Title: Challenging a classical theory of sensory specificity: inconsistency and instability of thermosensitive spots

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Abstract

Thermal sensitivity is not uniform across the skin, and is particularly high in small (~1mm^2) regions termed 'thermosensitive spots'. These spots are thought to reflect the anatomical location of specialised thermosensitive nerve endings from single primary afferents. Thermosensitive spots provide foundational support for "labelled line" or specificity theory of sensory perception, which state that different sensory qualities are transmitted by separate and specific neural pathways. This theory predicts a highly stable relation between repetitions of a thermal stimulus and the resulting sensory quality, yet these predictions have rarely been tested systematically. Here we present the qualitative, spatial and repeatability properties of 334 thermosensitive spots on the dorsal forearm sampled across 4 separate sessions. In line with previous literature, we found that spots associated with cold sensations (112 cold spots, 34%) were more frequent than spots associated with warm sensations (41 warm spots, 12%). Still more frequent (165 spots, 49%) were spots that elicited inconsistent sensations when repeatedly stimulated by the same temperature. Remarkably, only 13 spots (4%) conserved their position between sessions. Overall, we show unexpected inconsistency of both the perceptual responses elicited by spot stimulation and of spot locations across time. These observations challenge the traditional view that thermosensitive spots reflect the location of individual thermosensitive primary afferents serving as specific labelled lines for corresponding sensory qualities.

Keywords: Thermosensation // Thermoception // Thermal spots // Primary afferents // Innervation

New & Noteworthy. Thermosensitive spots are clustered rather than randomly distributed, and have highest density near the wrist. Surprisingly, we found that

thermosensitive spots elicit inconsistent sensory qualities and are unstable over time. Our results question the widely believed notion that thermosensitive spots reflect the location of individual thermoreceptive primary afferents, that serve as labelled lines for corresponding sensory qualities.

Introduction

Thermoreception is not uniform across the skin surface.[1-5] Even within a body part, there are small areas of unusually high thermal sensitivity, commonly referred to as 'thermosensitive spots'.[6-23] Early work reported that many spots were temperature-specific, eliciting either warm or cool sensations when the corresponding stimulus was applied.[6] Crucially, each spot was thought to indicate the presence of nerve endings from a single cutaneous afferent fibre, responding consistently to either warmth or cold.[17-23] Thus, thermosensitive spots have provided foundational support for theories of neural specificity – the view that specific sensory qualities are associated with specific classes of afferent fibre.[24] Later studies of the loss of sensation during pressure block and anaesthetic block showed that cold sensations were carried by thinly myelinated A-δ fibres, while warm sensations were carried by unmyelinated C-fibres, confirming the link between afferent fibre types and sensory qualities.[25]

Green and colleagues[11] developed a two-step search method to identify thermosensitive spots across larger skin areas. Briefly, they used a thermode with a contact area of 16 mm^2 to first identify broad thermosensitive sites, followed by a thermode with a contact area of 0.79 mm^2 to identify the smaller, classical spots within those sites. They applied this procedure in the human forearm, classifying

sites and spots according to the quality of the evoked sensations. They found that the quality of sensation evoked by a thermal stimulus could be inconsistent. Although 96.7% of sites remained sensitive over the experimental session, a surprising 31.8% were associated with different sensations across repeated tests, which presumably meant that their stimulations activated multiple thermosensitive primary afferents. In that case, smaller stimulation areas should produce more consistent sensory qualities – although this prediction was not tested in that study. In fact, the main focus of Green et al.'s investigation was their finding that innocuous heating and cooling of some spots could evoke painful sensations. Together, their results question the classical theories of neural specificity and labelled lines.

Such a study is required for two reasons. First, if thermosensitive spots are shown to be inconsistent and unstable over time, this might question the notion that each spot corresponds to a single afferent unit, since the skin locations of afferents' nerve endings can be assumed to be unchanging. Second, near-threshold stimulation of a single thermosensitive spot can be considered to cause a minimal afferent signal to the brain. Neural specificity theories predict that even minimal afferent signals should consistently evoke the same sensation, because the "line" carrying the signal bears a "label" that is read by the brain as defining the sensory quality.

Methods

Subject details

8 participants (5 females; 18-35 years) were recruited from an institutional participant pool and compensated for their time. The sample size was chosen based on previous studies mapping suprathreshold thermosensitivity in the forearm.[3,16,47,48]

Participants with skin conditions or sensitivity skin were excluded. The experiment was approved by the UCL Research Ethics Committee.

Participants gave written consent to video recording and photography of their arm during the experimental session. They were invited to review recordings and images after the experiment.

Experimental schedule

Our procedure to identify spots was based on the protocol described by Green et al.,[11] but included several extensions and modifications. The procedure was repeated 4 times on different days. Sessions 1 and 2 were separated by 24 hours. In these 2 sessions, thermosensitive spots were identified based on detection of a warming stimulus 2°C above individual baseline skin temperature, or detection of a cooling stimulus 2°C below baseline. Sessions 3 and 4 took place 30 days after sessions 1 and 2 respectively, and used ±4°C variations. We predicted that larger temperature changes should reveal more thermosensitive sites, so this factor acted as an internal validation that our methods correctly tracked human thermosensitivity.

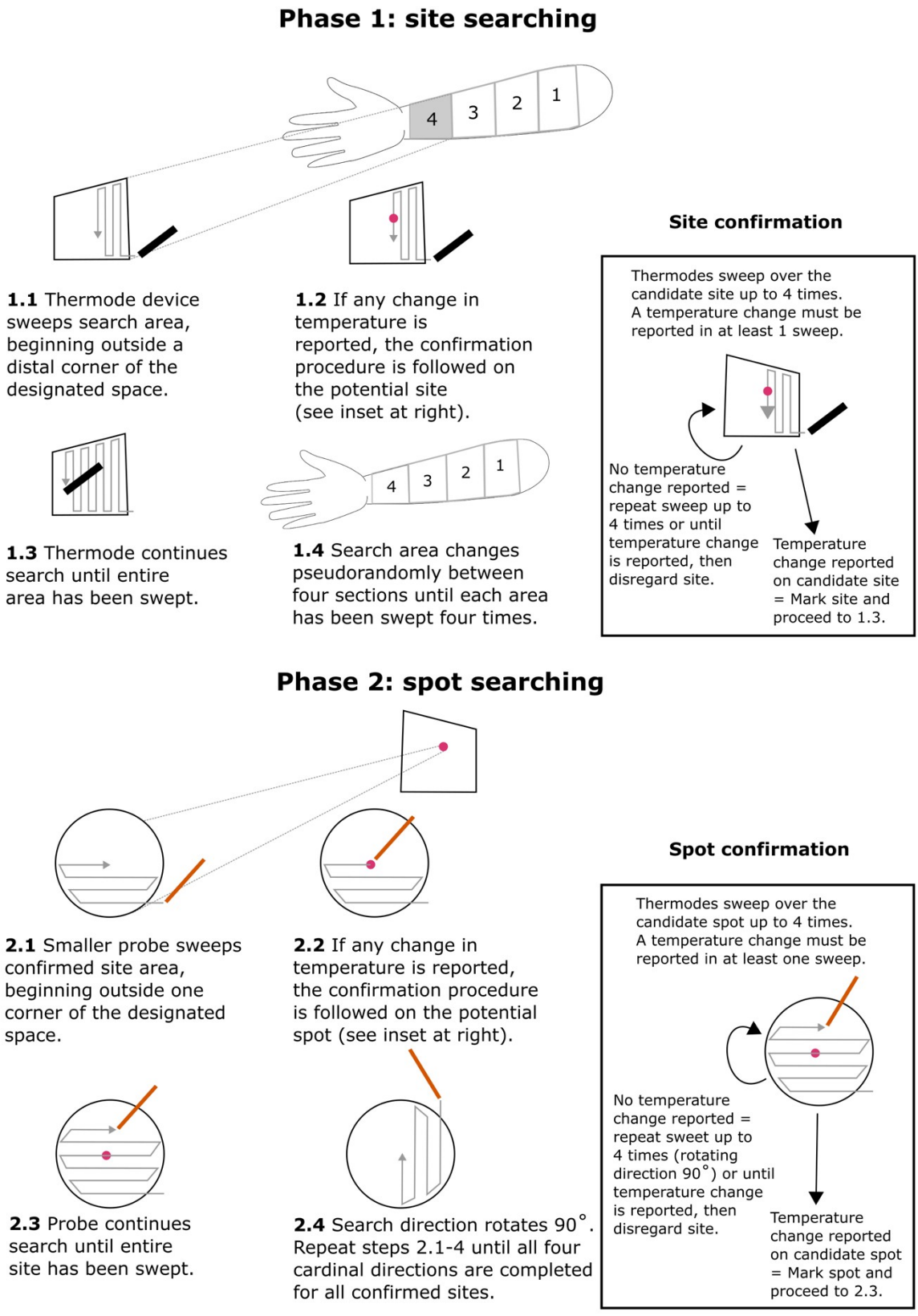

Figure 1. Spot searching method. In Phase 1, the dorsal forearm is divided into four equal segment and thermodes sweep each area to locate candidate thermosensitive sites. In Phase 2,

each confirmed site is swept with an aluminium wire (contact area: 0.79 mm^2) to locate thermosensitive spots.

In each session, we used a two-step systematic search and classification procedure to identify thermosensitive spots (Figure 1). In Phase 1, we used a circular Peltier thermode (Physitemp NTE2A, diameter: 12.7 mm, contact area: 126.68 mm^2) to search efficiently for general sites of high thermal sensitivity in the dorsal forearm. In Phase 2, we used blunted aluminium wires (diameter: 1 mm, contact area: 0.79 mm^2) to scan for smaller thermosensitive spots within these larger sites (Figure 1). The data of interest here are the spots, with sites being just an intermediate step for efficient identification of spots. The blunted aluminium wires were maintained in a water bath (Premiere XH-1003, C&A Scientific Company, Virginia, USA Premiere) at the desired temperature, then quickly dried and applied to the skin. The experimenter held one end of the wire via a custom-made thermoinsulating handle.

Laboratory room temperature was maintained at 23°C by an air conditioning unit. The experiment was recorded with a 720x720 pixel camera located 53 cm above the table, giving an effective spatial resolution of 0.33 mm/pixel. The table was covered with 1-mm graph paper allowing accurate repositioning of the arm, and thus comparison of spot locations across sessions.

Procedure

After obtaining informed consent, the right forearm was placed comfortably on the table, with the dorsal side upwards. To familiarise participants with the sensations they should report, we demonstrated and narrated the procedure for locating a single

site (Phase 1). Participants were instructed to report immediately by saying “warm” or “cold” if they felt any change in the temperature of the applied thermal probe.

Participants were then blindfolded. The tip of the middle finger and centre of the elbow were aligned to the graph paper. The distance from the wrist to elbow was measured and the forearm divided into four equal segments, which were marked on the paper and visible to the camera. The graph paper from the first session was kept to allow precise repositioning in future sessions, and standardisation of coordinates for image alignment and analysis.

Thermal stimuli were specified relative to each participant’s baseline skin temperature at the beginning of each session. Using a laser thermometer, skin temperature was measured adjacent to the wrist and elbow. The cooling stimulus was set to either 2°C (sessions 1,2) or 4°C (sessions 3,4) below the lower of the these and warming stimulus was set to 2/4°C above the higher of the same two temperatures. Cold and warm stimuli were tested in separate, counterbalanced blocks within each session.

In Phase 1, the four areas of the forearm were tested in pseudorandomised order to prevent both order effects and temporal summation.[49,50] In each area, thermosensitive sites were located by sliding the thermode over the skin. A silicone-based lubricating gel was applied to minimise friction and excessive mechanorecptor stimulation during movement of thermode. The weight of the thermode provided the downward force: the experimenter exerted no additional pressure. The thermode was placed in one corner of each area and systematically swept across it in a medio-

lateral direction (Figure 1). Each area was searched four times. At the end of each medio-lateral sweep, the thermode was moved proximally to begin the next sweep. The sweeps began and ended just outside the boundaries of each of the four area to prevent onset/offset effects (Figure 1).

If participants reported “warm” or “cold” sensations at any point during a search, this was considered a candidate thermosensitive site. We marked the location on the skin with coloured ink, and followed by sweeping up to four further times to confirm the site (Figure 1). These follow-up sweeps could help distinguish genuine thermal sensations from potential false-positive reports. If participants reported any thermal sensation during any follow-up sweep, then the location was marked as confirmed thermosensitive site, and the confirmation procedure was terminated. Importantly, the reported sensations did not need to be consistent with the actual stimulus temperature, nor with each other. If no thermal percept was reported in any of four confirmation sweeps, the candidate site was classed as unconfirmed.

In Phase 2, we then searched for smaller thermosensitive spots within each confirmed site, by repeating at a smaller scale the same process used to search for sites. This time we rotated the direction of each successive confirmation sweep by 90 degrees in order to discourage participants from responding simply on the basis of memory for elapsed time or for tactile location In place of thermodes, we now used much smaller warmed or cooled aluminium wire as stimulators (Figure 1).

When a spot was located and subsequently confirmed (Figure 1), it was marked on the skin. If a participant consistently reported a temperature sensation corresponding

to the stimulus temperature (i.e., 'cold' to temperature 2/4°C below baseline and 'warm' to temperature 2/4°C above baseline) both on initial identification and subsequent confirmation, then the spot was classified as cold or warm. If a participant reported different temperature sensations when the potential spot was first identified and in any of up to four confirmation attempts, then the spot was classified as inconsistent. Spots that elicited sensations to both stimulus temperatures in the separate blocks were classified as inconsistent. Occasionally, initial identification and subsequent confirmation responses were consistent with each other, but did not correspond to the actual stimulus temperature: these spots were classified as incongruous (Figure 2A). Warm, cold, inconsistent and incongruous spots were marked on the skin with four different ink colours. Some spots initially yielded a thermal sensation, but no further sensation was reported on any of four subsequent stimulation confirmation attempts with the same stimulus. These spots were considered unconfirmed and were identified with a different ink. At the end of each session, a final image was taken of the positions of all spots.

Analysis

The final images of each session were pre-processed. First, skin markings were annotated with a graphics editing program. Second, the images within each participant were aligned across sessions with DS4H Image Alignment[51] by defining a few fiducial points. Third, spot location data was extracted from these standardised images with a custom Python script (see software repository: https://github.com/iezqrom/publication-thermal-spots-quality-location-inconsistent). Briefly, the centre of the digital mark assigned to each spot was manually clicked and

an XY coordinate recorded. Forearm curvature was ignored. The classification of each spot was saved with the coordinates.

Spot classifications were compared across sessions and subjects. For some analyses, parametric or non-parametric tests were chosen depending on data normality. Unconfirmed spots were not included in this and subsequent analysis.

To assess spatial distribution of spots along the forearm, we used the Anderson-Darling test[52] to test for a uniform distribution of the spots' X-coordinates between elbow and wrist. The uniform distribution tested had a lower bound of 0 and an upper bound of 1200 pixels. We focussed on this spatial axis because thermosensitivity shows a proximo-distal gradient,[3,5] and because this axis was less affected by curvature distortions that would affect mediolateral position estimates. Data from each participant was tested separately, but data were pooled across sessions. Deviation from a uniform distribution would indicate that spots are more likely to be reported in certain locations on the dorsal forearm (for example, near the wrist, or elbow). Spot data were pooled across all four sessions. One participant reported only six spots, which was insufficient to estimate distribution, and was thus excluded from this test.

We also quantified spatial aggregation of spots. We compared the distance from each spot to its 'nearest neighbour' using the Clark-Evans Aggregation Index, R.[53] As there could be additional spots outside of our measured boundaries[13], we applied a correction for edge effects.[54] Spot data were pooled across all sessions.

To estimate stability and consistency of thermosensitive spots, we next compared the spatial positions of spots in each session with those in all other sessions within each participant. Repeatable repositioning of the arm is clearly crucial for this analysis, and we applied several strategies to standardise forearm positioning (see Procedure). Additionally, we performed image alignment. A spot was considered conserved if any spot in any other session was less than 2 mm (6 pixels) away. This criterion was based on twice the diameter of the aluminium wire used for stimulation.

Results and discussion

The sensory quality evoked by spot stimulation is variable

We have therefore extended Green's method[11] for studying thermosensitive spots (Figure 1; STAR Methods), using repeated systematic searches over a large skin region (the entire forearm), at extended timescales (days and months). We identified a total of 349 spots across participants of which 334 (mean = 10.44 ± 10.63 SD) were confirmed following the confirmation procedure (Figure 2A) and included in subsequent analyses. Crucially, we then distinguished between spots that consistently elicited a single sensory quality of warmth or cold on repeat testing, and inconsistent spots that evoked different sensory qualities when repeatedly tested with the same thermal stimulus.

Consistent with previous work,[6-8,10,11] spots eliciting 'cold' responses (n = 112, mean = 14.00 ± 13.55 SD) were more frequent than those eliciting 'warm' responses (n = 41, mean = 5.13 ± 6.81 SD W = 35.00, $p < 0.01$, r = 0.944, Wilcoxon signed-ranks test). We found 165 inconsistent spots, amount to 49% of all confirmed spots. Thus, the inconsistency of evoked sensory qualities reported by Green and colleagues[11] for

much larger thermal sites of 16 mm^2 was found also for much smaller thermosensitive spots of just 0.79 mm^2.

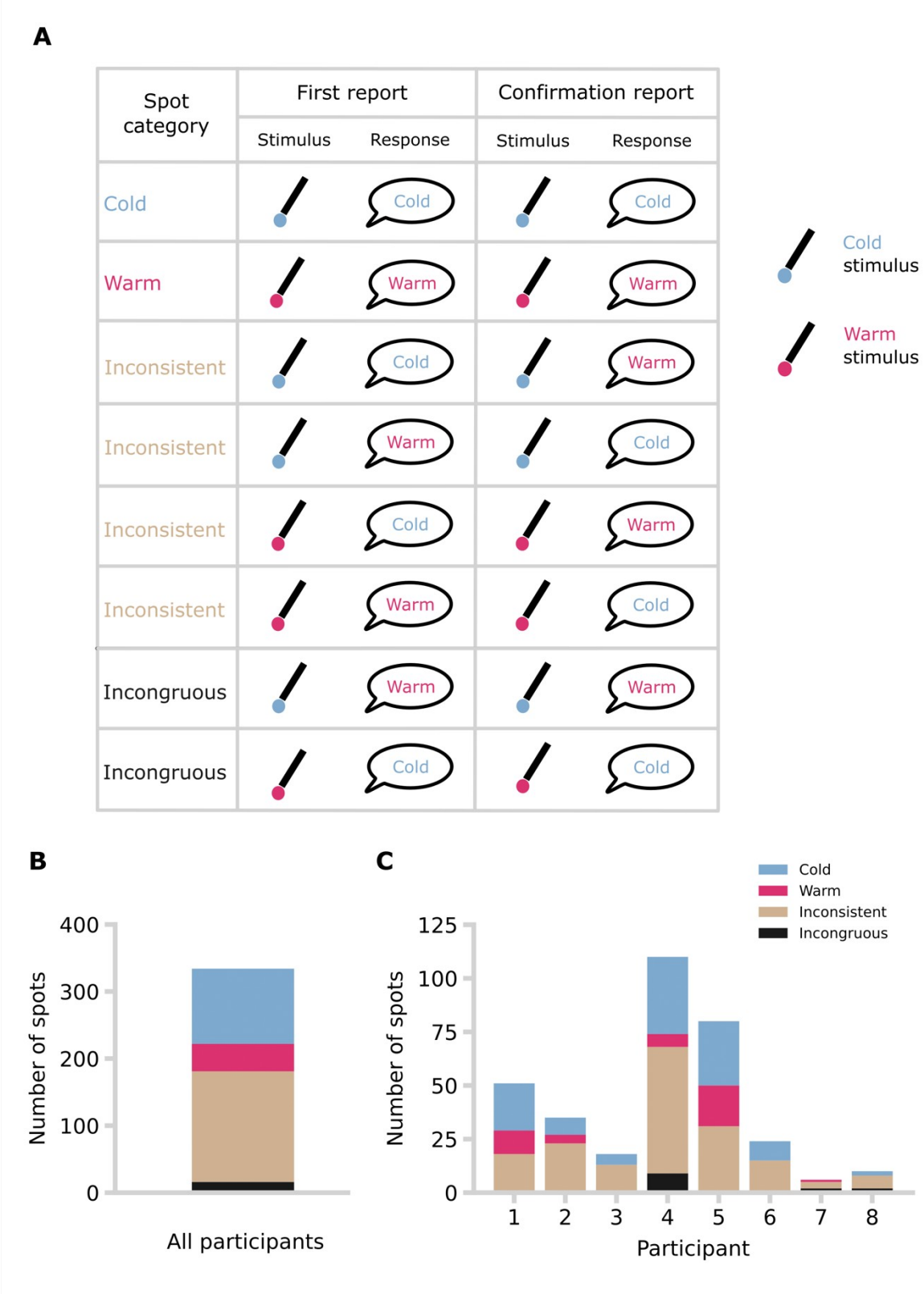

Figure 2. Classification and distribution of spots by sensation elicited, with respect to modality of stimulus. A) A table with the taxonomy of spots is shown. **B)** Total number of spots across participants by spot category. **C)** Total number of spots per participant and by spot category.

This high rate of inconsistency appears to contradict the classical picture that views thermosensitive spots as receptive fields of 'labelled-line' afferent fibres transmitting a specific sensory quality.[5,6,24] Our stimulator (contact area: 0.79-mm^2) might have stimulated a polymodal primary afferent, rather than a non-noxious thermoceptive afferent. Since polymodal fibres, by definition, are activated by multiple stimulus types and do not carry a distinctive stimulus quality, this could explain our inconsistent responses. Traditionally, innocuous cold sensations are thought to be mediated by Aδ-fibres, while innocuous warm sensations are mediated by C-fibres.[26-28] The responses of these fibres are driven by TRPM8 receptor channels in cooling-responsive afferents and by TRPV1 in warming-responsive fibres on warming.[27,28] However, this one-to-one mapping from receptors to sensations has been recently challenged by studies in mice. In fact, most primary afferents mediating themoreception are polymodal, and afferents that express TRPM8 (cooling-sensitive receptor) was found necessary for warm perception.[29-31] Thus, a specific sensory quality may depend on polymodal afferents, rather than specific afferents, contrary to labelled-line theories.[24] If sensory quality is mediated by polymodal afferents, this could be a source of variability in evoked sensations, particularly when a single afferent is stimulated.

Our experimental method is unnatural in targeting a single primary afferent. Population coding, in which sensory quality depends on a balance of activity across

many different afferents, potentially differing in physiological type as well as in location, may play a crucial role in robust and stable thermosensation.[32] Our results are thus in conflict with theories of neural specificity, but are compatible with recent behavioural, electrophysiological and theoretical work. For example, larger thermal stimuli produce psychophysical functions with higher precision than smaller stimuli, suggesting that averaging over multiple afferents reduces sensory noise.[33]

Spots are aggregated and non-uniformly distributed

Thermosensitive spots have classically been taken as a proxy of the anatomical distribution of thermosensitive afferent innervation. However, studies of spot spatial distribution have been limited to small subregions of the hand or forearm[6-18]. Green et al. (2008)[11] searched for spots across the entire forearm, but did not analyse their spatial distribution properties. This data would contribute to our understanding of the relationship between spots and thermosensitive afferent innervation.

Visual inspection of our data shows that spots were distributed unevenly across the forearm (Figure 3A). We applied three different analyses to describe the spatial properties of spots. First, the distribution of spots for four out of the seven participants included in this analysis deviated significantly from a uniform spatial distribution (Figure 3A). Second, dividing the forearm into four equal distal-proximal areas showed no significant main effect, nor interaction effect, in spot density ($F_{3, 28}$ = 2.14, p = .118, η_p^2 = 0.19) (Figure 3B), ruling out a simple spatial gradient hypothesis, though visual inspection shows a relatively high density of spots close to the wrist. Third, the Clark-Evans Aggregation Index was significantly below 1 for all participants tested, providing strong evidence of spot aggregation (Figure 3C).

Consistent with previous research on the insensitivity to warmth in subregions of the forearm,[10] we found that spots tended to aggregate (Figure 3) across the forearm. We also report significant non-uniformity in spatial distribution, with more spots observed closer to the wrist (Figure 3). This is consistent with the well-established proximodistal increase in tactile thresholds,[34,35] with recent reports of a similar gradient for nociception,[34] and with thermal sensitivity maps showing lower perceptual thresholds distally.[3,4] Our study is thus compatible with previous perceptual studies and shows for the first time the distribution of spots following a systematic search across a large skin region.

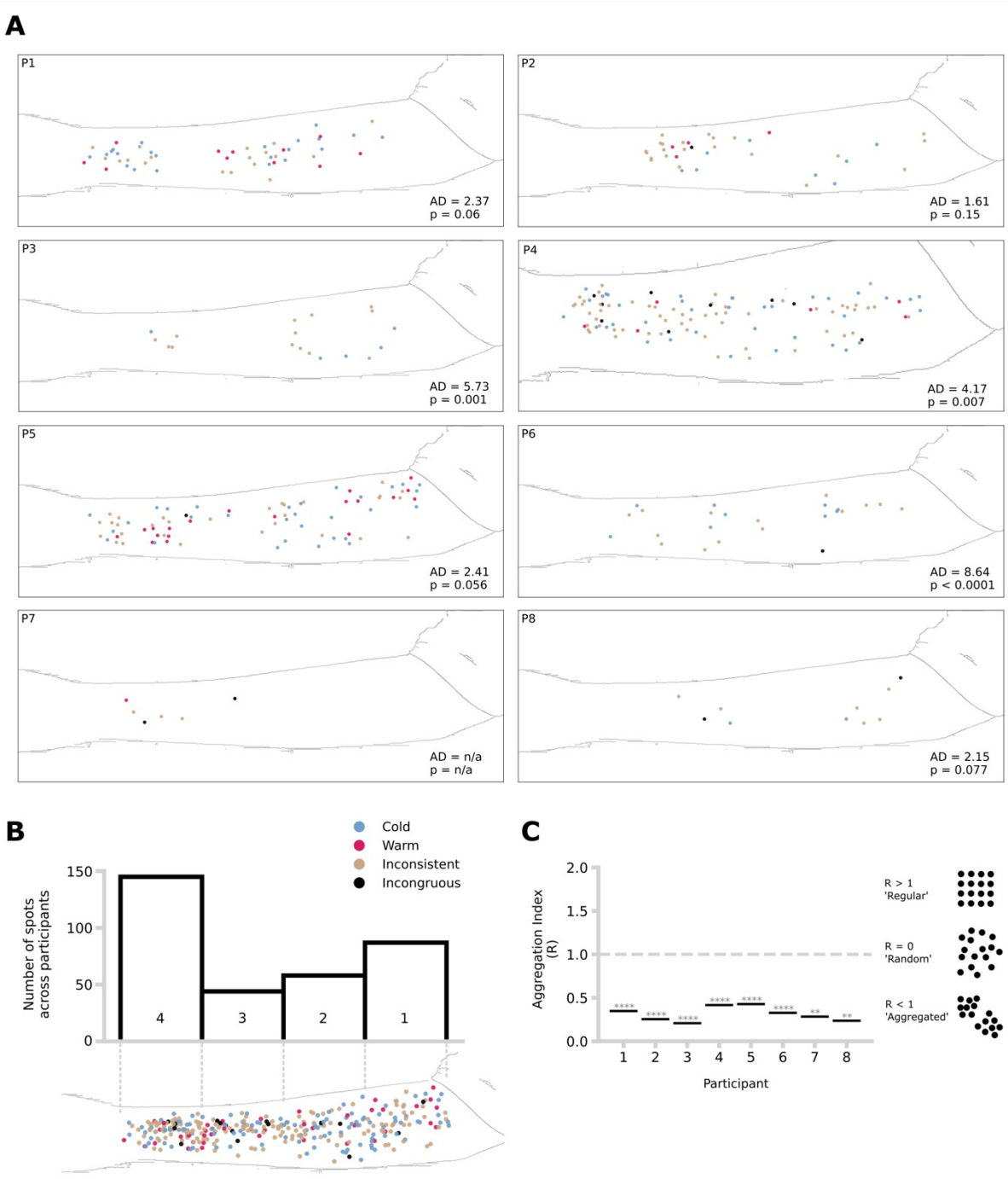

Figure 3. Spot spatial distribution. A) Spot distribution across participants. A single forearm silhouette has been placed in each box for visualisation purposes only. Anderson-Darling (AD) test results and associated p-values are shown in each panel at the bottom right corner. **B)** Total number of spots pooled across participants by search area. The top panel shows the number of spots per skin search area (1-4) across all participants and sessions. The bottom panel is a

visualisation of the distribution of all spots across participants and sessions in a template forearm silhouette. **C)** Aggregation index (Clark-Evans aggregation index, R) of confirmed spots per participant, with Donnelly correction. Illustrative examples are shown on the right. Asterisks indicate the p-values obtained from two-sided test statistics. ** p < .01, **** p < .0001.

The location of spots varies across testing sessions

If spots reflect the presence of nerve endings that are stable, then the same spots should be found across repeated searches.[8,12] However, no study has addressed this question with repeated systematic searches over large skin regions.

We found that conservation of spots across testing sessions was very rare (Figure 4). Just 13 of 334 confirmed spots were re-identified between sessions. Of the 13 conserved spots, 11 had the same classification (inconsistent/warm/cold) across sessions. No spot was conserved across 3 or more sessions.

If we had failed to reposition the arm or realign spatial data precisely, we might expect low rates of conservation. If our low conservation were due to these technical issues, visual inspection would show a common spatial pattern of spots within each session, which is simply shifted between sessions due to misalignment. We saw no evidence for this (Figure 4A). Similarly, mere misalignment would imply equal numbers of spots in each session. However, the number of spots varied across sessions as well as their locations (Figure 4B). The low conservation of spots across sessions is therefore unlikely to be due to limitations in arm positioning or data alignment.

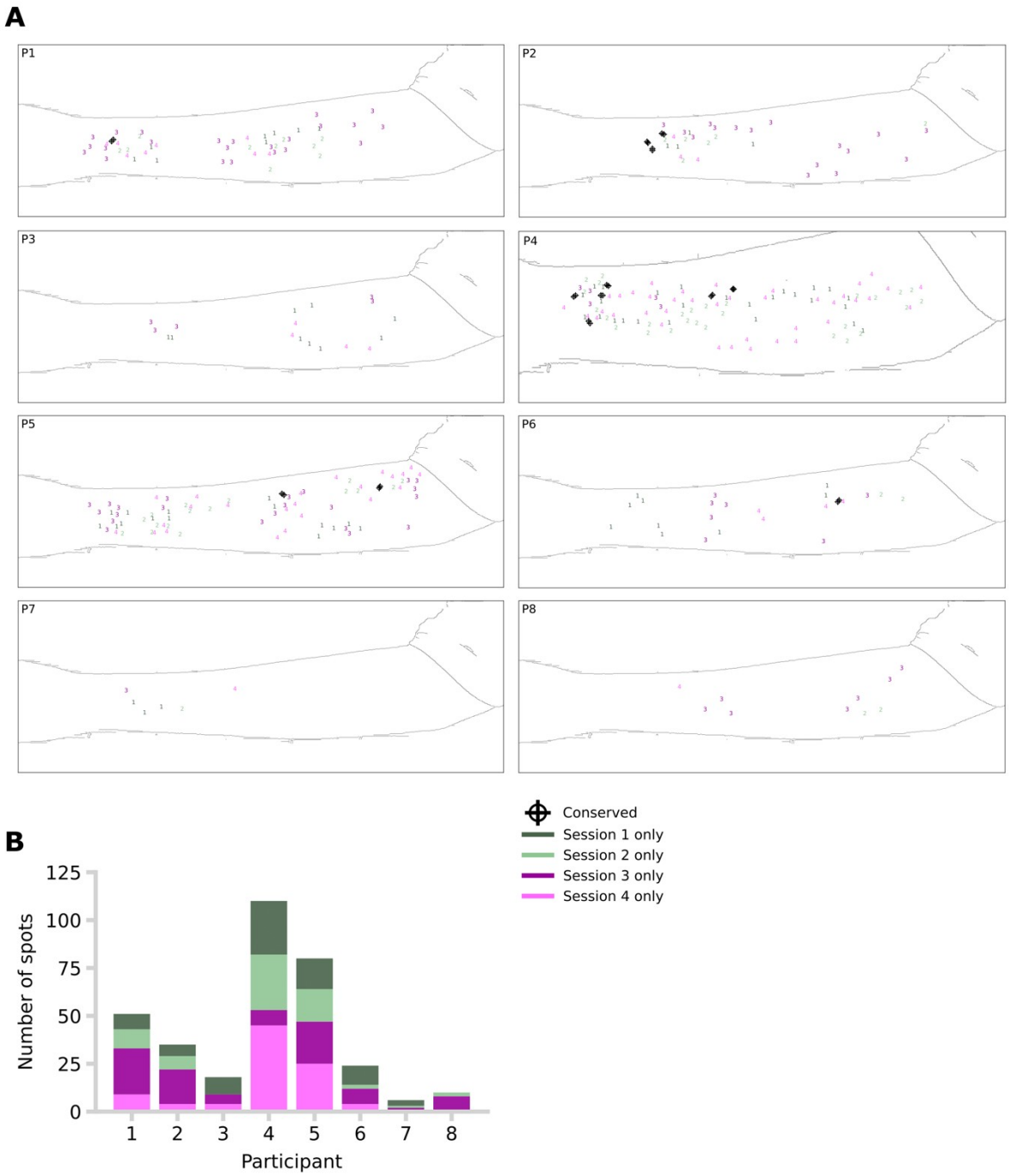

Figure 4. Conservation of spots. A) Position of spots per participant and session. The spots that were considered conserved across sessions are indicated with a black dot and cross. A single forearm silhouette has been placed in each box for visualisation purposes only. **B)** Total number of spots per participant and session.

A poor signal to noise ratio in thermal afferents would also lead to low measures of conservation. A spot might be identified on one session, but missed on another simply because of fluctuations in combined signal and noise reaching a central site for decision-making. However, high noise levels would imply a high false negative rate with stimulations of an afferent fibre often producing no thermal sensation (SDT misses). In our dataset, unconfirmed spots (see STAR methods) can be taken as a proxy for such false negatives. However, only 15 spots out of a total of 349 (4.3%) identified were classified as unconfirmed, a value similar to previous research.[11] Therefore, it is unlikely that methodological issues or sensory noise can account for low rates of conservation. We advance three possible alternative explanations for the surprising instability.

First, sensory detection reports may depend heavily on context, including experience prior to each session. Context-dependent sensitivity is known to be important in sensations at noxious temperatures,[36,37] but may also apply also to the non-noxious temperatures studied here. Second, fluctuations of peripheral excitability across time may also play a major role in thermoception.[38] For instance, thermal detection thresholds have been found to vary by 0.9°C in the hand of healthy young adults. Third, tactile afferent innervation renews throughout an animal's lifetime,[39] but the rate of renewal of thermosensitive innervation in humans is unknown. Our observations were necessarily limited to the roughly 90 minutes of individual sessions, and the 31 days that separated the first from the last session. However, we found minimal conservation of spots even between sessions separated by just 24 hours. Wholesale changes in the presence and location of receptor structures over such short timescales seem unlikely. Therefore, we suggest that non-conservation

reflects some process as yet unknown. A more comprehensive sensitivity profile over a longer period of time might reveal a clearer picture of time-varying sensitivity. Optical Coherence Tomography[40] promises the possibility of longitudinal imaging of sensory afferent fibres in vivo in future studies.

Revisiting a classical model of sensation

We found a high degree of sensory variability, across repeated stimuli, across locations, across testing sessions, and across individuals. These seems at odds with the way that thermosensitive spots have classically been interpreted. In particular, our results question the repeated notion that thermosensitive spots reflect the location of individual thermoreceptive primary afferents,[16-23] that serve as labelled lines for corresponding sensory qualities. At first sight, one might imagine that colocation of a warm and a cold fibre in very close spatial proximity might lead to inconsistent responses. However, this explanation requires the additional, unlikely assumption that a warm fibre might sometimes respond to a cold stimulus while the collocated cold fibre would not, and vice versa. Further, colocation would need to be very frequent to account for so many inconsistent spots. Alternatively, our stimuli might activate single thermosensitive afferents, but those afferents might not use a labelled line coding principle. For example, Campero et al (2001)[41] identified several unmyelinated fibres that responded both to cold and warm stimuli, but could not demonstrate that these fibres played any role in conscious sensation. Interestingly, recent models of tactile afferent coding[42,43] have relinquished the strong assumption of labelled-line coding that underlay classical models.[44] Our research is purely psychophysical, but it suggests that specificity of single thermosensitive afferents should also be re-examined.

Intraneural microstimulation provides perhaps the most direct test of the relation between specific afferents and a sensory quality. Near-threshold stimulation reliably produces a localised, distinct and pure sensory quality, though this conclusion is based on mechanosensitive rather than thermosensitive afferents.[45] Such stimulation bypasses the transduction process at the peripheral receptor, by stimulating the afferent directly. Contrasting microstimulation with our psychophysical data might suggest either that the inconsistency observed in our study arises in the process of transduction at the receptors, or that the labelled line concept is incorrect. While receptor noise may account for whether a stimulus is detected or not,[33] we are unaware of any receptor mechanism that could result in a switch to detecting a different quality.

Intraneural microstimulation also resembles our current design in focussing on *minimal* sensations. In our study, the stimuli were small, and close to threshold. Interestingly, a recent study of visual sensory qualities reported that simulation of a single retinal M-cone in vivo could often produce an achromatic percept[46] – a striking finding given that colour vision has been the paradigmatic evidence for labelled lines. This study, like ours, suggests that a minimal afferent signal may be insufficient to evoke a sensory quality. Presumably some element of evidence accumulation across time or across multiple afferent fibres is required for a stable sensory quality – a quantum for qualia. In that case, Muller's original metaphor of a *label*, i.e., a self-intimating sensory quality based on the origin of each neural signal, should be discarded.

Conclusions

Overall, our study confirms the existence of thermosensitive spots, consistent with previous studies.[6,7,11] However, we found that these spots often produced inconsistent sensory qualities, and were unstable over time. Our results call into question the widespread notion that thermal spots indicate the presence of individual thermosensitive primary afferents projecting centrally as labelled lines, and that minimal activation of an individual labelled line is sufficient for the distinct and reliable phenomenal experience of a specific sensory quality. Our results do not rule out some form of neural specificity theory at the level of fibre populations, but they do suggest that label metaphors for sensory quality should be revised.

Limitations of the study

Here, we highlight three limitations of our study. First, even though the sample size in our study is similar to previous studies suprathreshold thermosensitivity in the forearm.[3,16,47,48], the number of spots and participants in our dataset is limited. Second, our conclusions on conservation of spot location are limited to two time intervals (1 day and 30 days). Future studies should map thermosensitive spots over a wider range of time intervals, with a particular focus on repeat testing at regular intervals up to 1 day. Third, we adjusted the temperature of the thermal stimulus to each participant's baseline temperature after a period of acclimatization by measuring the temperature of two points in the skin. Given that skin temperature fluctuates over time and is not homogenous across the skin, our searching protocol could be improved by integrating thermal imaging to adjust stimulus temperature according to the baseline temperature of the stimulated skin region.

Supplemental materials

Raw data and source code can be found in the following repository:
iezqrom/publication-thermal-spots-quality-location-inconsistent: Code & data supporting academic publication "The sensory quality and spatiotemporal location of thermal spots are inconsistent." published at TBD (github.com)

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Disclosures

The authors declare no competing interests.

Author contributions

Conceptualization: P.H.; Methodology: I.E.R, M.F.C. and P.H.; Software: I.E.R; Validation: I.E.R and P.H.; Formal Analysis: I.E.R and M.F.C.; Investigation: I.E.R, M.F.C., S.C. and P.H.; Resources: P.H. and G.D.I.; Data Curation: I.E.R, M.F.C. and S.C.; Writing – Original Draft: I.E.R and P.H.; Writing – Review & Editing: I.E.R and P.H.; Visualization: I.E.R and M.F.C.; Supervision: S.C., G.D.I and P.H.; Project Administration: S.C. and P.H.; Funding Acquisition: G.D.I and P.H.

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