Acupuncture sensation during ultrasound guided acupuncture needling

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Abstract

Background Although acupuncture sensation (also known as de qi) is a cornerstone of traditional acupuncture therapy, most research has accepted the traditional method of defining acupuncture sensation only through subjective patient reports rather than on any quantifiable physiological basis.

Purpose To preliminarily investigate the frequency of key sensations experienced while needling to specific, quantifiable tissue levels (TLs) guided by ultrasound (US) imaging.

Methods Five participants received needling at two acupuncture points and two control points at four TLs. US scans were used to determine when each TL was reached. Each volunteer completed 32 sets of modified Southampton Needle Sensation Questionnaires. Part one of the study tested sensations experienced at each TL and part two compared the effect of oscillation alone versus oscillation + rotation.

Results In all volunteers, the frequency of pricking, sharp sensations was significantly greater in shallower TLs than deeper (p = 0.007); the frequency of sensations described as deep, dull and heavy, as spreading, and as electric shocks was significantly greater in deeper TLs than shallower (p = 0.002). Sensations experienced did not significantly differ between real and control points within each of three TLs (p > 0.05) except TL 4 (p = 0.006). The introduction of needle rotation significantly increased deep, dull, heavy sensations, but not pricking and sharp sensations; within each level, the spectrum of sensation experienced during both oscillation + rotation and oscillation alone did not significantly differ between acupuncture and control points.

Conclusion The preliminary study indicates a strong connection between acupuncture sensation and both tissue depth and needle rotation. Furthermore, the new methodology has been proven feasible. A further study with an objective measurement is warranted.

INTRODUCTION

When determining how to define acupuncture and quantify the dose of acupuncture needling, acupuncturists focus on acupuncture sensation (often referred to as de qi; we choose to use the term ‘acupuncture sensation’ to avoid any potential misinterpretation). Acupuncture sensation, a core concept in traditional acupuncture theory, is traditionally believed to be the action of gaining control of the flow of vitality in the body (qi) using acupuncture needles. Early classic Chinese medical texts state that an acupuncturist will know when the correct sensation has been reached when he/she feels the needle grasp; thus, the early definition focused on the acupuncturist’s perception. Yet, the term has, over time, come to connote the patient’s perspective as reported by several texts in the early 20th century, where patients report sensations of numbness, heaviness and deep soreness following acupuncture needling.

Despite this, acupuncture sensation has been inadequately investigated by the scientific community. While a limited body of research and conflicting results are certainly factors, a more significant barrier to progress in this field is the continued dependency on the classical notion of acupuncture sensation without adequate critical evaluation of the term’s validity or applicability. Since the seminal study by Vincent et al., much of the current research has stagnated on the single research focus of subjective needling sensations; most literature on the subject involves creating sub-subjective sensation scales. This focus limits scientific investigation of the physiological basis for acupuncture sensation and results in a disregard for anatomical structure. There is a pressing need for a scientifically based re-evaluation of acupuncture sensation, a rigorous investigation into its anatomical basis, and the quantification of needling techniques to improve acupuncture research and standardise acupuncture clinical practice.

A few studies have attempted to illuminate a potential physiological basis for needling sensations. A 1985 study found that certain relevant sensations corresponded with the activation of A8 fibres, while more recent studies used fMRI to study the attenuation of signal intensity displayed at multiple levels of the cerebro-cerebellar and limbic systems during acupuncture sensation but not during acute pain, the role of individual differences in studying reactions to acupuncture using fMRI, and the different patterns of activation.
and deactivation in the brain elicited during acupuncture sensation and acute pain. Nevertheless, the body of research on an anatomical basis remains insufficient.

Concerning needling techniques, several recent studies have concluded that deep needling is crucial for acupuncture sensation and is a more effective analgesic than superficial needling. On the other hand, Japanese acupuncture, which inserts needles superficially to depths just below the skin, is also said to elicit acupuncture sensation (hibiki). Other studies have found no difference in neural responses or therapeutic effect between shallow versus deep needling.

While this confusion certainly warrants further investigation, this review also highlights the lack of investigation into needle manipulation. Only a few studies have measured the mechanical parameters of needling techniques. Langevin et al. first established that the biomechanical phenomenon of needle grasp in acupuncture is measurable. Follow-up studies found that needle rotation increases pull-out force through connective tissue winding, which modifies the biomechanical behaviour of soft tissue.

The lack of an established definition for needling has made it difficult to design a proper control. Although several control methods have been developed for acupuncture, debate continues since the definition of acupuncture needling and the level of acceptable inertness of control methods have not been unequivocally resolved. Thus, a rigorous investigation into the physiological basis for acupuncture needling sensations will help illuminate the mechanisms of acupuncture needling and lead to the creation of better controls. Our study pairs a sensation questionnaire with ultrasound (US)-guided needling to create better controls. Our study pairs a sensation questionnaire with ultrasound (US)-guided needling to specific, quantifiable tissue levels (TLs). This article reports the findings of a preliminary study.

METHODS
Five healthy volunteers (three men and two women, aged between 28 and 36 years) were recruited in Chapel Hill. Inclusion criteria were being aged between 18 and 55, and having an education level of high school graduation or higher. Exclusion criteria included: a history of diabetes, having an education level of high school graduation or higher, being pregnant, suspected or known; would be taking medications such as narcotics or psychiatric medicines during the study; or had an insurmountable fear of acupuncture. Volunteers taking anti-inflammatory or antihistamine medications were asked to discontinue use 3 days before testing. Testing was not scheduled during menstruation to avoid possible discomfort due to the cessation of anti-inflammatory medication. The Institutional Review Board at the University of North Carolina at Chapel Hill approved this study.

This preliminary study consisted of two steps. Step 1: characterise the spectrum of sensation experienced during US-guided needling of four anatomical TLs (dermis, fascia, intramuscular) at acupuncture and non-acupuncture points (figure 1). Step 2: compare the effect of oscillation technique (vertical movement of needle approximately 5 mm at the frequency of 1 Hz) alone versus oscillation + rotation (approximately 360°) technique (corkscrew-like movement of needle approximately 5 mm deep at the frequency of 1 Hz) at skin penetration (TL 2) and perimuscular fascia penetration (TL 3) (figure 2). Although practitioners also use rotation alone, for this study we selected the two most frequently used simple stimulations. See figure 3 for a schematic of the study flow. The study team comprised an US specialist, a study acupuncturist and a study director. Each enrolled volunteer participated in one testing session, including both steps, lasting in total 1–2 h, during which time a total of four points (LI13 and LU4, on the lateral side of the upper arm (figure 4) and their corresponding non-points, 3 cm away from the classical meridians) on the body received acupuncture needling. There were four TLs tested in this study (figure 5): TL 1, needle pressed but not inserted into the skin surface; TL 2, needle inserted to the lower border of dermis; TL 3, needle inserted around 2 mm beyond the first perimuscular fascia; and TL 4, needle inserted around 15 mm beyond the first perimuscular fascia (figure 6).

Upon consent, we randomised subjects with a coin flip to see which side of the body would be needled first at which order of depth, either from level 1 to 4, or level 4 to 1. For the second experiment (LU4 and corresponding non-point), the randomisation determined which side would be needled first and the depth of TL (TL 2 or TL 3). This randomisation also served to eliminate the possible effect of a building-up of sensation. The order of needle technique was fixed, always oscillation first followed by oscillation + rotation.

Then, the acupoints were identified and marked by the study acupuncturist (JP) based on traditional methods. The approximate position was determined in relation to anatomical landmarks (eg, bones, tendons) and proportional measurements (eg, fraction of the distance between wrist and elbow creases). Within the area delineated by these landmarks, the precise position of each acupuncture point was determined by palpation, feeling for a slight depression or yielding of tissues. For each location, the right and left sides of the body were then randomly selected for acupuncture point and control point. On the side selected for the control point, a disk-shaped template 3 cm in radius was centred on the points to be acupunctured. The control point was marked on the perimeter of the disk at a 45° angle from the acupuncture point’s meridian and as far as possible from the nearest bone and joint. Each acupuncture point was therefore paired with a corresponding control point on the opposite side of the body. The term ‘acupuncture/control location’ is hereafter used to refer to a corresponding pair of acupuncture and control points. Throughout testing, subjects were neither told nor were able to see or hear any indication of which side was used for each point (acupuncture point and control point).
For each insertion at each point, a new sterile, disposable needle (Seirin, Shimizu, Shizuoka, Japan) 40 mm in length and 0.25 mm in diameter was used.

**Ultrasound imaging procedure**
A live US image guided the investigator to locate the correct needling depth. For each acupuncture/control location, target needle insertion depth relevant for the target level was determined based on US measurement of the lower border of the dermis and perimuscular fascia. With US imaging, the dermis is a visible gray area underneath the thin, white epidermis layer, and the perimuscular fascia is distinguishable as an echogenic line separating two tissues of different echogenicity and compressibility (subcutaneous tissue vs muscle) (figure 5). US imaging was performed with an LOGIQ e US machine (GE Health Care, Wauwatosa, Wisconsin, USA) equipped with a 6–12 MHz linear array transducer. The transducer was always held perpendicular to the skin. In order to disinfect the needled area during the US, we used Surgilube sterile surgical ointment (Fougera, Melville, NY, USA) while using Aquasonic US transmission gel (Parker Laboratories, Fairfield, NJ, USA) for the initial US scanning. Between test points for each subject, the transducer was disinfected by submerging in isopropyl alcohol for 30 s. Between subjects, all parts of the instrument that came in contact with the subject’s skin were disinfected.

**The Southampton Needle Sensation Questionnaire**
The Southampton Needle Sensation Questionnaire (SNSQ) consists of 17 sensation adjectives organised into...
two factors: aching sensations and tingling sensations. Patients were asked to record which of the sensations were experienced and the intensity on a scale of 0–3 (0=none; 1=slight; 2=moderate; 3=intense). The questionnaire can be completed in minutes, is user-friendly and has been shown in testing to be capable of measuring acupuncture sensations.30 For our preliminary study, we modified the SNSQ to better suit the American volunteer population of the study. Four sensations, ‘twinge’, ‘uncomfortable’, ‘bruised’ and ‘fading’, were removed from the questionnaire for a remaining total of 13 descriptors on the ground that these terms were confusing for the subjects, based on the recommendation of a college-educated native American English speaker (online appendix 1 and figure 3).

**Sensation descriptors**
The primary study outcome was the four-response (0–3) scale of the 13 descriptors of sensation comprising the majority of items redefined in the SNSQ: pricking, sharp, stinging, throbbing, electric shock, warm, spreading, dull ache, heavy, numb, tingling, deep ache and pressure. In order to simplify the principal analysis, we dichotomised the sensation points of 13 indicators as our primary outcomes, that is, recording all 1, 2 and 3 responses as a ‘yes’ response. We conducted exploratory correlation analyses with the scale values. The spectrum of sensation in each depth/anatomical TL at either an acupuncture or non-acupuncture point was then defined by a sequence of 13 proportions of sensations that were expressed by subjects during the needling procedures. However, extending upon previous work,7 we assumed that each of four different stimulations by epth/anatomical TLs would elicit a constellation of three to four sensation descriptors. We therefore used only those ‘indicating’ sensations for hypothesis testing purposes to avoid a large number of simultaneous tests. More precisely, we used the descriptors ‘pricking’ and ‘sharp’ as the indicating sensations for TLs 1 and 2, and ‘dull’, ‘deep ache’ and ‘heavy’ as sensations for TLs 3 and 4.

**Independent factors**
There are two sets of independent factors. The first set of factors includes the anatomical layer factor (four levels: epidermis, dermis, perimuscular fascia and intramuscular tissue), and the acupuncture point factor (real and control, locations (arm LI13), sides of body (left and right) and insertion sequence). The second set of factors includes techniques (oscillation alone or with rotation).

**Data analysis**
Our general strategy of quantifying the association between the independent factors (eg, anatomical TLs, acupuncture points and needling techniques) and research outcomes (eg, intensity of sensation and needling force) includes initial exploratory analyses followed by McNemar’s tests for paired outcomes or Fisher’s exact tests for independent outcomes to test the hypotheses. Due to the limitation of our sample size, we assume outcomes from different arms or acupuncture points are independent. Outcomes from different TLs in the same acupuncture point were otherwise considered dependent. To simplify the analysis, we aggregated outcomes from TLs 1 and 2 to an indicator for a certain constellation of sensations; likewise for TLs 3 and 4. Since these two outcomes occurred in the same acupuncture point, they were considered as paired data in our analysis. Test results with p values smaller than 0.05 were considered statistically significant. All analyses were conducted using SAS V.9.2.
RESULTS
All five healthy volunteers completed 32 sets of modified SNSQs. In all five healthy volunteers, the frequency of pricking, sharp sensations was significantly greater in TLs 1 and 2 than TLs 3 and 4 (p=0.007) (table 1 and figure 7); the frequency of sensations described as deep, dull and heavy, as spreading, and as electric shocks, was significantly greater in TLs 3 and 4 than TLs 1 and 2 (p=0.002) (table 1 and figure 7). The spectrum of sensation experienced during US-guided needling at acupoint (LI13) and that experienced at its corresponding non-acupuncture point did not significantly differ within each of three TLs (p>0.05) except TL 4 (p=0.006) (table 1 and figure 7 and summarised in table 2 and figure 8).

The introduction of needle rotation significantly increased deep, dull, heavy sensations at TL 3 (p=0.021), but not pricking and sharp sensations (p=1.00); this
increase occurred at TL 3 but not at TL 2 (p=1.00 for both indicating sensations) (table 3 and figure 9); and within each level, the spectrum of sensation experienced during both oscillation + rotation and oscillation alone did not significantly differ between acupoints and non-acupoints (table 3 and figure 9 and summarised in table 4 and figure 10).

**DISCUSSION**

The key findings of this preliminary study are as follows: (1) it is feasible to evaluate the sensations experienced by individuals following US-guided acupuncture needling at different TLs; (2) pricking, sharp sensations are more frequently sensed by needling at TLs 1 and 2 than at TLs 3 and 4, while sensations described as deep, dull and heavy, as spreading, and as electric shocks are sensed in reverse frequencies; (3) the spectrum of sensations by different TLs or different stimulations is not different between acupoint LI13 and its corresponding non-point at the same TLs; and (4) needle rotation increases deep, dull, heavy sensations, but not pricking and sharp sensations; and this occurs at TL 3 but not at TL 2.

US guidance, in our opinion, is the only non-invasive technique to offer real-time images of the tissue being stimulated by an acupuncture needle. This study reports that acupuncture guided by US is feasible and that it can establish at least the depth and anatomical TLs of needling.

The different frequencies of instances of experiencing a constellation of sensations caused by needling at TLs 1 and 2, and TLs 3 and 4 can lend support to the ideas that: (1) various subjective sensations involve different and relevant sensory receptors and needling at different TLs stimulates distinctive sensory nerves; and (2) pricking and sharp sensations are dominant in TLs 1 and 2, while those for deep, dull, heavy, spreading and electric shocks are prevalent at TLs 3 and 4. At this time, we must state that the sensations from TLs 3 and 4 overlap somewhat with those from TLs 1 and 2. From this observation, it is plausible that the traditional notion of acupuncture sensations refers to sensations stimulating or at least involving TLs 3 and 4.

Recently, the existence of distinctive acupoints has been challenged; some reported that needling itself is effective regardless of stimulating traditionally known acupoints or non-points. This leads to a further difficulty in using point aspect as a control method of acupuncture. Since the area that one acupuncture needle can stimulate is somewhat limited no matter the diameter of the needle and whether the stimulation takes place on acupoints or non-points, further clarification of where to needle as an experimental point or control point is urgently needed. In this respect, this study indicates that, at least from the viewpoint of the frequency of sensations, using a point 3 cm away from the LI meridian did not produce different sensations from those of LI13. With this notion, the authors emphasise we have not yet studied the difference between the clinical effects of stimulating these two points.

Langevin et al. reported that acupuncture with needle rotation can cause more tissue wrapping than insertion alone. However, questions such as whether or not this affects all TLs and if the TL in which the

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**Table 1** At acupoint (LI13) and non-acupuncture points, the spectrum of sensation experienced during ultrasound-guided needling of four anatomical tissue levels (TLs) (five subjects × 16 administrations of modified Southampton Needle Sensation Questionnaire)

<table>
<thead>
<tr>
<th>Sensations (frequency (n))</th>
<th>Acupoint</th>
<th>Non-acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL 1</td>
<td>TL 2</td>
</tr>
<tr>
<td>Prick, sharp</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Deep, dull, heavy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stinging, throbbing</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spreading, electric shock</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Frequency of pricking, sharp sensations in TLs 1 and 2 > TLs 3 and 4 (p=0.007). The spectrum of sensation at acupoint (LI13) ≈ that at its corresponding non-acupuncture point within TLs 1, 2 and 3 (p>0.05) except TL 4 (p=0.006).
acupuncture occurs makes any difference have been waiting for answers. This study hints that tissue wrapping and the sensations it induces may be more prevalent in TL 3, which includes the perimuscular fascia.

Based on this study’s promising results, we envision that further research through a larger-scale study will rigorously challenge the historical role of acupuncture sensation in acupuncture theory and practice. We seek to shift current research towards creating quantitative measures to gauge successful acupuncture treatments that are grounded not in the traditional definitions of Chinese medicine but in scientific understanding. Such a shift will improve acupuncture clinical practice by providing quantifiable standards by which to judge the success of acupuncture techniques and, in turn, will improve treatment quality. This research will also contribute greatly to a definition of acupuncture treatment for use in clinical trials in terms of criteria for adequate stimulation, for the first time based on scientific validation rather than clinical judgement. A larger-scale replication of this preliminary study will meet these goals by employing an innovative approach as well as improved methods and novel instrumentation measuring motion force and torque.

This study is innovative in its approach to needling depth. As noted above, there is much debate surrounding the relationship between acupuncture sensations and depth of needle penetration. However, previous research has examined needling at only two arbitrarily defined levels: ‘superficial’ and ‘deep’. The definition of these two terms varies among studies, but the majority describe ‘superficial’ as a set depth of 1–2 mm, the depth of insertion commonly used in Japanese traditional practice, and ‘deep’ to be 8–12 mm, the depth commonly used in Chinese traditional practice.14 18 21 By setting depth at predetermined measurements, these studies do not account or adjust for

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**Table 2 At acupoint (LI13) and non-acupuncture points (combined), the spectrum of sensation experienced during ultrasound-guided needling of four anatomical tissue levels (TLs) of five participants (five subjects × 16 administration of modified Southampton Needle Sensation Questionnaire)**

<table>
<thead>
<tr>
<th>Sensations (frequency (n))</th>
<th>TL by point</th>
<th>Acupoints + non-acupoint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL 1</td>
<td>TL 2</td>
</tr>
<tr>
<td>Prick, sharp</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Deep, dull, heavy</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Stinging, throbbing</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Spreading, electric shock</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The spectrum of sensation at acupoint (LI13) ≈ that at its corresponding non-acupuncture point within TLs 1, 2 and 3 (p>0.05) except TL 4 (p=0.006).
anatomical variation among study participants. To improve upon these methods, we are using a novel approach: Needling sensations are mapped at depths defined by tissue layers, beginning with pressing against the skin (non-penetrating), then penetrating to the lower border of the dermis, the perimuscular fascia and intramuscular tissue. This approach accounts for variability among participants and positions the findings in anatomy.

This study is also the first to use US to guide needling of different tissue layers in combination with the acupuncture sensation scale. This tight connection between anatomy and sensation has not previously been attempted in acupuncture research. Results can be applied to future research on acupuncture sensation and acupuncture needling.

To question and challenge the traditionally defined acupuncture sensation, we studied via US, using the previously validated SNSQ, the needling sensations experienced when different anatomical structures were stimulated. We are confident that this application will move acupuncture research toward creating measures to gauge successful acupuncture treatments that are grounded in a solid scientific understanding of the clinical practice of acupuncture. Such a shift will improve acupuncture’s clinical practice by potentially providing more quantifiable standards by which to judge the adequacy of acupuncture applications.

Regrettably, this study only measured the subjective sensations of needling, not any objective data such as force or torque of needling. It would be desirable to measure force and torque of needling at different TLs and with different manipulation skills in future studies.

**CONCLUSION**
The preliminary study indicates a strong connection between acupuncture sensation and both tissue depth and

<table>
<thead>
<tr>
<th>Sensations (frequency)</th>
<th>Acupoint</th>
<th>Non-acupoint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL 2 OS</td>
<td>TL 3 OR</td>
</tr>
<tr>
<td>Prick, sharp</td>
<td>6 4</td>
<td>4 4</td>
</tr>
<tr>
<td>Deep, dull, heavy</td>
<td>1 0</td>
<td>1 3</td>
</tr>
<tr>
<td>Stinging, throbbing</td>
<td>1 1</td>
<td>2 2</td>
</tr>
<tr>
<td>Spreading, electric</td>
<td>1 0</td>
<td>2 1</td>
</tr>
</tbody>
</table>

Needle rotation significantly increased deep, dull, heavy sensations at TL 3 ($p=0.021$), but not pricking and sharp sensations ($p=1.00$); this increase occurred at TL 3 but not at TL 2 ($p=1.00$ for both indicating sensations). The spectrum of sensation experienced during both oscillation + rotation at acupoint (LU4) ≈ that during oscillation alone both at acupoint (LU4) and its corresponding non-acupuncture point within TLs 2 and 3 ($p>0.05$).

OR, oscillation + rotation; OS, oscillation.

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Table 3 At acupoint (LU4) and non-acupuncture points, of five participants, the spectrum of sensation experienced during ultrasound guided needling of four anatomical tissue levels (TLs) (five subjects × 16 administration of modified Southampton Needle Sensation Questionnaire)

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Figure 9 At acupoint (LU4) and non-acupuncture point, the spectrum of sensation experienced during ultrasound-guided needling of four anatomical tissue levels (TLs). OS, oscillation; OR, oscillation + rotation; *$p>0.05$ when comparing acupuncture point and non-acupuncture point in each combination of TL and needle technique. The introduction of needle rotation significantly increased deep, dull, heavy sensations at TL 3 ($p=0.021$), but not pricking and sharp sensations ($p=1.00$); this increase occurred at TL 3 but not at TL 2 ($p=1.00$ for both indicating sensations).
Table 4  At acupoint (LU4) and non-acupuncture points (combined), the spectrum of sensation experienced during ultrasound guided needling of four anatomical tissue levels (TLs) of five participants (five subjects × 16 administration of modified Southampton Needle Sensation Questionnaire)

<table>
<thead>
<tr>
<th>Sensations (frequency)</th>
<th>Acupoints + non-acupuncture</th>
<th>TL 2</th>
<th>TL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS OR</td>
<td>OS OR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prick, sharp</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Deep, dull, heavy</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Stinging, throbbing</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Spreading, electric shock</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

The spectrum of sensation experienced during both oscillation + rotation at acupoint (LU4) = that during oscillation alone both at acupoint (LU4) and its corresponding non-acupuncture point within each levels of TLs 2, and 3 (p=0.05).

OR, oscillation + rotation; OS, oscillation.

Figure 10  At acupoint (LU4) and non-acupuncture points (combined), the spectrum of sensation experienced during ultrasound guided needling of four anatomical tissue levels of five participants. Within each level, the spectrum of sensation experienced during both oscillation + rotation and oscillation alone did not significantly differ between acupoints and non-acupoints.

Summary points

- Needle sensation is important but little studied
- In this preliminary study, we investigated the sensations induced by needling volunteers
- The sensation varied according to tissue level and stimulation, but not location

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Patient consent  Obtained.

Ethics approval  This study was conducted with the approval of the UNC-Chapel Hill IRB.

Provenance and peer review  Not commissioned; externally peer reviewed.

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