Experimentally-induced low back pain from hypertonic saline injections into lumbar interspinous ligament and erector spinae muscle

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RUNNING TITLE

Experimental low back pain

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ABSTRACT

Injection of hypertonic saline into back muscles or ligaments can induce acute low back pain (LBP). However, no study has systematically investigated pain characteristics from these structures. Further, induced muscle pain can change with stretching and contraction, which is problematic for studies into the effect of pain on sensorimotor control. However, it is unclear whether this occurs with experimental ligament pain. In separate sessions, ten healthy volunteers received a single bolus injection of hypertonic (0.2 ml, 5% NaCl) or isotonic saline (0.3 ml, 0.9% NaCl) into L4/5 interspinous ligament, or hypertonic saline into the left paraspinal muscle. Pain intensity, size and duration were recorded, and a body chart was completed for each injection. Changes in pain intensity and size with stretching or back muscle contractions were also assessed during muscle and ligament pain. Injection of hypertonic saline into the interspinous ligament produced central LBP that was longer in duration and greater in intensity and size compared to hypertonic saline injection into lumbar paraspinal muscles. Isotonic saline injection into the interspinous ligament yielded mild pain that was short-lasting (< 2 min). Intensity and size of muscle pain reduced with stretching and contraction, whereas these tasks did not affect ligament pain. Surprisingly, some participants pointed to a location of pain that was 1-2 segments above or below the injected level. The results highlight that injection into the interspinous ligament elicits consistent pain that is not influenced by trunk movements. These findings support the implementation of this experimental ligament pain model in research.

KEYWORDS: low back pain, hypertonic saline, interspinous ligament, experimental muscle pain

INTRODUCTION

Injection of hypertonic saline solution into the paraspinal muscles of the lumbar spine can induce acute low back pain (LBP) [12]. It is argued that hypertonic saline triggers depolarisation of small diameter nociceptive afferents [9; 14] and thus provides standard activation of the nociceptive system. Numerous studies have used this experimental pain model to examine the sensory and motor effects of spinal pain [1; 11; 23]. However, data from the forearm muscles suggest muscle pain induced by injection of hypertonic saline can change with contraction and stretching of the painful muscle [5]. Even with infusion or repeated injections, it is our experience that sufficient pain intensity from the back muscles is often difficult to maintain [11; 18]. Further, there is some limited evidence that hypertonic saline can excite other efferent and afferents neurones, including the large-diameter motor axons, which may be disadvantageous for studies of muscle function, even if there is slightest possibility of directly affecting these motor axons [20]. We investigated whether injections into other non-muscular structures may provide a more consistent model of acute LBP.

Acute pain can be induced by hypertonic saline injection into other non-muscular structures including the fat pad [2], tendon [8], periosteum [13] and ligament [13; 17]. At the spine, the interspinous ligament provides an accessible non-muscular structure for injection. Several studies have demonstrated that injections of hypertonic saline into the interspinous ligaments of the thoracic and lumbar spines can induce acute back pain [13; 17]. However, there is debate on the location of induced pain. Kellgren [13] showed that injections of hypertonic saline into the interspinous ligament induce lateralised spinal pain with referral to the buttock and leg. In contrast, Sinclair et al [17] utilised radiography to ensure the accuracy of injections and observed localised central back pain with minimal lateral referral. Others have reported consistent reproduction of localised pain but found variability in the presence and location of referred pain [10]. No study has systematically investigated the spatial and

temporal characteristics of experimentally-induced LBP from injection of the interspinous ligament, how this compares with pain from that of the back muscles, or whether these pain characteristics can be influenced by stretching or back muscle contraction.

Hypertonic saline injections into tissues induce an osmotic effect; that is, an influx of fluid into the injection site occurs to balance the change in salt concentration [9]. As the interspinous space is relatively small, it is possible that pain from hypertonic saline injections into this region may result from increased pressure rather than chemical activation of nociceptive fibres. Higher volume of isotonic saline can be injected to control for this effect. The current study aimed to investigate the characteristic of experimentally-induced pain from injection of hypertonic saline into the interspinous ligament, to compare this with pain from injection into the paraspinal muscles, and to examine the effect of stretching and back muscle contractions.

METHODS

Participants

Ten healthy volunteers (4 females and 6 males) with no history of back or pelvic pain were recruited (Age 23 ± 5 years (mean ± SD), height 175 ± 10 cm, weight 69 ± 14 kg). Participants were excluded if they had any major circulatory, orthopaedic, neurological or respiratory conditions, recent or current pregnancies or previous surgery to the abdomen or back. All procedures were approved by the Institutional Medical Research Ethics Committee and conformed to the Declaration of Helsinki.

Procedures

Participants were positioned in right side-lying on a custom-designed plinth with their arms folded, hips flexed to ~45° and knees flexed to ~90° (Fig. 1A). Pillows were placed

under the head and between the knees for comfort. The plinth consisted of two halves connected by a low friction hinge such that horizontal movement of the top half of the plinth was allowed. The L4/5 interspinous space was aligned to the hinge to maximise trunk flexion and extension movements at this spinal level. This interspinous space was identified through palpation and verified using ultrasound imaging.

Saline injection

All subjects attended four testing sessions separated by at least five days and received one of four injections per session in random order:

1. Hypertonic saline solution (5% NaCl) into the L4/5 interspinous ligament: The needle (25 G x 25 mm) was introduced through the skin with a slight superior angle to a depth where the tip of the needle contacted the inferior edge of the L4 spinous process (Fig 1B). Hypertonic saline solution (0.2 ml) was injected at this location.
2. An injection of 0.2 ml hypertonic saline (5% NaCl) into the L4/5 interspinous ligament with stretching and contraction tasks.
3. Isotonic saline solution (0.9% NaCl) into the L4/5 interspinous ligament: The needle was introduced in the same manner as hypertonic saline injections to L4/5 interspinous ligament. However, a greater volume of isotonic saline solution (0.3 ml) was injected compared to hypertonic saline injections to emulate the increased pressure in the interspinous space that would occur due to osmosis from injection at higher concentration of saline.
4. Hypertonic saline into the left lumbar longissimus muscle adjacent to L4/5 interspinous space with stretching and contraction tasks: The location and depth of the muscle was confirmed through palpation and ultrasound imaging. The needle (25 G x 38 mm) was

introduced perpendicular to the skin into the muscle, and 0.2 ml of hypertonic saline solution was injected [11] (Fig. 1C).

Subjects were instructed that they would receive an injection into either the muscle or the ligament of the back and that it may or may not induce pain. Each injection was delivered slowly over ~10 s.

Pain measures

Pain intensity was measured on a 10 cm electronic visual analogue scale (VAS) anchored with "no pain" at 0 and "worst pain imaginable" at 10. VAS data were sampled at 100 Hz and recorded using a Micro1401 Data Acquisition System with Spike2 software (CED, UK). Subjects were reminded to indicate the intensity of pain every 20 s. The duration of pain was also obtained and defined as the time period from the onset of pain to the cessation of induced-pain (i.e., where reports of pain had returned to zero). In addition, subjects were requested to indicate the size of the pain by selection of a series of ten circles (from 1 cm diameter to 10 cm diameter; [2]). Subjects were asked to rate the size of their pain every minute. At the end of each testing session, subjects drew their pain distribution on a body chart. As it is difficult for participants to associate their own spinal level to that on a body chart, they were first asked to point with their index finger to the location of their pain. The corresponding spinal level was marked on a body chart by the researcher and subjects traced the region of their perceived pain relative to this level. To assess the quality of pain, the short-form McGill Pain Questionnaire (MPQ) was also completed [15]. Subjects selected from a list of words that best described their pain, and rated each selected word on a three-point scale as (1) mild, (2) moderate or (3) severe.

Stretching/contraction tasks

The influence of stretching and contraction of the back muscles on pain intensity and size was also examined during experimental pain induced by hypertonic saline injection into the ligament and the injection into the muscle. This was performed immediately after the pain VAS had either plateaued or began to decline. Participants were informed that these tasks may or may not alter pain characteristics. Participants were reminded to report pain every 20 s. Three repetitions of stretching of the posterior structures of the lumbar spine and contractions of the paraspinal muscles were completed in random order, with 20 s rest between each. Participants rated the intensity and size of pain prior to and immediately after each trial of stretching or contraction.

For the stretching task, subjects remained relaxed and were slowly flexed to ~45° flexion by the researcher via movement of the plinth. This position was held for ~10 s. For the contraction task, subjects performed resisted isometric extension against a fixed cable attached anterior to the top half of the plinth. Subjects gradually extended their trunk to match a pre-determined force that was ~15% of their bodyweight. A strain gauge measured the force of trunk extension. The target and exerted force was provided to the subject as feedback through a computer monitor (Sampling rate: 100 Hz). Once the target force was reached, the contraction was held for ~10 s. To examine whether subjects contracted the back muscles to a similar level during ligament and muscle pain, electromyographic (EMG) activity of the back muscles (lumbar longissimus) were recorded bilaterally using surface electrodes positioned ~5 cm adjacent to the L4/5 interspinous space (Disposable Ag/AgCl electrodes with inter-electrode distance of 2 cm; Noraxon USA). EMG data were amplified 1000 times, band-pass filtered between 20 to 1000 Hz and sampled at 2000 Hz.

Data Analysis

For all injection conditions, the pain intensity (VAS) was averaged each minute. This averaged pain intensity and the size of pain were plotted against time. The mean and peak of the intensity (VAS) and size of pain, and the sum of these measures across time (i.e. measure of the total area under the pain curve) were calculated. As stretching and contractions may influence pain intensity/size, measures during these tasks were not included in the calculation of the sum during these sessions. Instead, the course of pain intensity and size were assumed to be linear from the time point before to the time point after the completion of three stretching/contraction tasks. For the MPQ, scores for each nominated word were summed across subjects for each condition. The three most frequently nominated words (greatest summed scores) were selected to describe the quality of the induced pain. In addition, images from the body charts were scanned and cropped (resolution: 553 x 634 pixels). The traced region of pain was identified and the area of pain (as distinct from the size of pain which was selected from the series of circles) for each injection condition (in pixels) was calculated using a custom program in Matlab 7 (The Mathworks, USA).

For stretching and contraction tasks, it is possible that changes observed may be related to natural decline in pain with time rather than due to performance of these tasks. To investigate this, we calculated changes in pain VAS and size during the task (i.e., difference between pre- and post-task), and changes between tasks where participants were at rest (i.e., difference between post-task and the subsequent pre-task). In addition, the root-mean-square (RMS) of the raw EMG from the back muscles during the contraction tasks was calculated over a 5 s window, immediately after the target angle was achieved. This was averaged across the three repetitions for both ligament and muscle pain conditions.

Statistical Analysis

Statistical analysis was performed using Statistica 7 (Statsoft, USA). The peak, mean and sum for the VAS and size of pain were compared between each injection condition using repeated-measures analysis of variance (ANOVA). The area of pain from the body chart was also compared between Conditions using repeated-measures ANOVA. For stretching and contraction tasks, changes in pain VAS and size were compared between Time (changes during stretching or contraction vs changes at rest) and Task (stretching vs contraction) for ligament and muscle pain conditions using repeated-measures ANOVA. The amplitude of back muscle EMG was compared between the ligament and muscle pain conditions using repeated-measures ANOVA. Post-hoc testing was undertaken using Duncan's multiple range test. Significance level was set at $p<0.05$.

RESULTS

Pain Measures

Fig. 2 shows pain intensity and size plotted against time. Injection of hypertonic saline into the interspinous ligament induced the greatest peak pain VAS and largest pain size compared to isotonic saline injection into the ligament or hypertonic saline injection into the muscle (Main effect: Condition: $p<0.001$; post-hoc: $p<0.001$; Fig. 3). This pain was described as "aching" (n=10; summed MPQ=19), "sharp" (n=8; summed MPQ=15) and "throbbing" (n=7; summed MPQ=12). No differences in pain intensity and pain size were observed between the two sessions of injection of hypertonic saline into the ligament (post-hoc: $p>0.22$). Hypertonic saline injection into paraspinal muscle induced greater pain VAS and size compared to isotonic saline injection into the ligament (post-hoc: $p<0.010$; Fig. 3). Most subjects described pain from the back muscle as "aching" (n=8; summed MPQ=17) "cramping" (n=8; summed MPQ=16) and "throbbing" (n=7; summed MPQ=15). The

duration of induced LBP from hypertonic saline injection into the ligament (pain duration without stretching/contractions: 10.1 ± 2.9 min; pain duration with stretching/contractions: 10.7 ± 2.4 min) was longer than that into muscle (pain duration 6.5 ± 2.7 min; Main effect: Condition: $p<0.001$; post-hoc: $p<0.001$). Isotonic saline injection to interspinous ligament induced minimal pain with comparatively shorter duration (pain duration 2.8 ± 0.4 min; post-hoc: $p<0.001$), and was described as "sharp" (n=10; summed MPQ=12), "stabbing" (n=7; summed MPQ=10) and "shooting" (n=7; summed MPQ=8).

Hypertonic saline injection into the paraspinal muscle resulted in pain that was located unilaterally on the side of the injection site (Fig. 4A). Injections of either isotonic or hypertonic saline into the ligament induced central LBP, except in one subject, in whom hypertonic saline injection into interspinous ligament produced right-sided pain consistently across two separate sessions. Furthermore, the area of pain was larger when ligament was injected with hypertonic saline compared to isotonic saline solution (Fig. 4B; Main effect for Condition: $p<0.001$; post-hoc: $p<0.001$). No difference was observed for the area of pain on the body chart between both sessions of hypertonic saline injection into the interspinous ligament (post-hoc: $p=0.39$). In contrast, injection of hypertonic saline into the left paraspinal muscle produced unilateral LBP that was similar in the area of pain compared to injection of hypertonic saline into the interspinous ligament (post-hoc: $p>0.075$).

Stretching/contraction tasks

Fig. 5 illustrates changes in pain intensity and size with either stretching or contraction, or in between these tasks when participants were at rest. For ligament pain, changes in pain intensity and size following stretching or contraction were not different to that at rest (Pain VAS: Main effect for Time $p=0.08$ and Task $p=0.10$; Pain size: Main effect for Time $p=0.13$ and Task $p=0.52$). However, pain intensity and size induced by hypertonic

saline injection into the paraspinal muscles were reduced by a greater extent by both stretching and contraction of the painful muscle compared to the natural changes during the rest period (Pain VAS: Main effect for Time $p<0.001$, Interaction for Time x Task $p<0.001$, post-hoc $p<0.001$; Pain size: Main effect for Time $p<0.001$, post-hoc $p<0.001$). In addition, there was no difference in RMS EMG amplitude of the longissimus muscles during extensor muscle contraction between the ligament (left: 17.9 ± 9 μV, right 19.6 ± 7 μV) and muscle pain conditions (left 17.7 ± 7 μV, right 18.1 ± 7 μV; Main effect for Condition: $p=0.30$). This suggests that the paraspinal muscles were activated to a similar intensity between the two pain conditions.

DISCUSSION

These data show that hypertonic saline injection into the interspinous ligament of the lumbar spine induces LBP that has a greater intensity and size, and is longer in duration than a similar volume of injection into the back muscles. Experimentally-induced ligament pain was largely confined centrally and did not modulate in intensity or size with stretching or contraction of the adjacent paraspinal muscles. The longer duration of pain and the lack of modulation by stretching or contractions suggest that hypertonic saline injections into the interspinous ligament may be a more ideal model for future research, particularly to study the effects of pain on muscle activity and function.

Injection of hypertonic saline into tissues induces an osmotic effect that increases fluid build-up and mechanical pressure. Given the relatively small volume of the interspinous ligament, it could be argued that increased pressure could explain the pain from injection into this structure. This possibility was not examined in previous studies [13; 17]. The exact increase in volume or pressure from osmosis following hypertonic saline injections remains unknown. Here we accounted for this fluid build-up by injection of 50% greater volume of

isotonic saline compared to hypertonic saline. The low levels of pain and short duration suggests that increased fluid pressure within the interspinous ligament from the injection and the related osmotic effect cannot explain the induced-pain from hypertonic saline injections. Furthermore, it is possible that pain induced by saline injections into the ligament is associated with a more acidic environment. However, as the pH of both hypertonic and isotonic saline solutions used in the current study were in a similar range (pH 4.5-7), it is unlikely that differences in pain intensity are related to variability in acidity of saline solution. Nevertheless, this may warrant further investigation.

Findings of centralised LBP following injection of hypertonic saline into the interspinous ligament of the lower lumbar spine support previous observations for the thoracic and upper lumbar spine [17]. However, this contradicts earlier observations of unilateral referred pain extending into the lower limb [13]. The method of injection in the latter study involved slight movement of the needle laterally to one side of the interspinous ligament until lateralised pain was felt by the subject. Thus, it is likely that this technique may have predominantly injected into the adjacent back muscles, which can produce referred pain into the lower limb [6; 19]. In contrast, the current study involved injection into the interspinous ligament of the lower lumbar spine, which is thicker than that of the thoracic or upper lumbar spines. In addition, the use of ultrasound imaging allowed confirmation of the location and anatomy of the interspinous ligament prior to needle insertion. Thus, we are confident that the potential for lateral deviation of the needle tip and hence injection into adjacent structures was minimised.

The current study involved an alternative method to evaluate the level of pain due to the difficulty expected for participants to extrapolate their spinal levels to a diagram. For this method, participants pointed to the location of their pain, which was then marked on the body chart by the researcher. We argue that this better orientates the subject to the body chart

figure and minimises the problem of identification of anatomical landmarks. However, despite this method, the level of reported pain was 1-2 segments away from the injected spinal level. This is not surprising as spatial acuity in the back is relatively low compared to other regions such as the hand or face [7], and this is associated with the density of sensory receptive fields [4]. In addition, structures in the lumbar spine including the interspinous ligament and paraspinal muscles are innervated by converging afferents from multiple spinal levels [3]. Thus, it is expected there should be a poor ability to accurately discriminate sensory information in the back. The poor accuracy of identification of the painful level was not evident in previous studies [6; 12; 13]. The absence of inaccuracy of patient reporting is surprising as earlier studies introduced the addition of error by expecting participants to identify spinal levels on a body chart. It suggests that those studies may have involved correction or normalisation. Thus, our study provides the first depiction of variations in the location of experimentally induced LBP.

When the concentration and volume of hypertonic saline is controlled, pain induced from the interspinous ligament was greater in pain intensity and size and longer in duration compared to injection into the adjacent muscles. Greater pain intensity and size from the interspinous ligament may be related to greater density of free nerve endings in this structure compared to that of the paraspinal muscles [3; 21; 22]. Previous studies that involved hypertonic saline injection into the paraspinal muscles have commonly injected a greater volume, which may induce greater pain intensity than that observed in the current study. Shorter duration of pain in muscle may be due to greater vascularity, increased rate of diffusion and/or other effects such as accommodation. Furthermore, it is possible that stretching and contraction tasks during muscle pain may have contributed to its shorter duration. These require further investigation.

The results show that pain induced by saline injection into the muscle is modulated with stretching or contraction of the painful muscle, which is consistent with previous findings from limb muscles [5]. Intensity and size of pain induced by injection into the interspinous ligament did not change during stretching of the ligament or contractions of the adjacent muscles (which would tend to compress the interspinous ligament). Notably, even when stretching and contraction movements were performed during ligament pain, the duration of induced-pain did not reduce compared to the ligament pain without stretch and contraction.

The reduction in pain intensity during stretching and contracting when pain was induced in the muscle pain could be explained by several mechanisms. It is possible that stretching or contraction may distract the subject's attention away from the induced-pain, hence result in a reduction in the intensity of pain perceived. However, if this was the case, changes in pain size and intensity should also be observed during ligament pain. Alternatively, stretching or contraction could activate large diameter afferents that may inhibit nociceptive afferent input through processes such as the gating of pain [16]. However, this would also be expected to influence pain from ligaments. Thus, the most plausible explanation for changes in pain intensity and size may be changes in the rate of diffusion of salt out of and water into the tissue during contraction and stretching manoeuvres. That is, as muscles are more vascularised than ligaments, it is possible that contraction or stretching could increase the rate of diffusion of salt out of and water into the muscle. This would reduce the saline concentration more rapidly and reduce the intensity, size and duration of induced-pain. In contrast, as ligaments are less vascular, the potential to influence the rate of diffusion of water into this structure could be limited. Further work is needed. Regardless of the exact mechanisms, the consistency of induced-pain from hypertonic saline injection into the

interspinous ligament suggests that it may be a better model to study effects of pain on sensorimotor function than injection into the paraspinal muscles.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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Fig. 1: *Experiment setup* showing patient position (A), injection site for interspinous ligament (B) and injection site for the paraspinal muscles (C). Note arrow and cross indicate location of injection on body chart. White circle represents the L5 spinous process.

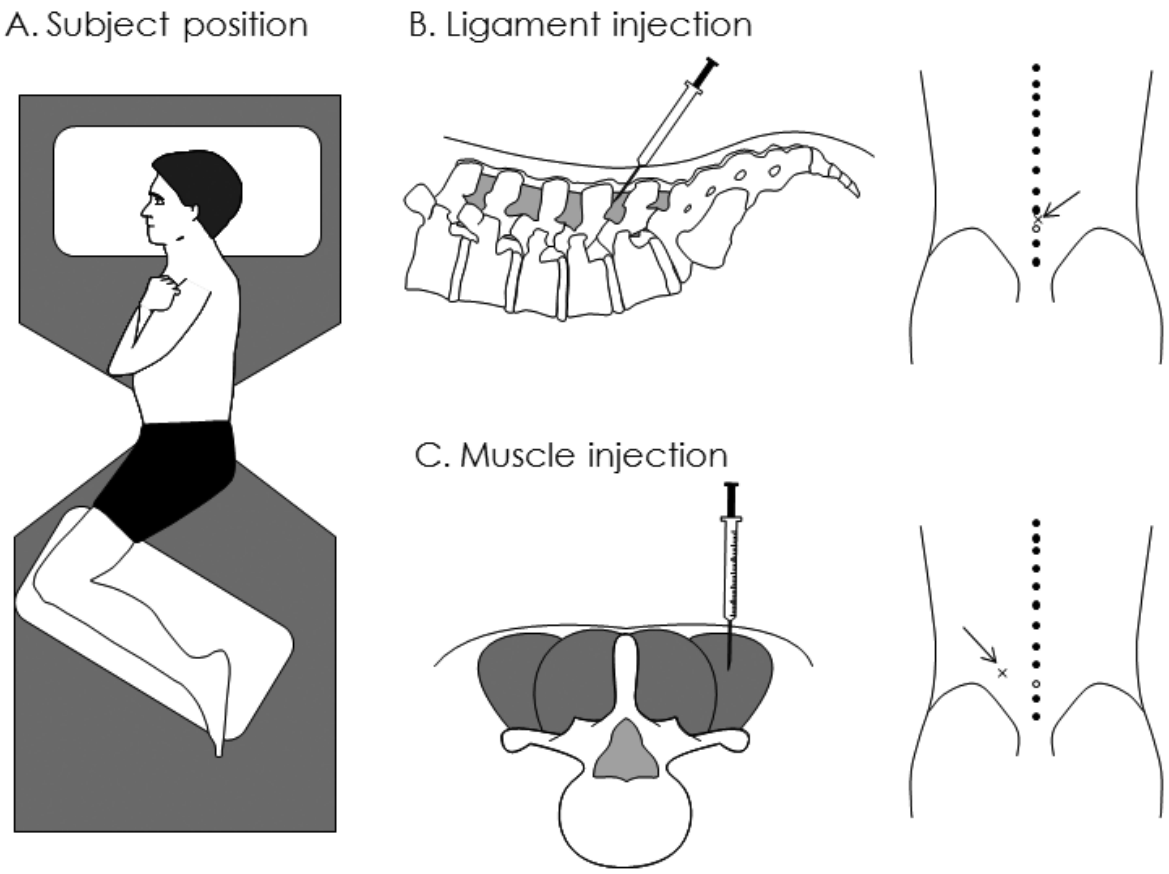

Fig. 2: Group mean and standard deviation of pain intensity and size plotted against time following ligament pain without stretching/contraction (Lig1) and ligament pain with stretching/contraction (Lig2), hypertonic saline injection into the paraspinal muscle (Muscle) and isotonic saline injection into the interspinous ligament (ISO). Note missing data for Lig2 (3-4 min) and Muscle conditions (4-5 min) due to stretching/contraction tasks. Lig1 and Lig2 induced greatest pain intensity and size, and showed the longest duration compared to muscle pain or isotonic saline injection.

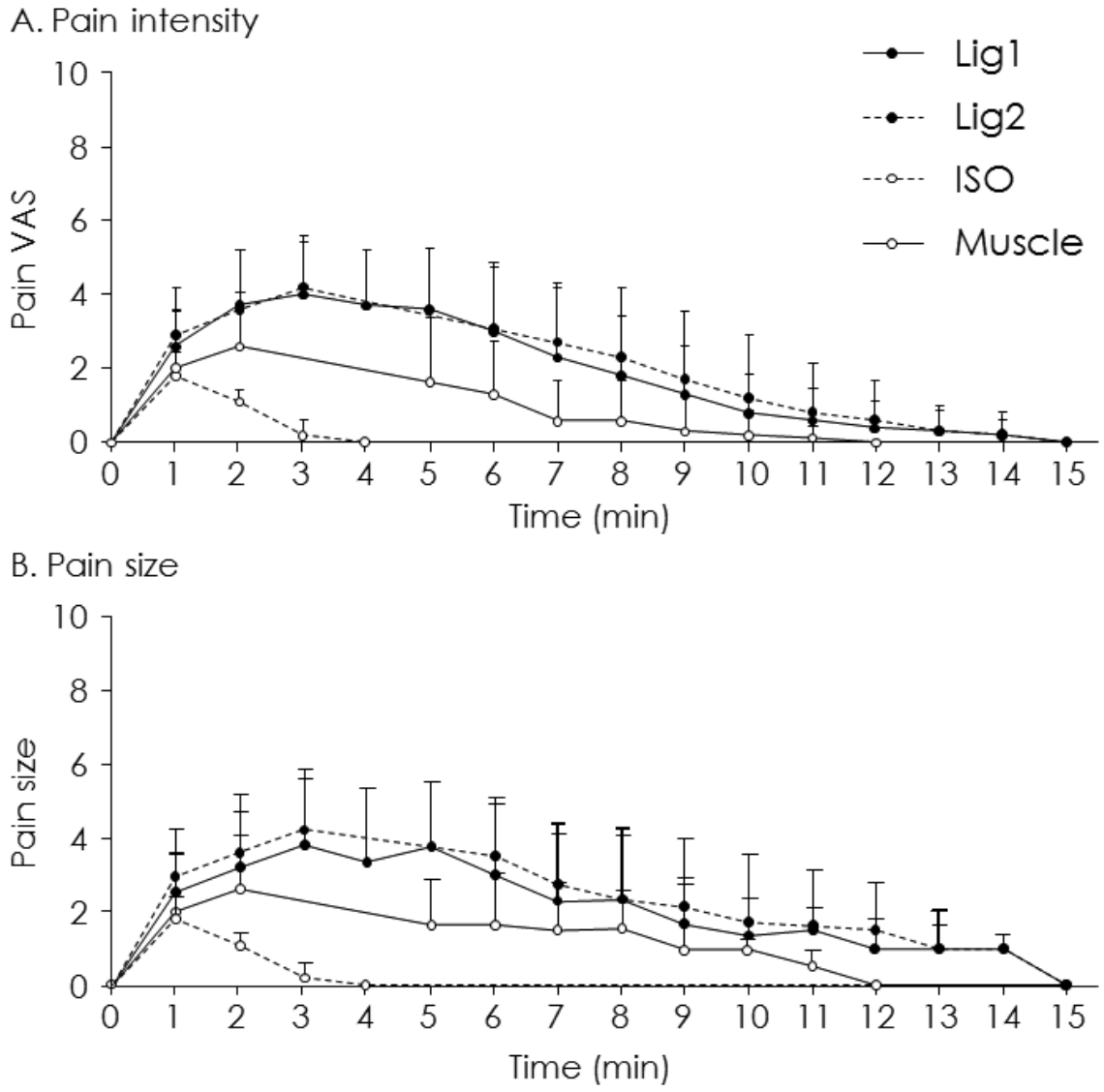

Fig. 3: *Peak, mean and sum of pain VAS (left panels) and size (right panels)* for injection of hypertonic saline into interspinous ligament without (Lig1) and with stretching/contraction tasks (Lig2), isotonic saline injection into the interspinous ligament (ISO) and hypertonic saline injection into the left paraspinal muscle (Muscle). Mean and 95% confidence intervals are shown. Note highest pain VAS and size were observed during Lig1 and Lig2 conditions. Asterix (*) indicates significant different (p<0.05) between bracketed items or compared with all other conditions.

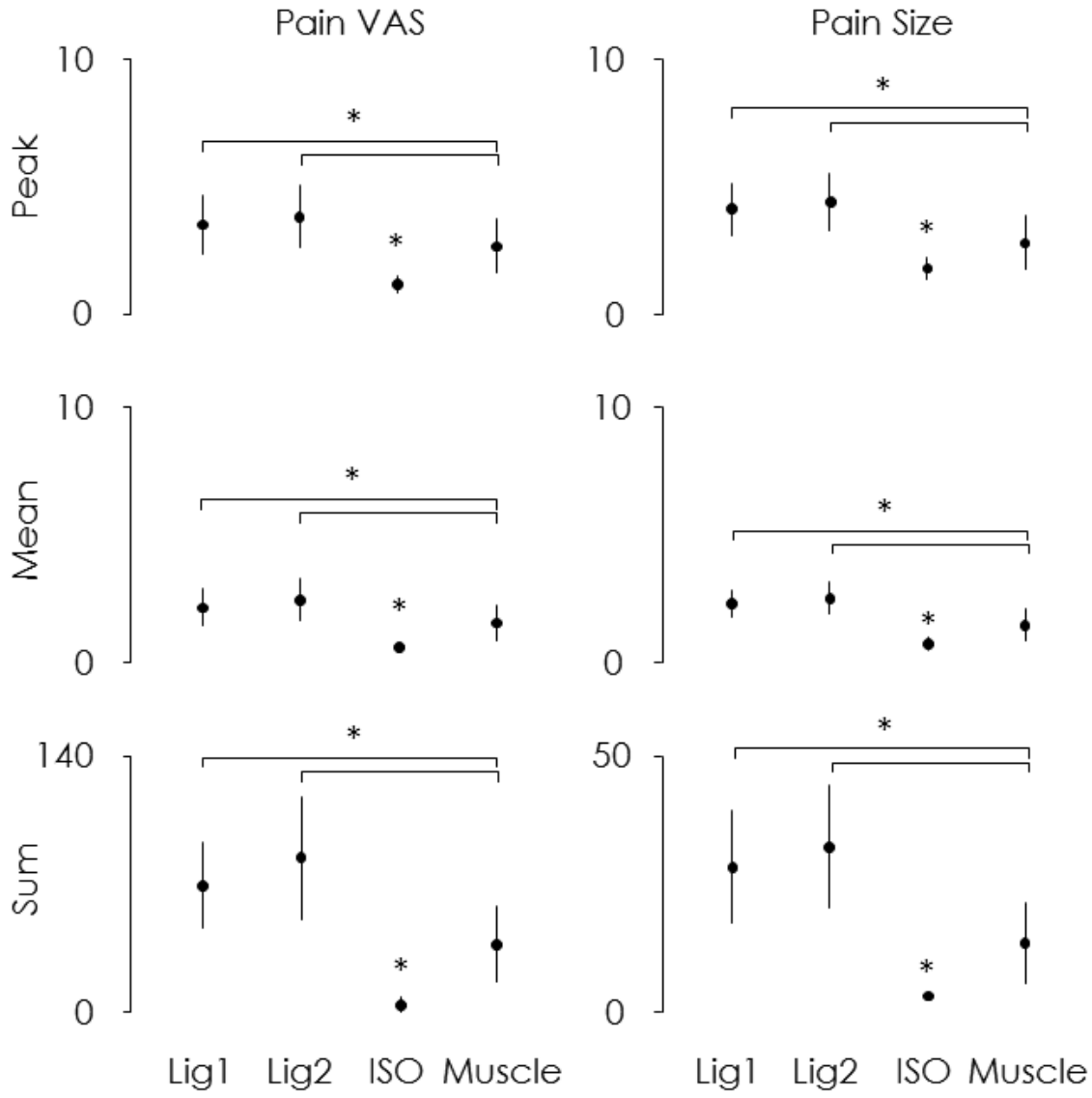

Fig. 4: *Body chart showing individual location of pain (A) and group data for area of pain (B; mean and 95% confidence interval) across the four injection conditions.* Note hypertonic saline injection into the interspinous ligament (Lig1 and Lig2) induced larger area of pain compared to hypertonic saline injections into the back muscle (Muscle) or isotonic saline injections into the interspinous ligament (ISO). * $p<0.05$.

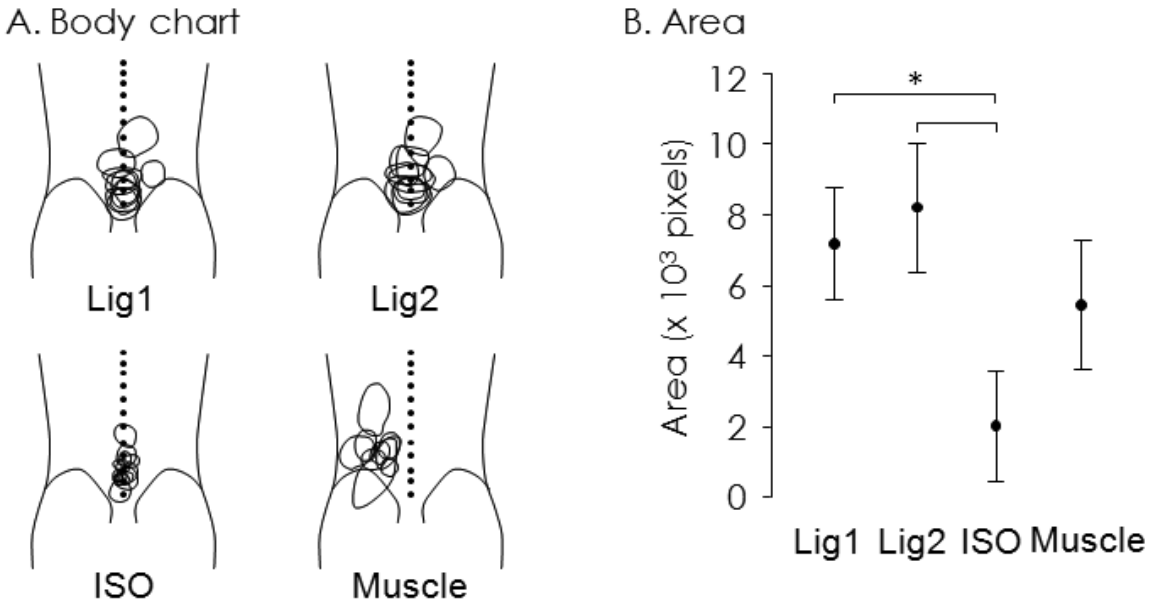

Fig. 5: *Changes in pain visual analogue scale (VAS) and size during stretching (S) and contraction of the back muscles (C), or at rest (R).* Horizontal dotted line represents no change, positive values denote a reduction in pain and negative values denote an increase in pain. Mean and 95% confidence interval are shown. Note reductions in pain VAS and pain size during these manoeuvres were greater than that at rest for induced muscle pain. * $p<0.05$.