been observed in more studies\textsuperscript{21, 35, 43, 74} and the absolute differences in RMS-EMG\textsuperscript{27} reflects the sum of changes indicating that bilateral pain generally had a stronger impact on the muscle activity in the trunk muscles. Although a trend towards minor decreased muscle activity was observed in all trunk muscles in the non-affected side during unilateral pain (Fig. 4A and 4B), these changes were not significantly decreased compared with control injections. Such changes may illustrate compensatory strategies by reorganization of the muscle activity to the non-affected side and thereby allowing the larger decreases in the affected side. In line with this, Hirata et al\textsuperscript{26} previously suggested that the area of pain could influence postural control to a greater extent than could pain intensity the postural control further than pain intensity. In the present study there were no significant correlation between the pain distribution and muscle activity. Since pain distribution only was collected after each series of 20 perturbations it is unknown if the time factor after pain induction is playing a role likewise the analysis of pain intensity when comparing the first and last time windows. The absolute changes in $\Delta$RMS-EMG
(Fig. 5A and 5B) after unilateral pain in the present study however showed identical values changes to control injections whereas bilateral pain resulted in generally more changes higher values. Compared with the correlation between pain intensity and muscle activity, this may indicate a stronger relationship between the pain intensity as well as the pain distribution and the functional aspects of the trunk muscles in a potential stiffening of the trunk during bilateral pain, although these mechanisms during functional motor tasks remains unclear.

Limitations
The non-randomized order of injections limited the possibility to discover if the impact of unilateral pain induction was influenced by preceding induction of bilateral pain. However, this might not be the case given that the level of pain intensity during unilateral pain was equivalent to the pain intensity level in studies based on similar pain induction methods.

Normalization of surface EMG measurements is important when comparing muscle activity between muscles and participants. The most widely used method is normalization to maximal voluntary contraction that is generally accepted as reliable, but encumbered with constraints related to the validity and participants’ ability to develop maximal exertion. Given the high variability in the motor strategy during pain, the individual differences in the muscle activity from a pain-free baseline condition were studied and additionally this method allowed calculating the absolute differences in the muscle activity.

Clinical implications
Increased trunk muscle activity after acute bilateral LBP with high intensity and large pain distribution was found present in the present study where muscle activity correlated with the pain intensity. This is a reasonable protective reaction during postural tasks as observed in pain-free recurrent LBP patients. Subsequent decreased muscle activity was present during unilateral pain...
in a similar series of postural tasks as reported in recent studies\textsuperscript{7,28}. It may therefore be suggested that it is clinically important to support intervention strategies aiming to reduce both the pain intensity and area. The results of the present study furthermore challenge the relevance of clinical examination of muscle activity during functional motor tasks since it would be difficult to know what constitutes impaired muscle function, due to the present complex and variable changes.

**CONCLUSION**

Pain intensity dependent reorganization of trunk muscle activity in healthy participants after experimental pain induction was observed after multi-directional surface perturbations in stance with generally increased muscle activity after bilateral and decreased activity after unilateral pain across the perturbations and functional muscle groups.
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FIGURE LEGENDS

**Figure 1.** Experimental induction of bilateral pain (black bars), unilateral pain (grey bars) and bilateral control (white bars) by injections of hypertonic saline and isotonic saline, respectively, into the longissimus muscle. (A) Average visual analogue scale (VAS) scores (+SEM, N=19) during the individual perturbations. Significantly higher VAS scores after bilateral pain than control injections (*, Bonferroni: P<0.01) and unilateral hypertonic saline injections (#, Bonferroni: P<0.05). (B) Superimposed perceived areas (N=19) of experimental pain following bilateral control (B1), unilateral pain and control (B2), and bilateral pain (B3) induction in the longissimus muscle. Significantly increased pain areas following bilateral compared with unilateral and pain.

**Figure 2.** Mean baseline (N=19) root-mean-square electromyographic (RMS-EMG) responses 500 ms following perturbation onset in the left side trunk muscles after an anterior perturbation. The muscle activity varied generally after the perturbation onset and peak values were reached between 150 and 300 ms after perturbation.

**Figure 3.** Mean (+ SEM, N=19) ∆RMS-EMG expressed as a percentage of the baseline RMS-EMG and averaged across the 10 post-perturbation epochs for 3 back (A, B, C) and abdominal muscles (D, E, F). Each muscle is illustrated separately for left and right muscles (X-axes, left and right) and the six different perturbations (Y-axes, 1 = anterior tilt, 2 = posterior tilt, 3 = left displacement, 4 = left tilt, 5 = right displacement, 6 = right tilt) showing ∆RMS-EMG values following bilateral control (white), unilateral pain (grey), and bilateral pain (black). Significant differences between conditions is illustrated (*, Bonferroni: P<0.05).

**FIGURE 4.** Mean ∆RMS-EMG after the 3 different injection trials. (A) Mean (± SEM, N=19) percentage change of ∆RMS-EMG across all perturbation in individual muscles. (B) Mean (± SEM, N=19) percentage change of ∆RMS-EMG across all perturbation in left and right back and abdominal muscles. (C) Mean (± SEM, N=19) percentage changes of ∆RMS-EMG across all perturbation in back and abdominal muscles. Significant differences (*, Bonferroni: P<0.05) with increased muscle activity during bilateral pain and decreased muscle activity during unilateral pain in muscles, across muscle groups and across sides and muscle groups.

**FIGURE 5.** Absolute changes in muscle activity across all 6 perturbations (Absolute ∆RMS-EMG) after the 3 different injection trials. (A) Mean (+ SEM, N=19) absolute changes of ∆RMS-EMG
across all perturbation in individual muscles. (B) Mean (+ SEM, N=19) absolute changes of ΔRMS-EMG across right and left back and abdominal muscles and (C) mean (+ SEM, N=19) absolute changes of ΔRMS-EMG across back and abdominal muscles bilaterally.

Significant differences (*, Bonferroni: P<0.05) with higher absolute changes in the muscle activity in muscles, across muscle groups and across sides and muscle groups during bilateral pain compared with unilateral pain and control injections.