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Title: Comparison of location, depth, quality and intensity of experimentally induced pain in six low back muscles

Authors: Kylie J Tucker[1] PhD, Matthew Fels[1] BPhy Hons, Scott R Walker[1] BPhy Hons, and Paul W Hodges[1] PhD.

[1] The University of Queensland, NHMRC Centre of Clinical Research Excellence in Spinal Pain, Injury and Health, School of Health and Rehabilitation Sciences.

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Corresponding Author Address:

Dr Kylie Tucker
NHMRC Centre of Clinical Research Excellence in Spinal Pain, Injury and Health
School of Health and Rehabilitation Sciences
The University of Queensland
Brisbane, Qld, 4072, Australia

Tel: +61 7 3365 4590
Fax: +61 7 3365 4567
e-mail: k.tucker1@uq.edu.au

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Abstract

The pattern of pain originating from experimentally induced low-back pain appears diffuse. This may be because: sensory information from low-back muscles converges; sensory innervation extends over multiple vertebral levels; or people have difficulty accurately representing the painful location on standardised pain maps. We aimed to provide insight into the perception of pain from noxious stimulation of a range of low-back muscles using novel depth and location measures. Hypertonic saline (1ml, 7%NaCl) was injected into bellies of longissimus (LO), quadratus lumborum (QL), superficial (SM) and deep multifidus (DM) at the level of the 4th lumbar vertebrae (L4) and in SM and DM at L5, using ultrasound guidance, over 6 sessions. Fifteen participants reported depth, location, intensity, size and descriptive quality of pain throughout the painful period (~14 min). Pain was reported deeper ($P<0.04$) for DML4/5 than SML4/5, LO and QL; more cranial for LO than DML4 and QL ($P<0.01$); more lateral for LO than DML4 ($P<0.02$) and for QL than all other muscles at L4 ($P<0.0001$). Pain intensity was higher in DML4/L5 than all other muscles ($P<0.04$) for ~3 min. Descriptive qualities varied little between muscles. Parameters such as depth and lateral position may be the most critical descriptors to determine the source of acute lumbar muscular pain. Overlapping regions of pain may be explained by convergence of receptive fields, innervation of multifidus fascicles at multiple lumbar segments and convergence of sensory input from different muscles to the same sensory cell bodies as demonstrated in the lumbar spine of animal preparations.

Key words: Low back pain; Hypertonic saline; Lumbar; Multifidus; Longissimus; Quadratus Lumborum

Introduction

Back pain is difficult for patients to localise and many indicate a distribution over a diffuse area of the back, buttock and leg on typical body charts in the context of both clinical back pain and experimental nociceptive stimulation [1-5]. This feature of back pain questions the utility of pain location for identification of the potential pain source. In contrast, studies of other body regions often highlight specific regions of pain with nociceptive stimulation (e.g. elbow [6], knee [7, 8] neck [9, 10]).

Back pain may be difficult to localise for several physiological reasons. First, animal data show convergence of inputs from discrete structures/receptive fields [11] onto single primary sensory neurons (Galea and Hodges, 2007, unpublished data). Hardwired convergence of afferents would make it impossible to differentiate the source of the nociceptive input. Second, sensory receptive fields overlap with sensory innervation extending over multiple vertebral levels, which would also render accurate localisation of pain source impossible. However, in the case of deeper back muscles it has been argued that muscle fascicles arising from a single vertebral level have innervation limited to the nerve root with same number as the segment from which they arise [12, 13]. Thus, differentiation of pain location between fascicles arising from different levels should be possible; but differentiation of pain from discrete fascicles from the same level may not.

Further complicating interpretation of pain location are issues related to extrapolation of the area of perceived pain (from a body region that cannot be seen) to a body chart. In addition to lack of view of the back, extrapolation may also have limited accuracy because the body chart may not represent the participant's own body shape, and difficulties extrapolating from a specific vertebral level to the same level on a body chart. This has been overcome in some studies by use of anatomically-trained participants [4, 5, 14-16], but this limits extrapolation to findings of a naïve patient population.

We aimed to systematically investigate the potential to differentiate the location of nociceptive inputs arising from lumbar muscles fascicles, which differed in terms of vertebral level, depth, lateral placement and innervation. To allow extrapolation of the findings to an anatomically naïve population, participants were not anatomically trained. Results show unique features of acute pain from nociceptive stimulation of discrete structures.

Materials and Methods

Participants

Fifteen healthy individuals (6 females, 9 males; mean(SD) age of 20.9(1.8) years) participated in this study. Individuals were excluded if they reported any history of chronic pain, had a history of neck, back or lower limb pain that had required treatment or time off work, any condition that may affect the nervous system and/or any current pain medication or narcotic use. This study was approved by the Institutional Medical Research Ethics Committee and participants provided written informed consent. All procedures conformed to the Declaration of Helsinki.

Procedure

We investigated whether patients could differentiate between nociceptive stimulation of:

Question i: Muscle fascicles that have similar anatomy (similar depth and location), but arise from adjacent vertebral levels with innervation from different spinal levels [deep short fascicles of multifidus (DM) at L4 vs. L5; superficial long fascicles of multifidus (SM) at L4 vs. L5];

Question ii: Different muscle fascicles at the same vertebral level that are spatially unique in terms of muscle depth and with innervation from different spinal levels [DM vs. SM at L4; DM vs. SM at L5];

Question iii: Separate muscle fascicles with the same innervation (same nerve branch likely to arise from same vertebral level) but with different spatial location in terms of depth [DM at L4 vs. SM at L5];

Question iv: Separate muscle fascicles at the same vertebral level that are spatially unique in terms of placement in the coronal plane (medial to lateral), and with innervation from branches of different peripheral nerves arising from different spinal levels [DM vs. SM vs. quadratus lumborum (QL) vs. longissimus (LO) at L4].

Participants attended six separate sessions for the induction of experimental pain at each of six test locations (Fig. 1A,B). In each session, pain was induced by injection of hypertonic saline into one muscle on the right side (SML4, SML5, DML4, DML5, and QL and LO at L4). Although not replicating features of ongoing clinical pain (e.g. spread of pain to multiple regions through mechanisms such as secondary sensitization) this technique provides information regarding the system's potential to differentiate peripheral sources of acute nociceptive input, which satisfies the aims of this study. The order of which muscles were injected was counter-balanced to minimize the potential effect of any carry-over effects between pain sessions on our data. Up to two sessions were completed within one day with at least 30 min (after cessation of pain) between trials. All trials for an individual were completed within seven days.

In the first session a standardized photograph was taken of the subject's back from the axilla (arm pit) to the gluteal fold, from a height of ~1 m with the subject lying prone. This photograph was printed twelve times in grey-scale on standard A4 white paper for recording location of pain (see *Pain Measurements*, below). The locations of the spinous processes of the lumbar vertebra were determined by palpation and ultrasound imaging (Logic e, GE Healthcare) and were marked with ink on the subject's skin. The skin was also marked with

ink 4 and 8 cm to the right of L4 and then superiorly every 3 cm for a total of 10 locations. The ink remained visible in subsequent sessions. A standardized photograph of the subject's back with the ink marks was taken and printed six times (as above). This photograph, with clear landmarks allowed the investigator to accurately transcribe the area of most intense pain indicated by the participant (see *Pain Measurements*, below: Fig. 1A-C).

Pain was induced by a single 1 ml bolus injection of sterile hypertonic saline (7% NaCl) into the middle of the belly of the target muscle. Injections were performed with hypodermic needles of 23-25G and length of 19-70 mm, depending on the muscle. Prior to the saline injections the vertebral level and location of test muscle were determined using the pre-marked vertebral locations (described above) and the skin was cleaned with an antiseptic swab. The SML4/L5 injection depth was ~19 mm, and LO was ~25 mm, which corresponded with the belly of the respective muscles. The needle was inserted adjacent to the spinous process and perpendicular to the skin. Needle insertion into the bellies of DM and QL was guided by ultrasound imaging [17, 18]. For DM the needle insertion was directed towards the lamina of the corresponding vertebrae. For example, for DM at L4 the ultrasound transducer was placed parasagittally along the spine and the most dorsal relfection of the L4 lamina was identified. At this point the transducer was turned 90° and the location adjusted to optimize the image. The needle was directed towards the lamina within view of the ultrasound image in a similar manner to that described for insertion of fine-wire electrodes [19]. The average depth of needle insertion into mid muscle belly of DML4/L5 and QL was 34.2 (5.0) mm and 36.9 (5.3) mm, respectively (measured using ultrasound in seven participants). Because of the position of the ultrasound transducer relative to the muscle, the needle was inserted ~15º from perpendicular. The subject was not informed which muscle was injected.

Pain Measurement

Pain was reported in six ways. Participants reported these measures while lying prone. All reporting cues were positioned at eye level ~50 cm below a face hole in the table upon which the participant was lying and were clearly visible when needed. Pain intensity was measured with a 10-cm visual analogue scale (VAS) anchored with 'no pain' and 'worst pain imaginable'. Participants marked the VAS with a vertical line at 30-s intervals throughout the painful period. Participants were asked to estimate the size of the painful area by comparing the area of painful sensation with a standardized diagram depicting a series of ten circles that increased in size from 1 to 10 cm in diameter [20, 21].

Given our interest in the ability to report the depth of pain in the lumbar muscles, we developed a method for measurement of perceived depth of pain. A diagram representing the participant's back consisted of concentric semicircles designed to provide a reference. Each equally spaced semicircle band represented 10% of the depth between the surface and centre of the back (e.g. Fig. 2B). The depth of pain was measured on a line from the surface, through the marked point, to the center point of the diagram, and reported as a "% of depth to centre". The average radius from the centre of the trunk to the skin in the posterior midline was estimated to be ~10-12 cm from people of similar weight, and waist diameter to those included in this study, therefore each equally spaced semicircle also represents approximately 1 cm depth.

As it would be expected to be difficult for anyone, particularly people who are anatomically naïve, to associate a specific spinal vertebral level with a specific location on a standardized body chart, we developed a new technique to identify pain location that used a digital photograph of the participant's own back and transcription of the location of most intense pain to the photo as a reference. Participants were asked to point (on their body) with their index finger to the location of their 'most intense pain' [22]. This location was marked

on a standardized photograph of the participant's back by an investigator naïve to the injection site (in some cases the needle insertion point remained visible to the investigator). The location indicated by the participant was marked on the participant's back, then transcribed to the standardized photograph by the investigator using the standardised ink marks for accuracy. This point was then copied onto a 'clean' photograph with no additional marks, and the photograph was provided to the participant to complete the pain drawing by indicating their perceived area of pain with reference to the point they had indicated. Each photograph was scanned, and imported into Matlab (version 7.5, The Mathworks, USA) for analysis. Matlab was used to identify co-ordinates of the location of most intense pain, scaled to the distance between the L4 and L5 spinous processes (as marked on the photograph); and referenced from the L4 spinous process (see Fig. 1B,C).

To determine the quality of the pain, participants indicated all of the words that described their pain on a McGill Pain Questionnaire (MPQ) [23, 24]. Participants recorded the region(s) of referred pain on a 23 cm standardized body chart (that partially replicate the surface anatomy/physical dimensions the participants from the study). This reporting method enables comparison of the presence and location of referral, but may not be ideal for consideration of absolute size of the referred pain area as it requires participants to scale their pain area, which may not be done systematically.

The order of reporting the intensity (VAS), depth, size, location, MPQ descriptors and pattern of referred pain was standardized between participants (Fig. 3).

Statistical analysis

Results were analyzed in two steps. The first compared pain measures for muscles DML4, DML5, SML4 and SML5 to investigate Questions i-iii. The second compared the pain measures for muscles DML4, SML4, LO and QL to investigate Question iv. In both cases, the intensity of reported pain (VAS) was compared with a repeated-measures analysis

of variance (ANOVA) with independent variables of Muscle and Time. The mode of the measure of depth of pain, the location of most intense pain (in the cranial-caudal and medial-lateral directions with respect to L4, separately), and the maximum reported size of pain for each muscle were also compared with ANOVAs, with Muscle as the independent variable. Post-hoc analysis involved Duncan's multiple range tests. Data from the McGill questionnaire were reported as frequency of use of each word. The percentage of participants who experienced referred pain was compared between muscles using a Cochrans Q test and, where necessary, post-hoc pairwise comparisons were conducted using the McNemar test with Bonferroni adjustment of P values (post hoc P value adjusted to $P< 0.007$ for 7 pair wise comparisons). The area of referred pain was presented visually. Significance was set at $p<0.05$. Data are shown as mean (SD) throughout, except as indicated.

Results

All participants experienced pain following hypertonic saline injection, but with variable intensity within and between participants. Two individuals were aware of a mild discomfort (not pain) at the injection site on the day following the QL injection. This resolved within 48 hours. These participants chose not to complete the remaining sessions. No other participants reported increased sensitivity during subsequent testing sessions. Complete data for comparison of muscles at L4 was available for 14 participants, and data for comparison between DM and SM at the same level, and between L4 and L5, was available for 13 participants.

Average peak pain intensity in any muscle was 3.8(1.8)/10 cm (range - 0.6 to 8.1 cm, Fig. 4). Peak pain intensity was reported between 0 and 6.5 min after injection. The longest recorded duration of pain was 14.5 min in one participant following injection into the SML5 and LO. The pain descriptors chosen by participants were similar for all muscles (Fig. 5).

Comparison of DML4, DML5, SML4 and SML5

The intensity of pain varied between multifidus muscles and over time (interaction - Muscle x Time, $p<0.001$). Although depth of pain differed between muscles ($p<0.001$), the location (Fig. 1C: cranial-caudal: $p=0.093$; medial-lateral: $p=0.386$), and size of the painful region (Fig. 1C: SML4: 13.5(8.5) cm^2; DML4: 15.7(9.8) cm^2; SML5: 11.1(11.2) cm^2; DML5: 15.6(10.1) cm^2: $p=0.260$) did not. The percentage of participants (from n=13) who reported referred pain was not significantly different between muscles [$Q = 7.3$, $df = 3$, $p = 0.06$]. In addition to the referred pain reported with nociceptive stimulation of DML5 (54%), DML4 (46%), SML5 (23%), and SML4 (15%), one participant described a distant "numb" sensation that accompanied the local pain in DML5, L4 and SML4 (see grey shaded areas in Fig 6). The incidence and size of the area of referred pain, although not formally quantified, appears greatest for DML5, followed by DML4, SML5 and SML4 (Fig. 6). The size of the area of referred pain, although not formally quantified, appears greatest for DML5, followed by DML4, SML5 and SML4 (Fig. 6).

Question i: Similar muscle fascicles arising from adjacent vertebral levels with separate innervation (DML4 vs. DML5; SML4 vs. SML5)

There was no difference in the intensity of pain at any time when saline was injected into the same muscle (either SM or DM) at adjacent vertebral levels (L4 and L5), [post hoc: DM: both $p>0.42$; SM: both $p>0.50$: Fig. 4A]. The depth of reported pain (Fig. 2A) was not different between adjacent vertebral levels for DM [post hoc: $p=0.75$], but pain in SML5 was reported deeper than SML4 [post hoc: $p<0.001$].

Question ii: Separate muscle fascicles at the same vertebral level innervated as same spinal level (SML4 vs. DML4; SML5 vs. DML5)

Pain intensity (Fig. 4A) was higher in DM than SM at L4 [post hoc: from 0 to 90 s, $p<0.05$], and L5 [post hoc: from 0 to 30 s; $p<0.05$]. Pain was also perceived deeper in DM than SM at both L4 and L5 [post hoc: both $p<0.005$; Fig. 2A].

Question iii: Separate muscle fascicles with the same innervation (DML4 vs. SML5)

Pain intensity (Fig. 4A) was higher in DML4 than SML5 [post hoc: from 0 to 120 s; all $p<0.05$]. Pain was also perceived deeper in DML4 than SML5 [post hoc: $p<0.001$; Fig. 2A].

Question iv: Comparison of separate muscle fascicles at the same vertebral level that are spatially unique (DML4 vs. SML4 vs. QL vs. LO)

The intensity of pain was greater in DML4 than LO (from 0 to 180 s), QL (from 0 to 150 s) and SML4 (from 0 to 120 s) [interaction - Muscle x Time $p<0.001$; post hoc: all $p<0.04$: Fig. 4B]. Pain in DML4 was perceived deeper than in LO, SML4 and LQ [main effect - Muscle $p<0.001$; post hoc: all $p<0.05$; Fig. 2B], more cranial in LO than QL and DM [main effect - Muscle $p=0.009$; post hoc: both $p<0.01$], and more lateral in QL than SM, DM and LO [main effect - Muscle $p<0.001$; post hoc: all $p<0.001$; Fig. 1C]. Pain was also more lateral in LO than DM [post hoc: $p=0.014$]. There was no difference in the size of the pain area between any of the muscles at the level of L4 [$p=0.697$; SML4: 13.5(8.5) cm2; DML4: 15.7(9.8) cm2; LO: 11.7(9.8) cm2; QL: 14.5(1.6.7) cm2].

The percentage of participants (from n=14) who reported referred pain differed between muscles [$Q = 13.0$, $df = 3$, $p < .005$]. A greater proportion of participants reported pain referral following the injections into QL (50%) and DM4 (43%), than SM4 (14%) and LO (7%). Further, a greater proportion of participants reported pain referral from SM4 than LO [all post hoc $P <0.001$]. As described above, one additional participant described a distant “numb” sensation that accompanied the local pain in DML4 and SML4; another participant described “tingling” in the posterior and anterior-lateral thigh during the QL painful period

(see grey shaded areas in Fig 6).

Discussion

The potential for participants to differentiate location of nociceptive inputs arising from muscles fascicles, which differ in level of vertebral attachment, depth, lateral placement and afferent innervation was investigated. Depth and lateral position appear the most useful descriptors to determine the source of acute lumbar nociceptive input between the muscles tested. Descriptive qualities of pain varied little between muscles. The presence of referred pain did not exclude a probable nociceptive source, but frequency and spread of referred pain differed between muscles. Issues related to extrapolation of the area of pain to a body chart, that may arise when using naïve populations with variable body shapes, were overcome by asking participants to point to the painful location, and transcribing this location onto a photograph of the participants' back.

Comparison of pain experiences following nociceptive stimulation of deep and superficial multifidus fascicles at L4 and L5

When nociceptive stimulation was applied to muscle fascicles that are innervated by nerve branches arising from different spinal levels (i.e. DM at L4 and L5; and SM at L4 and L5), none of the reported pain measures provided an indication of which fascicles of the multifidus were the source of pain. The only exception was that pain was perceived deeper for SML5 than SML4, despite the similar anatomical depth of these muscle fascicles. In contrast, the intensity and depth of pain was greater for DM than SM at both lumbar levels (i.e. separate muscle fascicles located at the same vertebral level, spatially unique in muscle depth, with innervation from different spinal levels); and in DML4 than SML5 (i.e. separate muscle fascicles with innervation from the same spinal level and spatially unique in muscle depth).

The medial branch of the dorsal rami of the vertebral level from which the fascicle originates innervates the multilayered fascicles of multifidus [13, 25]. This means the deepest fascicles that lie adjacent to the L4 spinous process but arise from L3 are innervated by the dorsal rami of L3, whereas the most superficial fascicles adjacent to the same spinous process that arise from L1 are innervated by the dorsal rami of L1. The distinct innervation of the multifidus fascicles is likely to explain the ability to discriminate the depth of pain. However, it did not permit discrimination of the source of nociceptive stimulation of DM at adjacent levels. This contrasts evidence of overlapping sensory receptive fields over multiple vertebral levels in superficial but not deep paraspinal muscles [12, 13].

The inability to distinguish the source of nociceptive stimulation between adjacent fascicles of DM (in caudal-cranial and medial-lateral directions) may also be related to the method of noxious stimulation and close proximity of injection sites. The use of hypertonic saline injections to provide localized nociceptive stimulation is well established [4, 5, 14, 26-28]. However, saline can spread through tissue [29] which may have compromised localization of the stimulus. The ability to differentiate between depths of stimuli provides some evidence that the region of nociceptive stimuli was sufficiently different between test locations.

The intensity of reported pain was greater for DM than other muscles in the initial period following nociceptive stimulation. As the volume of saline injected into each muscle was kept constant an important consideration is that the smaller muscle volume and its bony boundary may cause a greater increase in intra-muscular pressure, and additional mechanical nociceptive irritation from the injection, than muscles bounded by soft tissues on all sides. The similar time-course of pain for all muscles implies pressure or reduced diffusion from the muscle is unlikely to explain our results. It is also possible that the density and/or sensitivity of nociceptive afferents differ between tissues. A difference in nociceptive density between

human back muscles has not been shown, but motor points of human limb muscles have greater sensitivity to nociceptive stimuli than other regions of the muscle [30]. Saline injection may have been closer to the motor point(s) of smaller and deeper muscles than the larger more superficial muscles. In addition, animal [31], and human [32] studies show higher densities of nociceptors in connective tissue than muscle, and more tissue (connective and muscle) will have been penetrated during deeper needle insertions.

Comparison of spatially unique muscle fascicles at the same vertebral level

Participants identified differences in the location and intensity of pain when hypertonic saline was introduced into separate back muscles/fascicles with differences in depth, proximity and innervation, albeit at the same vertebral level. The intensity and depth of pain were greater following injection of hypertonic saline into DML4 than LO, QL and SML4. This does not concur with the differences in depth of the injected saline (QL injection was ~3mm and ~15mm deeper than DM and SM/LO injections, respectively). One interpretation is that perception of depth of nociceptive stimulation is less accurate (underestimated) for lateral regions than locations close to the spine.

The medial-lateral and caudal-cranial location of pain was similar for DM and SM at L4. Pain location was reported more lateral for LO and QL than DM (and more lateral for QL than SM). This correspond to the muscles' anatomical location (mid-muscle belly of DM/SM, LO and QL are ~11(1), 22(4) and 75(6) mm lateral the midline of the spine, respectively [33]). Location of pain from LO was also perceived more cranial than DM and QL, despite injection at the same vertebral level. This may be because LO is innervated by intermediate branches of the dorsal rami at multiple lumbar levels [13, 34] and its long muscles fibres extend further in the cranial direction than other muscles.

The ability to differentiate the source of nociceptive stimulation in discrete muscles at a single lumbar level confirms localization of irritation of separately innervated muscles is

possible. However, localization was not observed between some muscles (e.g. LO and SML4). This is consistent with the convergence of afferent inputs (i.e. from LO and SM (Galea and Hodges 2007, unpublished data)), and other sensory afferents [35, 36] to a single primary cell body) and/or branching of free nerve endings [37] as observed in animal preparations.

Pain referral from noxious stimulation of back muscles

Somatic referred pain is common in back pain. Referral of pain, particularly into the anterior pelvis, was possible from noxious stimulation of any of the muscles investigated, although most common following DM and QL irritation. The pattern of referral did not appear to follow dermatomal patterns. Referred pain from L2-L4 and L5 nerve roots is expected to run along the lateral-anterior and lateral-posterior thigh, respectively [4, 38]. Although pain in the present study was unlikely to be caused by nerve root irritation, referred pain/sensation areas did not match those innervated by the same root as the injected muscle: i.e. muscles at L4 were more commonly associated with posterior and lateral thigh pain, and that for L5 in the lateral and anterior thigh (opposite to expected anteroposterior arrangement). This implies a diffuse mechanism for referred pain rather than a simple explanation of "confusion" of the source of inputs to a spinal segment.

Methods for reporting pain location

The new methods for reporting the location of pain in this study provide advantages over those in common use in clinical and experimental work. First, the new depth measure provided one of the most informative parameters of all those included in the study. Most differences in identification of location of nociceptive input related to this measure, and even noxious input arising from closely approximated fascicles of multifidus could be discriminated on the basis of this measure. There was some correspondence between the actual depth of saline injection and the reported depth of pain perception. The depths of

injection of saline into SM, DM, LO and QL were ~19 mm, ~34 mm, ~25 mm, ~39 mm, respectively and the average perception of depth of pain was 10-15%, 35%, 15% and 25% of the diameter of the trunk depicted in the depth measure, respectively. If the average radius from the centre of the trunk to the skin in the posterior midline is ~10-12 cm (estimated from people of similar weight, and waste diameter to those included in this study), this means the perception of depth was a reasonable estimation of the actual depth of nociceptive stimulation.

Second, the use of transposition of an indicated locus of pain to an image of the participant's own back by the investigator has distinct advantage over extrapolation of a perceived location to a black and white drawing by the participant. Using the latter conventional method it is unclear how a participant can accurately indicate a spinal vertebral level. This is because there is no clear reference to spinal level on most pain drawings and people do not know where their spinal levels are located in their body, relative to any specific landmark indicated on the drawing. In most previous work [1, 3-5] pain location is generally shown relative to a very specific spinal level. As this is unlikely to be possible when relying on the participant's extrapolation to the body chart, it would seem that most work involves some adjustment of an indicated pain location to the spinal level that is injected. Our method, which enables accurate indication of the exact location of pain and exact transposition to the spinal level on the body image provides the first evidence that people do not necessarily experience pain at the spinal level that is injected. For example, although needles for injection of hypertonic saline into multifidus at L4 and L5 were inserted into the muscle adjacent to the respective spinous processes, and separated by several centimetres, participants pointed to an almost identical area between the L4 and L5 spinous process (Fig. 1C). Thus, indication of pain at this location does not appear informative of origin of nociceptive stimuli.

Conclusions

The findings of this study provide important insight into the perception of pain in response to nociceptive simulation of a range of back muscles. Although this information reinforces the belief that differential diagnosis of the source of pain in the back is difficult, the data provide four key outcomes of importance for clinical and experimental work. First, the study highlights the diverse locations where pain can be perceived in response to nociceptive stimulation of back muscles and shows likely areas of referral and variation that are not predicted based on dermatomal patterns. Second, new measures of depth of perceived pain and mediolateral location provided greater insight into the site of acute nociceptive stimulation than the traditional body chart, and may assist the clinician to differentiate the source of acute lumbar muscular pain. Third, data confirm that an individual may not experience pain at the spinal level that directly corresponds to the nociceptive stimulation. Fourth, regardless of the region of nociceptive irritation, pain was most often reported between the L4 and L5 spinous process and an indication of pain at this level may not be informative of origin of nociceptive stimuli. In summary, although distinction between muscles as a source of nociceptive input is difficult, this paper provides new measures that can assist this process and highlight specific complexities in this decision-making process.

Abbreviations

LO: longissimus

QL: quadratus lumborum

SM: superficial multifidus

DM: deep multifidus

L4: 4^{th} lumbar vertebrae

L5: 5^{th} lumbar vertebrae

MPQ: Magill Pain Questionnaire

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Fig. 1 (A) Example of the standardized photo from gluteal fold to arm pits. Approximate anatomical landmarks and relative muscle locations have been overdrawn on this figure (not provided during the experiment). (B) The approximate injection site for each muscle is shown (location was confirmed with ultrasound for each subject). (C) Mean (SD) location of most intense pain from the low back muscles for all participants in the caudo-cranial and medio-lateral directions. Pain in longissimus was reported more cranial than quadratus lumborum and deep multifidus L4 ($P<0.01$) and more lateral than deep multifidus L4 ($P=0.014$). Pain in quadratus lumborum was reported more lateral than superficial multifidus L4, deep multifidus ML4 and longissimus ($P <0.0001$).

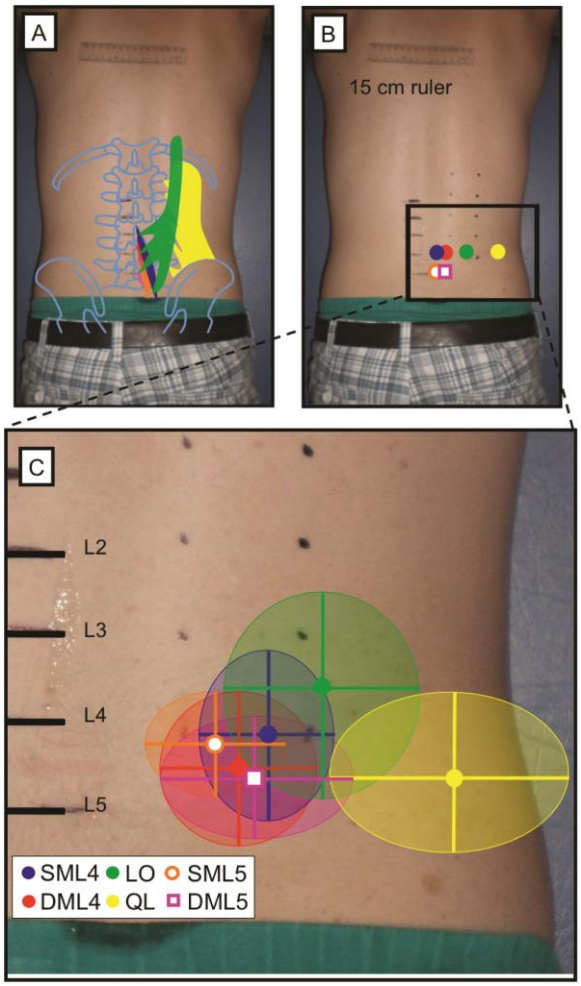

Fig. 2 Depth of pain in the lumbar paravertebral muscles following hypertonic saline injection. Data is laterally displaced for ease of interpretation, but the lateral placement of the mark was not measured from the participant's marks on the depth drawings. Mean (SD) are shown; *P<0.05.

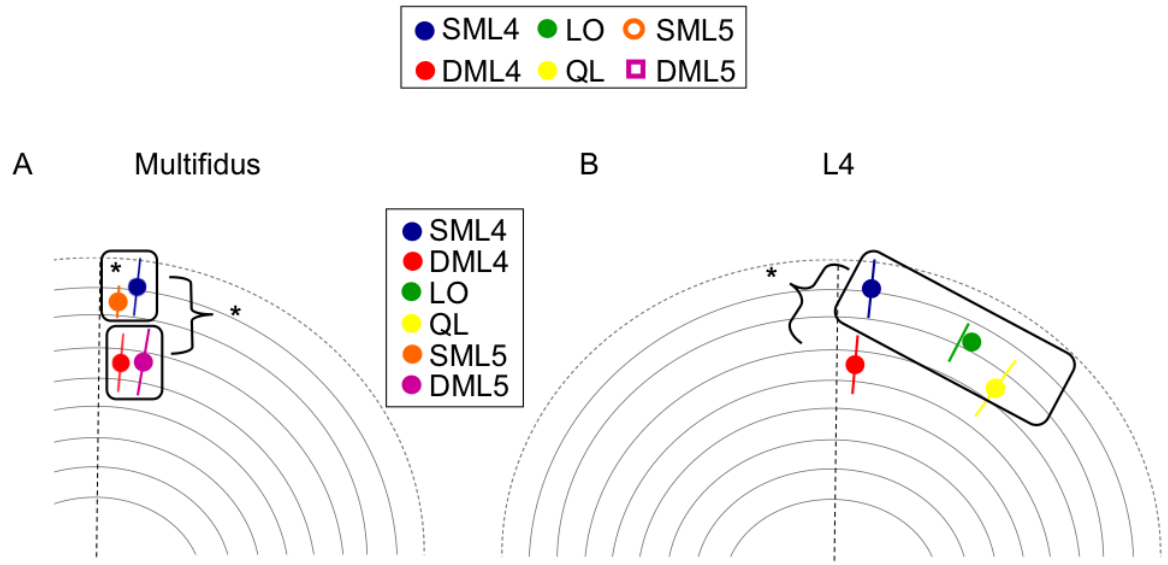

Fig. 3 The timing of measurements during each session. The 60-s cycle was repeated throughout the duration of for which pain was reported by the participant. The Magill Pain Questionnaire (MPQ) and body charts were completed only once after the pain had ceased.

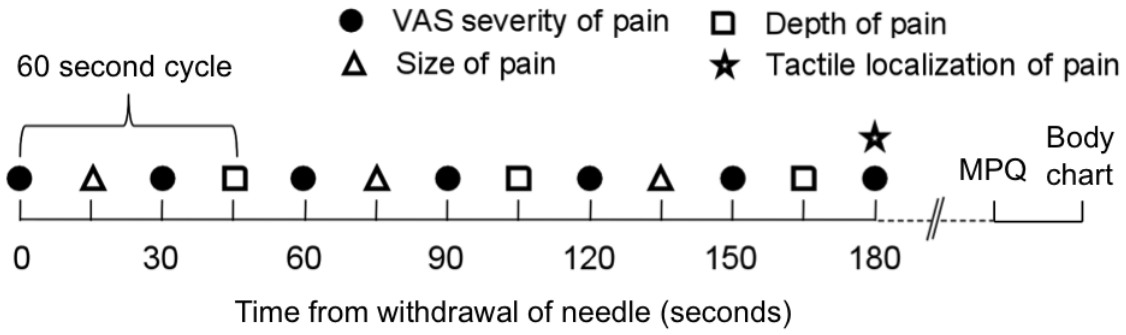

Fig. 4 Time course of pain intensity (mean (SEM)) after hypertonic saline injections. (A) Deep and superficial fascicles of the multifidus muscle at L4 and L5. (B) Muscles at the level of the 4th lumbar vertebrae. *P < 0.05, **P < 0.01. In addition to the differences shown in the figure, deep multifidus L5 was more painful than superficial multifidus L4 (0 – 30s*); and deep multifidus L4 was more painful than superficial multifidus L5 (0 – 90s**; and at 120s*).

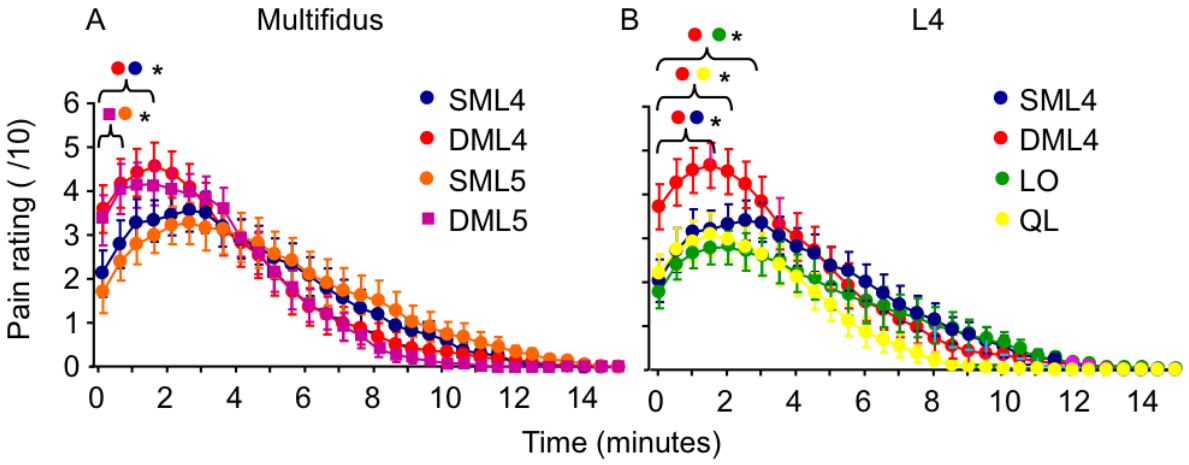

Fig. 5 Percentage of participants that chose McGill Pain Questionnaire qualities (percentage is indicated by the size of the circle relative to 100% shown at bottom left). Only qualities chosen by >2 participants are shown.

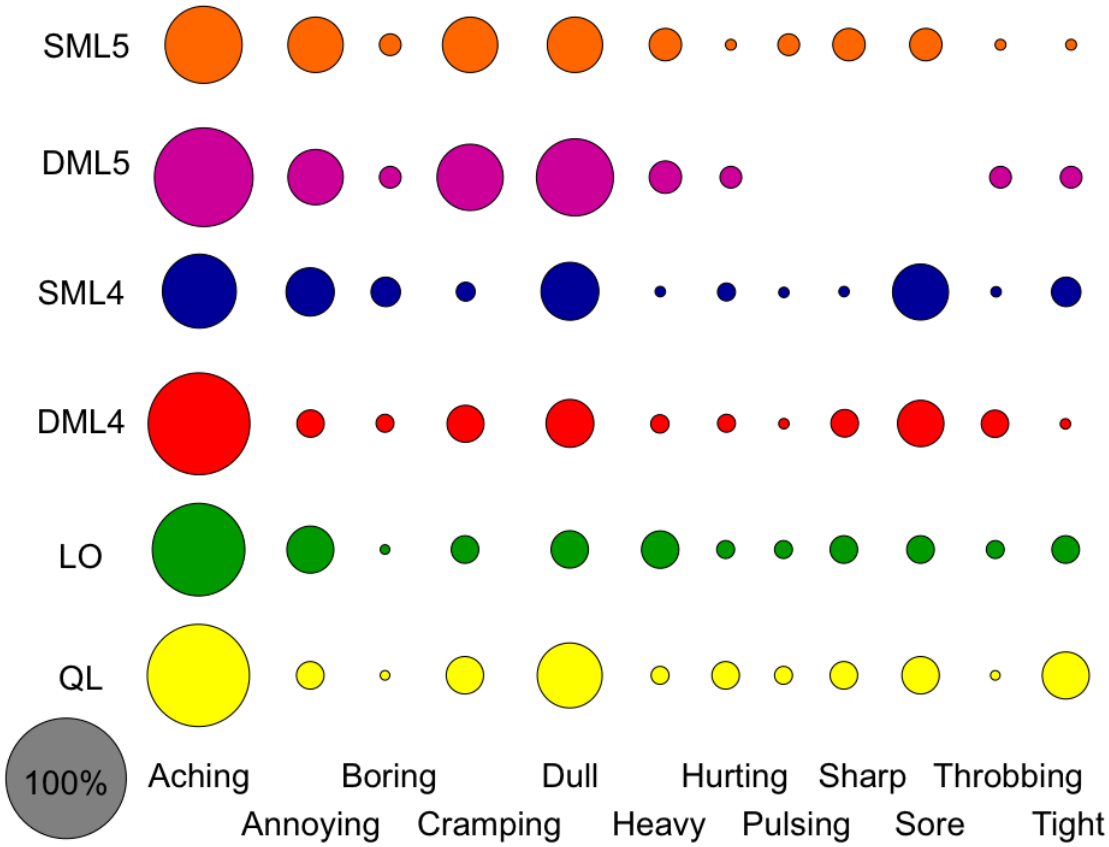

Fig. 6 Location of local and referred pain during each experimental session for all participants as reported on the standardized pain map. Each outline represents the pattern of referred pain experienced by a single subject, in some cases more than 1 area was indicated (by an individual subject) and each location is represented. The areas highlighted in grey were described as tingling or numb but not painful.