Pressure-induced referred pain is expanded by persistent soreness

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Published in:
Pain

DOI (link to publication from Publisher):
10.1097/j.pain.0000000000000497

Publication date:
2016

Document Version
Accepted author manuscript, peer reviewed version

ABSTRACT

Several chronic pain conditions are accompanied with enlarged referred pain areas. This study investigated a novel method for assessing referred pain. In 20 healthy subjects pressure pain thresholds (PPTs) were recorded and pressure stimuli (120%PPT) were applied bilaterally for 5-s and 60-s at the infraspinatus muscle to induce local and referred pain. Moreover, PPTs were measured bilaterally at the shoulder, neck and leg before, during and after hypertonic saline-induced referred pain in the dominant infraspinatus muscle. The pressure and saline-induced pain areas were assessed on drawings. Subsequently, delayed onset muscle soreness (DOMS) was induced using eccentric exercise of the dominant infraspinatus muscle. The Day-1 assessments were repeated the following day (Day-2). Suprathreshold pressure stimulations and saline injections into the infraspinatus muscle caused referred pain to the frontal aspect of the shoulder/arm in all subjects. The 60-s pressure stimulation caused larger referred pain areas compared with the 5-s stimulation ($P<0.01$). Compared with pressure stimulation, the saline-induced referred pain area was larger ($P<0.02$). After saline-induced pain, the PPTs at the infraspinatus and supraspinatus muscles were reduced ($P<0.05$) and the 5-s pressure-induced referred pain area was larger than baseline. PPTs at the infraspinatus and supraspinatus muscles were reduced at Day-2 in the DOMS side ($P<0.05$). Compared with Day-1, larger pressure and saline-induced referred pain areas were observed on Day-2 ($P<0.05$). Referred pain to the shoulder/arm was consistently induced and enlarged after one day of muscle soreness indicating that the referred pain area may be a sensitive biomarker for sensitization of the pain system.

PRESSURE-INDUCED REFERRED PAIN IS EXPANDED BY PERSISTENT SORENESS

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Original paper for: PAIN

Running title: *Pressure-induced referred pain is expanded by persistent soreness*

Key words Pressure Pain Threshold · Delayed onset muscular soreness · Referred Pain · Infraspinatus · Shoulder Pain · Muscle Pain

Number of pages: 11
Number of figures and tables: 3 figures, 3 tables.

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INTRODUCTION

Chronic musculoskeletal pain affects a significant part of the population. The clinical presentation varies greatly between patients with respect to symptoms where variables such as pain intensity, distribution, and quality combined with the pain duration are commonly used for diagnostic purposes. One particular feature is referred pain which is defined as pain located distant to the site of primary tissue insult [14]. Referred pain from sore musculoskeletal structures is well-known in various clinical conditions [5,37,49,53] where the affected structures have a fairly distinct pattern of pain referral [52]. Referred pain is likely driven by a central mechanism as it can be evoked in areas where sensory input has been removed [30] and it has also been shown that experimentally-induced referred muscle pain can be reduced by ketamine; an NMDA-antagonist [16,48]. This is supported by findings from animal studies demonstrating that new receptive fields develop and an expansion in dorsal horn neuron activity occurs following a nociceptive stimulus from muscle [21,22]. Such hyperexcitability may be involved in the referred muscle pain mechanism [39], potentially explaining the widespread pain and hyperalgesia commonly found in patients [1,2,16,29,43,49], suggesting that the efficacy of central processing is facilitated by ongoing or previous localized tissue insult [40]. Thus, referred muscle pain may be a useful biomarker for assessing sensitivity of central pain mechanisms.

In experimental settings, different types of stimuli have been used to assess pain referral. The most common types are chemical and mechanical stimulation [10,11,14]. An intramuscular injection of hypertonic saline induces a deep sensation of pain, locally and distally, where a correlation is found between the localization, duration and intensity of the nociceptive stimulus and the area of referred pain [11]. Another characteristic is that the referred pain is delayed compared with the local pain [14,36]. Furthermore, muscle pain caused by eccentric exercise (delayed onset of muscle soreness, DOMS) has been demonstrated to be a useful model in experimental settings for inducing deep-tissue pain hypersensitivity developing over 24 h to 72 h [33]. DOMS results in enlarged and increased number of pain areas following chemical [10] and mechanical stimulation (e.g increased temporal summation of pain to repetitive painful pressure stimulations) [41]. Both phenomena have been linked with sensitization of central pain mechanisms [23]. Thus, the DOMS model allows studying the efficacy of both chemical and mechanical models to assess local and referred or expanded pain in response to the persistent soreness.

In this study, a novel non-invasive model of referred muscle pain was developed to evaluate the similarities in referred muscle pain patterns evoked by chemical and mechanical stimulations before and after persistent pain. The hypotheses were that 1) a painful pressure stimulation induces areas of referred pain dependent on the stimulus duration, 2) pain referral patterns from saline and pressure-induced pain are similar, and 3) the referred pain areas are expanded after 1 day with muscle soreness. This model will be described in detail in the following section.

MATERIALS AND METHODS

Subjects

Twenty-one healthy volunteers (10 female) were recruited for this study, through an advertisement on social networks, public announcements, and from the university campus. Subjects had no current or previous history of persistent musculoskeletal pain specific to the neck, shoulder, arm, and/or in general. Pregnant women were not included in the study. All subjects were asked to refrain from intense physical exercises on the days of participation. The participants gave informed consent prior to participation after receiving a detailed description of the study protocol. The study was approved by the local Ethics Committee (VN 20130060) and performed in accordance with the Helsinki Declaration.

Experimental protocol

This study was a cross-sectional, randomized study which was carried out in two sessions, separated by 24 hours (Day-1 and Day-2, respectively). In both sessions, pressure pain thresholds (PPTs) were recorded and suprathreshold pressure stimuli relative to the PPT were applied bilaterally at the infraspinatus muscle to induce local and referred pain at different time-points. Moreover, PPTs were recorded bilaterally at baseline, during, and after experimental pain induced in the infraspinatus muscle of the dominant side by injection of hypertonic saline. The post-pain assessment was performed five minutes after the saline-induced pain had subsided. The local and referred pain areas were assessed at baseline (bilateral pressure stimulation), during pain (unilateral hypertonic saline) and in the post-pain state (bilateral pressure stimulation). One of the aims of this study was to investigate the time-dependent aspects of suprathreshold pressure stimulation (STPS). Therefore, the pressure stimulation was given with two different durations, 5 and 60 seconds. The second session (Day-2) was intended to investigate how and if tissue hypersensitivity caused by

DOMS would result in a facilitated pain referral from pressure and saline stimulation and since DOMS requires a minimum of 24 hours to appear [49] the Day-2 was performed approximately 24 hours after the Day-1 session. The protocol for Day-1 and Day-2 was identical apart from the eccentric shoulder exercises which were only performed on Day-1. All assessments were performed with the subjects lying in prone position except when performing the exercise, which was done in sitting.

Assessment of pressure pain sensitivity

A handheld pressure algometer (*Somedic, Hörby, Sweden*) with a 1 cm^2 probe covered by a disposable latex sheath was used to record the PPTs bilaterally at 4 locations in the shoulder region (Fig. 1). The assessment sites were identified using manual palpation using distinct anatomical landmarks: 1) The infraspinatus muscle site was defined as the equidistant point between the medial point of the scapular spine, the inferior angle of the scapula and the midpoint of the medial border of the scapula. 2) The supraspinatus site was found 1 cm above the midpoint of the spine of the scapula. 3) The lower trapezius site was found 4 cm lateral to the spinous process of the seventh thoracic vertebra. 4) The gastrocnemius site was located on the distal third on a line connecting the popliteal line with calcaneus. The sites were marked on the skin with a semi-permanent marker allowing for easy identification in the second session. PPTs at the 8 sites were assessed in random order at baseline, during saline induced pain, and post pain. Regional and distant sites were included in the PPT assessment to account for potential spread in sensitization (regional and widespread). The supraspinatus muscle was included as it has shared innervation with infraspinatus (via the suprascapular nerve) but does not have a primary function as an external rotator of the glenohumeral joint, particularly when the joint is in flexed position [54]. Thus, since supraspinatus does not contribute to external rotation of the shoulder, any potential differences in sensitivity on Day-2 might not be due to tissue effects produced by eccentric external rotations, but rather a central mechanism causing hypersensitivity. The lower trapezius was selected for similar purposes as it shares neither innervation or function with the infraspinatus muscle. The medial gastrocnemius was chosen as a distant site to control for any possible widespread changes in pain sensitivity.

The pressure was increased gradually at a rate of 30 kPa/s until the point where the pressure became painful upon which the subject pressed a button and the stimulation was stopped. This was defined as the PPT and each site was assessed three times at baseline and twice in the ´during´ and

´post-pain´ conditions. A minimum interval of 30 s was kept between assessing each site. The average value of the two or three PPT recordings was used for analysis.

Referred pain evoked by pressure stimulation

Pressure-induced referred pain was evoked by constant pressure stimulation with the pressure algometer (*Somedic, Hörby, Sweden*). In both sessions, pressure pain thresholds (PPTs) were recorded at the infraspinatus muscle and based on these values from each respective day, suprathreshold pressure stimulation (STPS) was calculated as 120%, similar to a previous study using suprathreshold pain stimulations to evoke referred pain [11]. This pressure intensity was selected for two reasons; first, it lies slightly above the pain threshold but can still induce referred pain in healthy volunteers (pilot study, data not shown) and second, the method can be utilized in future studies including clinical populations as it is non-invasive and cause minimal discomfort. The STPS-5s, STPS-60s and the PPT after saline-induced pain were assessed in random order (PPT or STPS, although STPS were always performed in the order of 5-s and 60-s). This was done to reduce the risk of the longer stimulation would sensitize the area. The effect of pressure stimulation was not tested during saline-induced pain as this would have imposed two competing painful stimuli which would have made it difficult for the subjects to accurately report pain intensity and pain referral. The subject drew the pressure-induced pain area on a body chart immediately after the pressure stimulation.

Referred pain evoked by hypertonic saline

Sterile hypertonic saline (0.5 ml, 5.8%) was injected into the mid-portion of the muscle belly of the infraspinatus muscle site on the dominant side using a 1-ml plastic syringe and a disposable needle (27G). Only the dominant side was stimulated to reduce the invasiveness of the protocol as side differences were not expected to be seen. This enabled an investigation of additional effects of hypertonic saline on top of the bilateral DOMS by comparing the dominant and non-dominant sides. The injection depth was measured using real time ultrasound (*Logiq-S7, General Electric, USA*) where the subject was asked to externally rotate the shoulder causing a contraction of the infraspinatus muscle allowing a differentiation from subcutaneous adipose tissue, the muscle and the posterior surface of the scapula deep to the muscle. The needle was angled perpendicular to the skin and inserted carefully until it reached the injection depth previously measured on ultrasound.

The participant indicated the pain intensity on a 10-cm electronic visual analogue scale (VAS) with an external handheld slider. The VAS was anchored with "*no pain*" (0 cm) and "*maximal pain*" (10 cm). The signal from the electronic VAS was recorded continuously (sampling frequency 20 Hz). For analysis, the peak pain (VAS peak) and the area under the VAS-time curve (VAS area) were extracted. The duration of pain was calculated as the difference between the first time the VAS exceeded 0 cm and the last time the VAS was above 0 cm. If no pain was perceived the VAS duration was defined as 0 s. The hypertonic saline injection given on Day-1 was not expected to cause any changes in pain sensitivity on Day-2 based on what has previously been reported [17].

After the saline-induced pain had subsided, the quality of pain was assessed by completion of the Danish [7], English [38], or the Spanish [31] version of the McGill Pain Questionnaire, depending on the native language of participants. Words chosen by more than 30% of the participants were registered for later analysis as previously reported [44,50]. Moreover, subjects were asked to draw the area of pain after the saline injection using a body chart.

Assessing the size and distribution of referred pain

Referred pain evoked from the infraspinatus muscle was chosen as it is easily accessible and commonly used in previous experimental pain studies [27,32,35], and it has been considered the source of symptoms in clinical shoulder pain populations [6,8]. The size of the pain area was extracted using VistaMetrix (v1.38) in arbitrary units (a.u.) after scanning the body charts. Furthermore, the body chart was subdivided into 15 different regions (Fig. 1): 1) The posterior head and neck area from the occipital process above to the cervicothoracic junction below. 2) Supraspinal area; limited by the base of the neck in C7 and the spine of the scapula. 3) Injection site area, overlying the infraspinatus muscle. 4) Posterior shoulder, corresponding to the posterior deltoid muscle. 5) The back area, consisting of the ipsilateral part of the thoracic and lumbar spine below the infraspinatus area. 6) The posterior arm; the area between the posterior deltoid and the line joining the lateral and the medial epicondyles at the elbow. 7) The posterior forearm area was limited proximally by the olecranon and distally by the line joining the radial and ulnar styloid processes. 8) The posterior hand area, comprising the dorsal aspect of the hand. 9) The anterior head and neck area, from anterior craniofacial region, including the anterior part of the neck down to the level of C7. 10) The supraclavicular area overlying the area from the clavicle to C7. 11) The chest area was marked by the sternum medially, the clavicle above, a vertical line from the axilla laterally, and the inferior part of m. pectoralis major below. 12) The anterior shoulder area

corresponding to the anterior deltoid. 13) The anterior arm area was defined as the area between a horizontal line inferior to the anterior deltoid muscle and a line joining the lateral and the medial epicondyles at the cubital fossa. 14) The anterior forearm was limited proximally by the cubital fossa and distally by the line joining the radial and ulnar styloid processes. 15) The anterior hand area, comprising the volar side of the hand. The frequency of pain occurring in each region was used for data analysis. In line with Gibson et al. [11] referred pain was defined as being pain isolated and distinct from the local pain caused by injection or pressure. An expansion of the area of pain beyond the local stimulation region may overlap with what is defined as referred pain, making it difficult to differentiate between the two. Therefore, in this study referred pain was defined as symptoms experienced outside the stimulation region in accordance with what has been done previously [10].

Exercise-induced shoulder pain

At the end of the first session, the subjects performed an eccentric exercise for the external rotators of the glenohumeral joint in the dominant side to induce DOMS to be assessed at Day-2. The exercise was performed in sitting position with the elbow of the dominant arm supported so the glenohumeral joint was in 70-80 degrees of flexion in sagittal plane and the elbow in 90 degrees of flexion as previous findings show maximal activation of the infraspinatus in this position [19]. Subjects were asked to sit upright in a chair with support to the upper body. This position was chosen to minimize compression of subacromial structures [46]. In this position the participants performed repeated shoulder external rotations against the resistance of a firm elastic band which they held in their hand and was fixed to the wall on the other end. When fatigued and unable to actively perform concentric contraction of the external rotators, the subjects were instructed to use the contralateral arm to assist the concentric phase of external rotation and increase the load of the eccentric phase. In this way, although initially concentric contraction was performed, the maximal effort was done in an eccentric way. Four sets of this exercise were completed with 1 minute of rest between sets. Failure in performing the exercise was defined as when the subject was 1) unable to perform the exercise, 2) unable to reach full range of motion, or 3) unable to maintain a stable upper body when performing the exercise. Similar procedure of exercise-induced fatigue has previously been used for inducing DOMS in external shoulder rotators muscles [9]. This method incorporated primarily the external rotators of the shoulders but not other muscles which are normally active during shoulder movement (e.g. m. biceps brachi and m. triceps brachi). Although these muscles are

active during the exercise, they only perform an isometric contraction as no movement occurs around the elbow joint. Therefore, DOMS was only expected to occur in external shoulder rotators muscles. Pain experienced outside the region of the rotator cuff muscles on Day-2 could therefore be determined as being referred pain and not merely an expansion of local pain due to facilitated peripheral pain mechanisms. The following day, the degree of muscle soreness was evaluated using a modified 6-point Likert scale [12] anchored with 0: "a complete absence of soreness", 1: "a light soreness in the muscle felt only when touched (minimal pain)", 2: "a moderate soreness felt only when touched (a slight persistent ache)", 3: "light muscle soreness when lifting objects or carrying objects (a fair amount of pain)", 4: "severe pain, stiffness or weakness when moving the arm", 5: "unable to perform any task or movement due to pain".

Statistics

Data are presented as mean, standard error of the mean (SEM) and for non-parametric data as median and interquartile range (IQR). The referred pain area and frequency of the body areas affected by referred pain did not pass the Kolmogorov-Smirnov test for normality and were therefore analyzed first with Friedman´s analysis of variance (ANOVA) with the Wilcoxon's paired test used post-hoc combined with a Bonferroni correction to account for multiple comparisons. The PPT and VAS data passed the Kolmogorov-Smirnov test for normality and were therefore analyzed with repeated measures ANOVA and T-test, respectively, performing two within-day analyses for Day-1 and Day-2, and another 2-way ANOVA to analyze between-days effects. Repeated factors were "session" (*Day 1 or Day-2*), time (*baseline, during pain, and post-pain*), "site" (*4 sites for PPT assessments*). Gender (*male or female*) was set as an independent factor. This analysis was performed for both the injection (dominant) side and the contralateral side. The Newman-Keul's (NK) test was used for post-hoc comparisons incorporating correction for multiple comparisons. To assess the likelihood of producing referred pain, frequencies of pain in each body region at baseline (Day-1) and at Day-2 were extracted and the odds ratios (ORs) and 95% confidence intervals were calculated. Finally, a correlation analysis was used to investigate a possible relationship between pressure pain sensitivity and pain referral. A statistical significance level of 5% was accepted.

RESULTS

One male subject endured an elbow injury between the two sessions and was therefore excluded from further participation leaving data from 20 subjects being available for data analysis. The mean age was 26 years (range 20-36 years), weight was 69 kg (range 46-88 kg), and height was 173 cm (range 165-195 cm). No differences were found when gender was set as a factor in any of the analysis presented below.

Soreness level provoked by eccentric exercise was registered for the participants in the study. The participants rated the level of DOMS when moving the shoulder using a 6-point Likert scale and reported a pain score of 2 [1-2, IQR] in the shoulder region of the dominant side. In the contralateral side they reported a score of 0. One participant did not develop DOMS according to pain scores after the eccentric exercise although PPT values were lower for that individual. The data was nevertheless included in the data analysis as it was considered to give a more conservative estimate on the measurements on Day-2.

Saline-induced muscle pain model

The following results show the influence of saline-induced pain in both experimental sessions (Day-1 vs Day-2).

VAS-area, VAS-peak and duration of saline-induced pain: No difference was found between days when comparing the VAS-area (27.7 ± 4.1 cm·s in Day-1 versus 24.4 ± 3.5 cm·s in Day-2) or VAS-peak (6.0 ± 0.7 cm in Day-1 versus 6.5 ± 0.7 cm in Day-2) after the hypertonic saline injections. However, the duration of saline-induced pain was longer on Day-1 compared with Day-2 (410.1 ± 23.9 s versus 354.3 ± 30.9 s, *t*-test $P < 0.02$).

Quality of pain: The pain caused by hypertonic saline on Day-1 was described as "taut" (35% of participants) and "heavy" (40%) whereas when injected into the sore muscle (Day-2) the most frequent words were "sharp" (45%) and "pressing" (40%).

Areas of referred pain: Saline-induced pain was felt in multiple areas at varying frequencies (Table 2), but mainly in the anterior and posterior shoulder, the supraspinal, and posterior arm areas (Fig. 2). For Day-1 and Day-2, the majority of subjects perceived saline-induced pain locally and also referred to at least one upper limb region besides the infraspinatus area.

Size of the area of pain: The area of saline-induced pain was larger on Day-2 compared with Day-1 (Wilcoxon: $P<0.01$; Table 3).

Number of pain referral regions (of a maximal of 14): No differences were found on Day-2 compared with Day-1. The frequency of referred saline-induced pain (expanded or referred to any area outside the infraspinatus area) were 75% on Day-1 and 85% on Day-2.

STPS-induced pain model

The following results show the influence of pressure-induced pain in both experimental sessions (Day-1 vs Day-2).

Quality of pain: The pain caused by STPS was described both in Day-1 and Day-2 as "pressing" (45% of participants), "annoying" (35%), and "drilling" (35%)

Areas of referred pain: The STPS-5s stimulation mainly caused pain referral to the anterior and posterior shoulder and supraspinal regions (Fig. 3 and Table 2) whilst the STPS-60s stimulation caused pain referral also to the anterior and posterior arm regions. In some cases, pain was also felt in the anterior and posterior forearm and hand regions.

Size of the area of pain: On Day-1, in the saline-induced post-pain condition, the area of pain was increased for the STPS-5s compared with baseline assessments (Wilcoxon: $P<0.005$; Table 3) whilst no differences were found for the STPS-60s. However, the STPS-60s pressure stimulation produced a larger area of pain than the STPS-5s stimulation at baseline and post-pain (Wilcoxon: $P<0.0001$, Table 3). In contrast, on Day-2, significant differences were only found for the baseline condition for both STPS-5s and STPS-60s (Wilcoxon: $P<0.005$) but not in the saline-induced post pain condition. When comparing Day-1 and Day-2, the STPS-5s produced a larger area of pain on Day-2 than the same STPS-5s on Day-1, at baseline (Wilcoxon: $P<0.005$), whilst no differences were found for the STPS-60s.

Number of pain referral regions affected (of a maximal of 14): On Day-1, in the saline-induced post-pain condition, the number of pain referral regions affected was increased for the STPS-5s compared with baseline assessments (Wilcoxon: $P<0.005$; Table 3), whilst no changes occurred with the STPS-60s. However, the STPS-60s produced pain in more areas than the STPS-5s only at baseline (Wilcoxon: $P<0.0005$, Table 3). On Day 2, the number of affected regions did not change for the STPS-5s or the STPS-60s when baseline and post pain conditions were compared. However, the STPS-60s produced a larger number of affected regions than the STPS-5s at both baseline (Wilcoxon: $P<0.0005$) and saline-induced post-pain condition (Wilcoxon: $P<0.005$).

When compared with the baseline condition on Day-1, the odds ratio for experiencing pain in the anterior shoulder region on Day-2 after 60-s baseline pressure stimulation, was higher (OR: 4.3; 95%CI: 1.6 - 16.3; Table 2).

Comparison of STPS and saline-induced referred pain models

The STPS-5s area was significantly lower at baseline and saline-induced post pain condition, and affected fewer regions only at baseline when compared to saline-induced area at Day-1 (Wilcoxon: $P<0.005$; Table 3). On Day-2, STPS-5s and STPS-60s areas were significantly lower only at baseline (Wilcoxon: $P<0.005$).

Pressure pain thresholds

A significant interaction was found between the factors *time (baseline, during and post-saline)* and *sites (8 locations)* on Day-1 (RM-ANOVA: $F(6,114)=4.44$, $P<0.001$) and Day-2 (RM-ANOVA: $F(6,114)=6.62$, $P<0.001$). The post-saline measurement showed reduced PPTs compared with baseline and during saline-induced pain at the ipsilateral m. infraspinatus (NK: $P<0.0001$ on Day-1 and NK: $P<0.005$ on Day-2), m. supraspinatus (NK: $P<0.001$ on Day-1 and NK: $P<0.005$ on Day-2), and lower trapezius muscle (NK: $P<0.05$ on Day-1) assessment sites (Table 1).

Independent to the assessment time (*baseline, during and post-saline)*, PPTs were significantly reduced on Day-2 compared with Day-1, at ipsilateral m. infraspinatus (NK: $P<0.0005$), m. supraspinatus (NK: $P<0.0005$), and lower trapezius (NK: $P<0.005$) muscle (RM-ANOVA $F(3,57)=5.49$, $P<0.01$). For the contralateral side, no significant changes between Day-1 and Day-2 were found at any assessment site.

Correlation between pain, pressure pain sensitivity and pain referral

On both days, in the post pain condition a correlation was found between the VAS-peak and the pain area (Spearman = 0.52, $P<0.05$) and between the VAS-peak and the number of pain-affected regions (Spearman = 0.48, $P<0.05$). On Day-2, a correlation was found between the saline-induced VAS-area and the size of the STPS-60s area of pain at baseline (Spearman 0.59, $P<0.05$). No correlation was found between pressure pain sensitivity (PPT) and pain referral.

DISCUSSION

This study introduces a novel method for assessing pain referral from skeletal muscle which is sensitive to expansion of referred pain areas due to prolonged muscle soreness. The pressure-induced referred pain provided referred pain characteristics comparable with the classical approach based on hypertonic saline injections. Moreover, the results indicated a time-dependent effect on pain referral patters which was further facilitated by prolonged muscle soreness. The findings demonstrate that suprathreshold pressure-induced referred pain may be a useful biomarker for investigating clinical pain states.

Pressure and saline-induced referred pain

In the present study, healthy individuals with no current or previous history of shoulder pathology participated, suggesting that the pain referral caused by the experimental stimuli on day one is a normal response to a painful stimulus. Early findings have demonstrated that a painful stimulation of musculoskeletal structures may result in a pain area extending beyond the stimulation site [26] which has later been replicated in several experimental and clinical studies [14]. In the current study the size of the pain area, the referred pain frequency and pain intensity were dependent on the duration of the local stimulation, which is in accordance with previous findings [11,15,45] indicating involvement of a central mechanism where integration of the nociceptive input and/or time are essential [41]. When investigating the infraspinatus muscle in particular, the muscle has been shown capable of causing an extensive pain referral pattern in healthy individuals [28,32], similar to the present findings; a response which is facilitated in patients suffering from pain in anatomically non-related areas such as the low back and neck [29,43]. These findings are in line with referral patterns from myofascial trigger points located in the infraspinatus muscle which give similar pain referrals to the shoulder and arm on the stimulated side [52]. Furthermore, by stimulating different elements of the muscle-tendon-bone unit results in varying pain referral [11] but the current findings show that stimulating the same structure in different pain states does the same.

The present results demonstrate that referred pain from a somatic structure is a time-dependent process originating from an intense local stimulus which is in line with previous findings [11,14]. This may relate to the time it takes for nociceptive input to converge actively onto neighboring levels of dorsal horn neurons and an activation of brainstem and supraspinal mechanisms [14]. The sensitivity of these central pain mechanisms seems to play a role in the size

of the painful area but it is less clear whether a sensitized system demonstrates a shorter delay between the onset of a nociceptive stimulus and the onset of pain referral [10].

Saline-induced muscle pain generally induces a delay between onset of pain referral distal to the stimulated site and local pain [14] with a comparable number of affected areas seen after pressure-induced pain referral [11]. This is in accordance with the current findings where no difference was found between the numbers of affected areas from chemical stimulation and 60 seconds pressure-induced pain. Additionally, it is interesting to consider that STPS-5s and STPS-60s show no difference in the size of the area of pain that they produce where the muscle is expected to be in its most sensitized state, which is the post saline-induced pain condition on Day 2. These findings indicate that with sensitized peripheral or central mechanisms, the responses to the different stimuli start to resemble each other.

Expanded referred pain areas due to persistent soreness

The protocol used eccentric training involving primarily the external rotators of the shoulder against resistance which has been shown to cause a local inflammatory response and a systemic up-regulation of inflammatory biomarkers [25]. Pain caused by such a pain model is usually transient, lasting only a few days, but it is worth considering that long lasting peripheral input from nociceptive fibers to the dorsal horn can produce short- and long term neuroplastic changes at this site of the central nervous system [20]. This may involve facilitated synaptic transmission between dorsal horn neurons and descending facilitation of the afferent signals along with impaired central pain inhibition [51]. Thus, tissue injuries may affect the central nervous system in a way that it becomes more susceptible to a new nociceptive stimulus to the region.

The present results indicate pain hypersensitivity at the infraspinatus, supraspinatus and lower trapezius regions in the DOMS condition, suggesting that the exercise only affected the structures related to the shoulder. However, it is interesting to consider that supraspinatus and lower trapezius muscles are not synergists of the infraspinatus in glenohumeral external rotation when the shoulder is in flexion, indicating that the increase in pain sensitivity of these regions on Day-2 may not be explained with tissue affection but rather with an increased contribution of central mechanisms. The applied protocol for exercise-induced soreness predominately induced localized sensitization but a previous study found that it can also result in a facilitated nociceptive withdrawal reflex [23] which is considered indicative of hyperexcitability of the spinal nociceptive system [4]. Likewise, temporal summation to pressure stimulations is known to be facilitated in DOMS-induced pain

[3,11]. Such facilitated central mechanisms are also potentially an underlying cause for the expanded referred pain areas to the same pressure stimulus on Day-2 compared with Day-1. Interestingly, the expanded referred pain areas were demonstrated when adjusting the stimulus intensity to the pressure pain threshold of the day (i.e. the absolute stimulation intensity was reduced on Day-2). Thus, it is assumed that the same relative nociceptive stimulus is provided on the two days, but yet the referred pain area was expanded. As this study wanted to assess the method of pressure-induced referred pain and comparing it with the saline-induced referred pain, the minimal amount of pressure stimulation was used to reduce the complexity and increase tolerability of the method. As a powerful peripherally-driven nociceptive stimulus can lead to a greater involvement of spinal [18] and supraspinal [34] structures, the enhanced pain referral pattern on Day-2 can be explained by the central nervous system being sensitized as a result of the prolonged muscle soreness.

Clinical implications

Assessment of pain sensitivity is considered relevant especially when differentiating between widespread and regional pain hypersensitivity [42]. Somatic structures are capable of causing an extensive pain referral pattern in different patient groups [5,37,49,53] which are commonly known to have increased pain sensitivity in pain-free areas, indicating involvement of facilitated central mechanisms [13,24,47]. The odds for experiencing pressure-induced pain in the anterior shoulder region on Day-2 was increased which is interesting for two reasons. First, this mimics what is found in a clinical population when investigating pain referral patterns from infraspinatus [5] and second this finding demonstrates the feasibility of using suprathreshold stimulation as a surrogate model for investigating pain referral patterns in soft-tissue.

As suprathreshold pressure intensities cause a pain referral pattern comparable to what hypertonic saline does, the utility of it for diagnostic purposes in clinical practice is obvious. However, this study used an experimental pain model to investigate pain referral expansion in healthy subjects and therefore warrants a similar investigation in a clinical population. As discussed above, patterns of pain referral may be a relevant marker for the sensitivity of central pain mechanisms but to enable the clinicians to monitor the irritability and progression of a given musculoskeletal pain condition more effectively, a more thorough assessment of the temporal as well as spatial characteristics of the pressure-induced pain referral pattern would be valuable.

Conclusion

This study introduced a novel method to assess referred pain patterns which is sensitive to expansion of referred pain areas due to prolonged muscle soreness. The results showed similarity between chemically and mechanically induced referred pain patterns which were dependent on the stimulus duration. Assessment of the pain referral pattern includes valuable information regarding involvement of sensitized central pain mechanisms. Further studies including clinical groups are warranted to further understand the usefulness of the methodology in clinical practice.

Acknowledgements

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). The authors have no conflict of interest to report.

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FIGURE LEGENDS

Figure 1. The figure represents 15 pre-defined body regions for quantifying experimental pain expansion for both pressure and saline stimulations. The stimulation site was always in the infraspinatus muscle. Assessment sites of PPT were located bilaterally at the infraspinatus, supraspinatus, lower trapezius, and gastrocnemius muscles.

Figure 2. Pain drawings illustrating the superimposed areas of saline-induced pain on Day-1 and Day-2. Note that 1) injections were always in the dominant side, but pain areas are mirrored when not located on the right side and 2) all individual areas of 20 subjects are included in the figure. See Table 2 for a specific description of the frequency of pain at each region.

Figure 3. Pain drawings illustrating the superimposed areas of pain produced by 5-s and 60-s pressure stimulations to the infraspinatus muscle with referred pain to the shoulder, arm and forearm areas, and performed on Day-1 and Day-2. Each day shows baseline and post-saline pressure-induced pain areas.

Mechanically-induced referred muscle pain was facilitated in size and intensity by persistent muscle pain. Pain referral evoked by suprathreshold pressure stimulation may be an important biomarker for determining sensitivity of central pain mechanisms.

Figure

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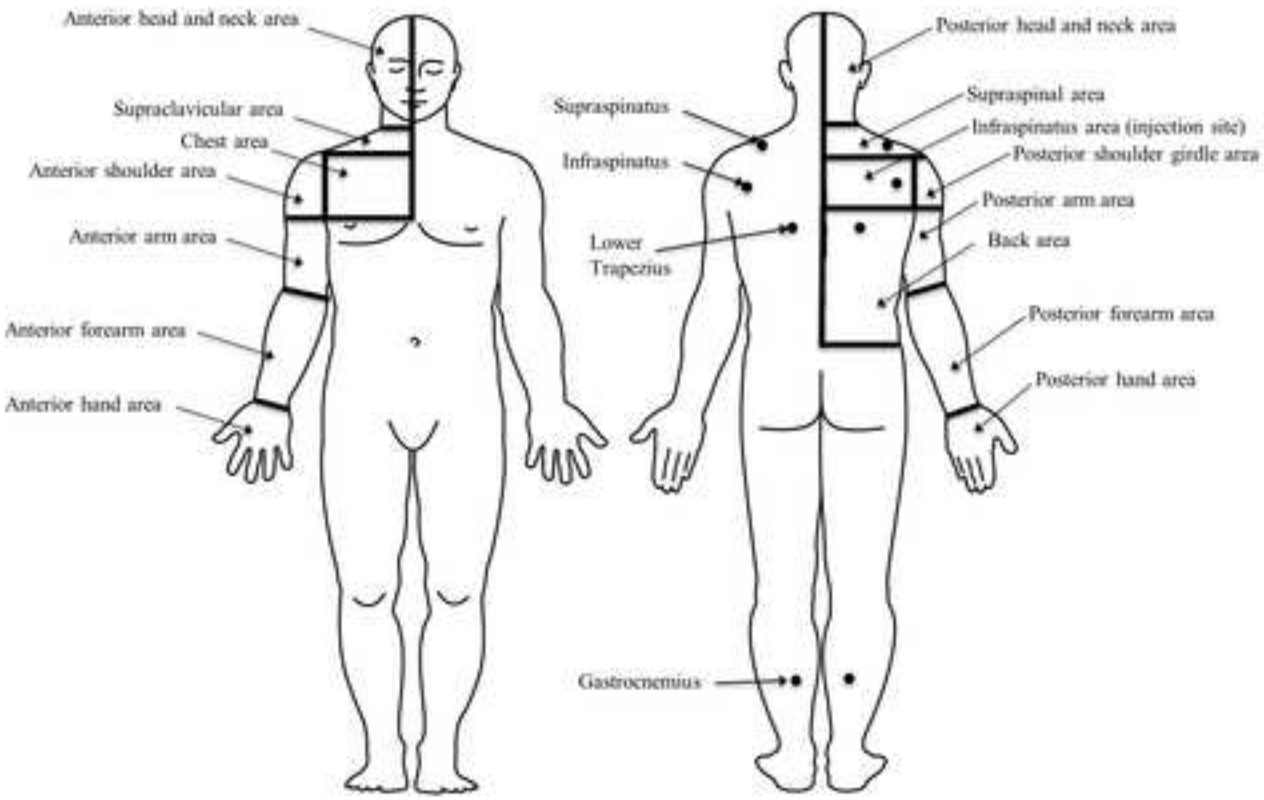

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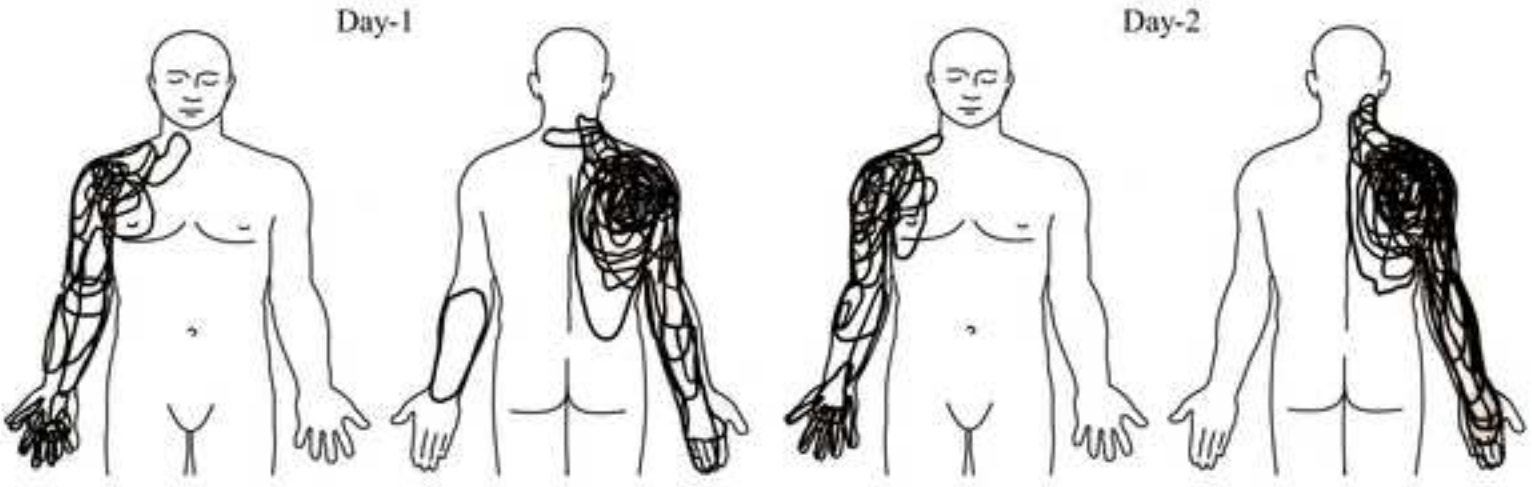

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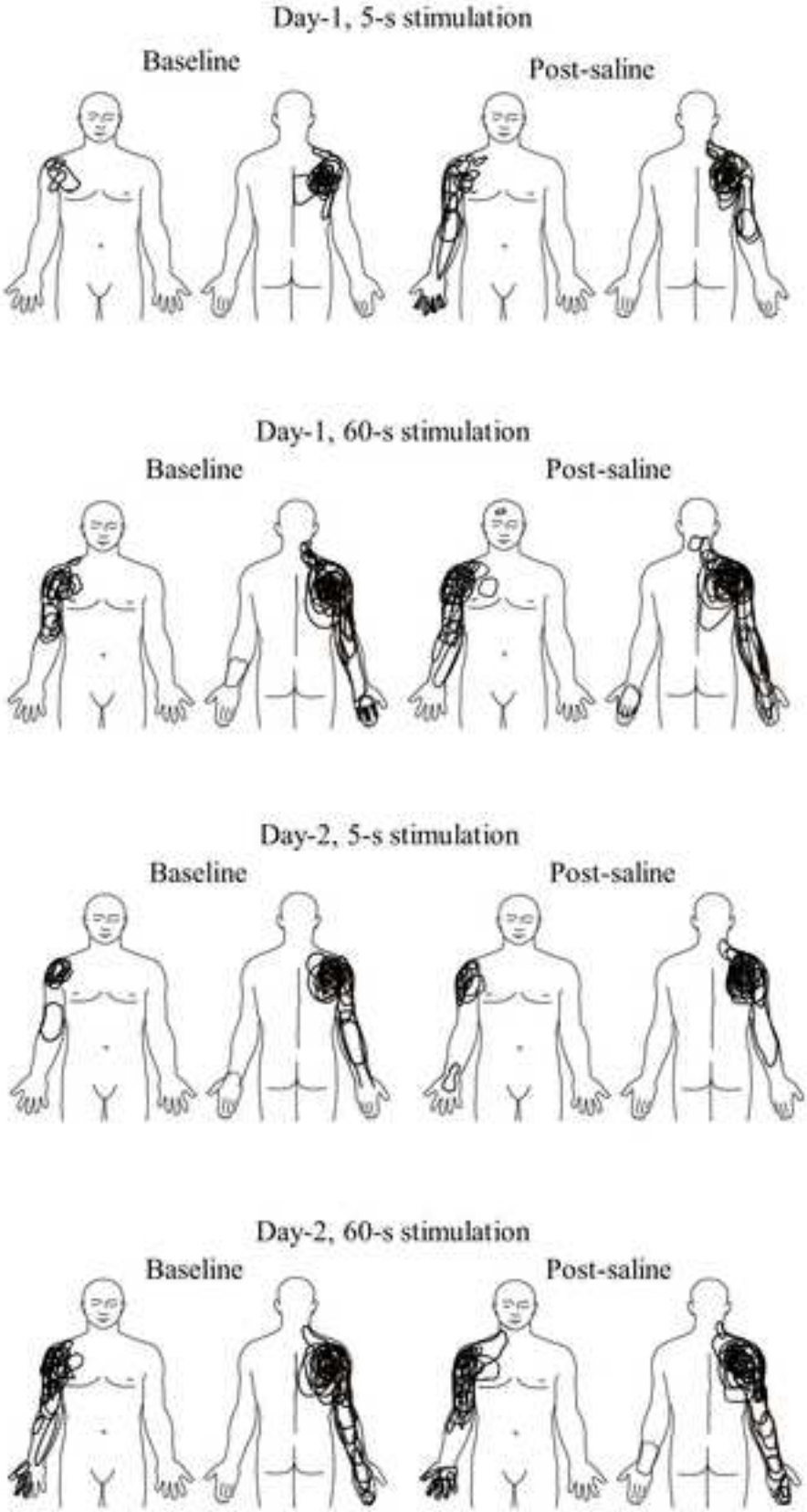

Table

PPT (kPa)		Day 1			Day 2		
		Baseline	During pain	Post pain	Baseline	During pain	Post pain
Ipsilateral	Infrapinatus **	344 ± 24	340 ± 28	224 ± 21*	211 ± 21	248 ± 25	165 ± 21*
	Supraspinatus **	407 ± 23	415 ± 31	331 ± 22*	354 ± 22	331 ± 24	298 ± 21*
	Lower trapezius **	373 ± 34	396 ± 30	344 ± 27*	306 ± 27	358 ± 30	337 ± 23
	Gastrocnemius	377 ±26	406 ±27	352 ± 21	323 ± 21	372 ± 22	346 ± 24
Contralateral	Infrapinatus	316 ± 20	391 ± 21	315 ± 18	267 ± 18	321 ± 22	296 ± 18
	Supraspinatus	375 ± 26	414 ± 22	389 ± 23	352 ± 23	382 ± 19	368 ± 19
	Lower trapezius	364 ± 31	398 ± 24	363 ± 29	341 ± 29	386 ± 24	355 ± 28
	Gastrocnemius	362 ± 27	402 ± 25	359 ± 21	313 ± 21	359 ± 22	356 ± 26

Table 1: Mean (±SEM, N = 20) pressure pain thresholds (PPTs) recorded at baseline, during, and post saline-induced pain on the ipsilateral and contralateral bilateral sides at: m. infraspinatus, m. supraspinatus, m. lower trapezius, and m. gastrocnemius. Significantly different compared with baseline within the same day (*, $P < 0.05$) and between days (**, $P < 0.05$).

Table

Pain region	Baseline pressure stimulation				Hypertonic saline		Post-pain pressure stimulation			
	5 s		60 s				5 s		60 s	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Posterior head/neck	0	0	20	5	10	10	5	5	15	5
Supraspinal area	10	10	35	35	45	60	20	25	35	35
Infraspinatus area	100	100	100	100	100	95	95	100	100	100
Posterior back	5	0	0	10	20	20	0	0	5	10
Posterior shoulder	5	30	30	45	50	60	30	35	35	40
Posterior arm	0	10	25	35	35	40	15	10	20	30
Posterior forearm	0	10	10	15	30	35	10	5	20	25
Posterior hand	0	5	5	10	10	15	0	5	10	15
Anterior head/neck	0	0	0	0	5	0	0	0	5	5
Supraclavicular area	0	0	10	5	15	5	10	0	10	5
Chest area	10	10	15	20	10	20	15	10	15	15
Anterior shoulder	15	30	30	65*	55	65	30	50	45	65
Anterior arm	0	5	20	30	35	35	20	20	25	35
Anterior forearm	0	5	5	20	15	20	10	0	20	10
Anterior hand	0	0	0	5	15	10	5	10	0	15

Table 2: Percentages of participants (N=20) reporting pain in the various regions after suprathreshold pressure stimulation and injection of hypertonic saline into the infraspinatus muscle before (Day-1) and after the induction of delayed-onset muscle soreness (Day-2). Significant Odds Ratio (*, OR 4.3 95% C.I. 1.6-16.3) with respect to the same stimulation on Day-1. Grey color: pain areas with the higher pain frequencies.

Table

	Baseline		Post-pain	
	Area (a.u.)	Number of pain regions	Area (a.u.)	Number of pain regions
Day-1				
Saline	126 [41-281]	3 [2-7]	-	-
Pressure (5 s)	14 [10-44] $^{\#\#}$	1[1-2]$^{\#\#}$	49 [26-115]$^{*,\#\#}$	3 [3-5]*
Pressure (60 s)	67 [25-163]$^{\#}$	3 [2-5.5]$^{\#}$	115 [46-205]$^{\#}$	3 [1-10]
Day-2				
Saline	188 [119-414]**	5 [3-8]	-	-
Pressure (5 s)	58 [30-105]$^{**,\#\#}$	2 [1.5-3]**	66 [44-121]$^{\#\#}$	3 [2-3.5]
Pressure (60 s)	83 [39-196]$^{\#,\#\#}$	4 [3-6]$^{\#}$	108 [33-170]$^{\#\#}$	4 [3-6.5]$^{\#}$

Table 3: The median [IQR] area of pain and number of the regions in which pain was felt by suprathreshold pressure stimulation and injection of hypertonic saline into the infraspinatus muscle at Day-1 and during delayed onset muscle soreness (Day-2). Pressure-induced pain was assessed at baseline and after saline-induced pain had vanished. Significantly different from baseline (*, $P < 0.005$), Day 1 (**, $P < 0.005$), hypertonic saline (##, $P < 0.01$) or comparing 5-s and 60-s stimulation (#, $P < 0.005$). A.u.: arbitrary units.