Paradoxical heat sensation in healthy subjects: peripherally conducted by Aδ or C fibres?

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Summary
Paradoxical heat sensation upon cooling of the skin has been reported in central as well as in peripheral neurological conditions. In our study, we examined this phenomenon in 35 naive healthy test subjects, of whom 23 experienced paradoxical heat sensation under test conditions. We measured the peripheral conduction velocities of cold sensation, warm sensation and of paradoxical heat sensation by using a quantitative sensory testing model of indirect peripheral conduction velocity measurement. This was based on comparison of measurements at a proximal and a distal site using two measurement methods, one inclusive and the other exclusive of reaction time. We found that the conduction velocity of paradoxical heat sensation (0.70 m/s) was similar to that of warm sensation (0.68 m/s), and that the conduction velocity of cold sensation (7.74–8.01 m/s) was considerably faster. Thus, we conclude that paradoxical heat sensation in healthy subjects is conducted peripherally via slow unmyelinated C fibres and not via the faster Aδ fibres. Consequently, we propose that paradoxical heat sensation is encoded via the heat sensing pathway, in accordance with the labelled-line code theory. The mechanisms proposed suggest a malfunctioning cold-sensing pathway disinhibiting the heat-sensing pathway, at peripheral, central or both levels, thus facilitating a paradoxical heat sensation.

Keywords: paradoxical heat sensation; quantitative sensory testing; conduction velocity; reaction time

Abbreviations: ANOVA = analysis of variance; HPC = heat, pinch, cold; PHS = paradoxical heat sensation; QST = quantitative sensory testing

Introduction
Paradoxical heat sensation (PHS) upon cooling of the skin was reported by Goldsheider as early as 1912, and has been found in 10–12% of healthy individuals (Hämäläinen et al., 1982; Hansen et al., 1996). Preheating was found to increase the incidence of this phenomenon markedly, to 35% of healthy subjects (Hämäläinen et al., 1982). More recent studies have demonstrated the phenomenon of PHS in apparently diverse neurological disorders, of both peripheral and central origin. It was found in 42% of patients with uraemic polyneuropathy (Yosipovitch et al., 1995) and in 63% of patients with probable or definite multiple sclerosis (Hansen et al., 1996), and experimentally in normal subjects under compression ischaemia block (Wahren et al., 1989; Yarnitsky and Ochoa, 1990).

Other studies have further demonstrated this ostensible aetiological diversity. The triple cold syndrome (Ochoa and Yarnitsky, 1994) is a syndrome of cold hypoaesthesia, cold limbs and cold hyperalgesia, described in patients with peripheral polyneuropathies or mononeuropathies of various aetiologies. The cold hyperalgesia has been described as a burning sensation induced by cooling stimuli, a description compatible with PHS. The Thunberg grill illusion (Craig and Bushnell, 1994), demonstrated in healthy subjects, describes the sensation of paradoxical strong or painful heat elicited by touching interlaced mildly warm and cool bars. This apparent aetiological diversity suggests different hypotheses pertaining to the mechanism of PHS. Primarily, it is necessary to investigate the way in which the stimulus is encoded. PHS could be transmitted via Aδ cold-specific fibres, but would be sensed as heat due to central modulation, thus behaving in accordance with the ‘pattern code’ theory of stimulus encoding. Conversely, PHS could be transmitted through C fibres which normally mediate sensations evoked by high temperature, thus behaving in accordance with the ‘labelled-line code’ theory of stimulus encoding.

We investigated PHS in healthy subjects by using a quantitative sensory testing (QST) model of indirect measurement of peripheral conduction velocity. We established the peripheral conduction velocities of PHS, warm sensation and of cold sensation. Consequently, by comparing these results...
we were able to determine whether PHS is conducted peripherally via C fibres or via Aδ fibres.

Methods
Subjects
The study was performed on 35 paid healthy subjects (16 men and 19 women) aged 18–36 years (median 22 years). All subjects filled in a health questionnaire and gave informed consent according to the Declaration of Helsinki. The study was approved by the Ethics Committee of The Technion Medical School, Haifa. Subjects with any sign of a neurological disorder, diabetes, renal disease or neoplastic disease were disqualified, as were subjects taking any medication which might have interfered with the study, e.g. analgesics.

Quantitative thermal sensory testing
All tests were performed using a thermal sensory analyser (TSA 2001; Medoc, Ramat Yishai, Israel). This consists of a probe (of interchangeable size) including a Peltier element and two thermistors, a subject feedback unit and a computer. The Peltier element cools and heats the contact plate while its temperature is measured by the two thermistors in the probe. The adaptation temperature in all tests was set to 32°C. The temperature range was limited to 0–50°C in order to prevent skin injury. Measurement accuracy of the methods employed was 0.1°C.

In order to calculate the reaction time indirectly for each site, we used two methods of threshold measurement: the method of limits, inclusive of reaction time, and the method of levels, excluding it (Yarnitsky, 1997). The method of limits as performed in this study was a modification of the classical method of limits.

Testing algorithms
Levels
A series of temperature ramps was administered to the subject’s skin, and after each one the subject replied whether he sensed anything, and if so, of what quality (hot or cold). The first predetermined temperature ramp was a step of 3.0°C (+3.0°C for warm sensation, –3.0°C for cold sensation and PHS), and this was halved at each turn temperature (the peak temperature of the stimulus at which the step-to-step stimulus change switches its direction, i.e. both the preceding and succeeding stimuli were either higher or lower than the one at the turning point). Thus, if the subject replied that the step was sensed (Y), the next step was halved (i.e. in this case to 1.5°C). However, if the subject replied that he sensed nothing (N), the next temperature ramp was increased to twice the initial step (i.e. in this case to 6.0°C), until the stimulus was sensed. At that point, the stimulus was lowered by a step half the size of the previous step, until the next turn point, where the step size was halved again, and so on. This went on until the step reached a predetermined size, in our case 0.1°C. The average of the last N and Y responses was taken as the equivalent sensation threshold.

Limits
A ramp of increasing or decreasing temperature was given, and the subject, using his feedback unit, pressed a button the instant he sensed something. After pressing the button, he told us what the sensation was. Such transients were given five times for each test, and their average was taken as the threshold. Thresholds obtained by this method are higher than those by the previous one, due to reaction time.

Two probes were used, a large one with an area of 3.0 × 4.5 cm (13.5 cm²) and a small one with an area of 1.6 × 1.6 cm (2.56 cm²). The large probe was used for testing the modalities of cold sensation and warm sensation, the rate of stimulus temperature change being 1.0°C/s, as routinely done in clinical QST applications. The small probe was used for PHS and cold sensation, and differed from the routine clinical application in having a high rate of temperature change (4.0°C/s). For PHS only, preheating was employed, giving five stimuli of heat pain at threshold intensity immediately prior to the measurement. Each probe was placed on two sites on the subject’s dominant lower limb: (i) anterior and superior to the lateral malleolus; and (ii) the proximal anterior thigh. The distance between these two sites (d₁₋₂) was measured.

The testing sequence
Each set of tests consisted of measuring the thresholds of the above sensations using limits and levels at both sites. The subjects were blinded to the type of sensation that was being tested. When tested for PHS, the sites were preheated prior to each test, as previously described. We defined PHS as a subjective feeling of heat upon cooling in >67% of all cold stimuli given with the small probe (on average around 25 such stimuli). The test sequence was randomized for the factors of probe size, starting site and starting method, except that (i) cold sensation was always tested before warm sensation in order to avoid the preheating effect, and (ii) small-probe warm sensation was not tested. The overall test time was 1.5–2 h per subject.

Determination of reaction time
Reaction time was determined indirectly for each site by calculating the difference between the thermal thresholds obtained by the two methods. This temperature difference was divided by the predetermined rate of stimulus temperature change (rate), thus yielding the time between the administration of the stimulus of sufficient intensity and eventual perception of the sensation (Yarnitsky and Ochoa, 1991) (equation 1).
Conduction velocity was calculated according to the following formula (equation 2):

\[
RT = \frac{|\text{levels (°C)} - \text{limits (°C)}|}{\text{(°C/s)}}
\]  

(1)

where RT = reaction time in seconds.

**Determination of conduction velocity**

The peripheral conduction velocity was determined using a model of indirect measurement, which was based on the work of Fowler et al. (1988), incorporating the reaction time determination method reported by Yarnitsky and Ochoa (1991), as described above.

We determined the reaction time of both sites as described above in equation 1, and the distance between the two sites \(d_{1-2}\). We then extracted the peripheral conduction time by calculating the difference between \(RT_1\) and \(RT_2\) \((\Delta t)\). Thus, conduction velocity was calculated according to the following formula (equation 2):

\[
\text{Conduction velocity} = \frac{d_{1-2}}{\Delta t}
\]

(2)

where conduction velocity is expressed in metres per second, \(d_{1-2}\) in metres and \(\Delta t\) in seconds.

The calculation might slightly underestimate the velocity because of the normal curvature of nerves. This model assumes equal central processing time for the two sites.

**Statistical analysis**

We calculated the mean values of \(\Delta t\) and \(d_{1-2}\) for all subjects, and their respective standard deviations. We then calculated the conduction velocity by dividing the mean \(d_{1-2}\) by the mean \(\Delta t\).

The statistical analysis consisted of a repeated measures (mixed model) analysis of variance (ANOVA) run under SAS (SAS Institute, Cary, NC, USA) Procedure MIXED, with sensory modality as the repeated variable and peripheral conduction time as the dependent variable. An appropriate covariance structure was chosen, based on Akaike’s Information Criterion, from among the following: compound symmetry; heterogeneous compound symmetry; Huynh-Feldt; and Variance Components/Simple. Scheffe’s test was used for post hoc comparisons among all pairwise combinations of modalities.

**Results**

**Paradoxical heat sensation**

PHS was reported by 23 subjects (66%) using the previously described protocol. All these subjects reported sensations of heat upon cooling of the skin in >67% (mean 90%) of the stimuli given.

Upon further analysis of the results regarding factors such as probe size, rate of stimulus temperature change and preheating, we found that PHS occurred in only 6% of the tested subjects using a large probe, a slow rate of stimulus temperature change and without preheating. This percentage increased to 26% when we used a smaller probe and a faster rate of stimulus temperature change, but still without preheating. When preheating was applied to the tested area using the smaller probe and the faster rate of stimulus temperature change, this percentage further increased to 66%.

The need to control for non-paradoxical sensation under these circumstances led to the measurement of cold sensation conduction with the small probe at the fast rate, without preheating.

For a small number of subjects, outlying results (>4 SD above mean) were excluded: one outlying result was excluded for cold sensation and two for warm sensation. Other exclusions consisted of two more subjects who had PHS upon attempts to produce cold sensation.

**Thresholds, reaction times and conduction velocities**

The mean thresholds of the tested sensations are shown in Table 1, as are the distances and the calculated reaction times of the tested sites. As expected, the thresholds obtained by the method of limits were higher than those obtained by the method of levels, and the calculated reaction times for the distal site were longer than those for the proximal site. Mixed model ANOVA, using a heterogeneous compound symmetry covariance structure (which Akaike’s Information Criterion indicated was the most appropriate for these data), indicated an overall effect of sensory modality on peripheral conduction time \(F(3,65) = 15.95, P = 0.0001\). Scheffe’s test indicated that the extracted peripheral conduction time \(\Delta t\) of PHS was significantly longer than that of cold sensation \((P < 0.0001)\) and of small-probe cold sensation \((P = 0.03)\). In addition, there was no difference between cold sensation and small-probe cold sensation and between PHS and warm sensation, while cold sensation and warm sensation did differ statistically (Scheffe’s test, \(P = 0.0001\)). The mean \(\Delta t\) of cold sensation was 0.07 s and that of small-probe cold sensation 0.08 s, while that of warm sensation was 0.86 s and that of PHS was 0.84 s. Thus, the subsequent calculated peripheral conduction velocities of cold sensation (8.01 and 7.74 m/s for large and small probes, respectively) were significantly faster than those of the similar and slower conduction velocities of warm sensation (0.68 m/s) and PHS (0.70 m/s). We consequently concluded that the peripheral conduction velocity of PHS is similar to that of warm sensation.

Analysis of the sex subgroups did not show any significant differences throughout the study.

**Discussion**

It is commonly accepted that cold sensation is conducted peripherally by myelinated cold-specific Aδ fibres and that
### Table 1  Thresholds, rate, calculated reaction times, % distances and conduction velocities between the sites with respective standard deviations

<table>
<thead>
<tr>
<th>Sensation tested</th>
<th>No. of subjects</th>
<th>Surface area of probe (cm²)</th>
<th>Rate (°C/s)</th>
<th>Site 1 (lateral malleolus) (thresholds and calculated RT ± SD)</th>
<th>Site 2 (proximal anterior thigh) (thresholds and calculated RT ± SD)</th>
<th>( d_{1-2} ) (m)</th>
<th>( \Delta t ) (s)</th>
<th>CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td>32</td>
<td>13.5</td>
<td>1.0</td>
<td>-0.85 ± 0.88, -1.82 ± 0.99, 0.97 ± 0.59</td>
<td>-0.90 ± 0.95, -1.80 ± 0.97, 0.90 ± 0.60</td>
<td>0.58</td>
<td>0.07</td>
<td>8.01</td>
</tr>
<tr>
<td>Small probe, cold</td>
<td>15</td>
<td>2.56</td>
<td>4.0</td>
<td>-3.61 ± 2.84, -9.29 ± 4.00, 1.42 ± 0.79</td>
<td>-4.21 ± 2.93, -9.58 ± 3.90, 1.34 ± 0.62</td>
<td>0.59</td>
<td>0.08</td>
<td>7.74</td>
</tr>
<tr>
<td>Warm</td>
<td>33</td>
<td>13.5</td>
<td>1.0</td>
<td>1.84 ± 2.26, 4.44 ± 2.34, 2.60 ± 0.88</td>
<td>0.93 ± 1.45, 2.67 ± 2.05, 1.74 ± 0.77</td>
<td>0.58</td>
<td>0.86</td>
<td>0.68</td>
</tr>
<tr>
<td>PHS</td>
<td>23</td>
<td>2.56</td>
<td>4.0</td>
<td>-3.29 ± 2.36, -11.22 ± 4.17, 1.98 ± 0.89</td>
<td>-3.60 ± 2.47, -8.15 ± 3.35, 1.14 ± 0.68</td>
<td>0.59</td>
<td>0.84</td>
<td>0.70</td>
</tr>
<tr>
<td>Preheating values for PHS*</td>
<td></td>
<td></td>
<td></td>
<td>-49.07 ± 1.28, 48.81 ± 1.48</td>
<td>47.51 ± 4.47, 47.01 ± 3.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Preheating was done by giving five stimuli of heat pain threshold using the method of limits. This was done prior to each test. The maximum temperature was limited to 50°C to avoid skin damage.
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warm sensation is conducted by unmyelinated warm-specific C fibres (Darian-Smith, 1984; Fowler et al., 1988; Martin and Jessell, 1991; Yarnitsky and Ochoa, 1991). Recent studies have demonstrated that C polymodal nociceptors participate in the mediation of painful low temperature stimuli (cats: Craig and Bushnell, 1994; primates: Simone et al., 1994; man: Campero et al., 1996).

The conduction velocity of these fibres has been studied by several strategies. Neurophysiological studies have shown the conduction velocity of Aδ cold fibres to be 6.3 m/s (primates: Hensel and Iggo, 1971) and 14.5 m/s (monkey: Darian-Smith et al., 1973), and the conduction velocity of C warm fibres to be 0.7 (primates: Hensel and Iggo, 1971) and 0.8 (monkey: Duclaux and Keshalo, 1980). By using microneurography in humans, Torebjörk (1974) found velocities of 0.5–1.2 m/s for C nociceptor fibres, and Adriaensen et al. (1983) found a range of 19.2 ± 7.2 m/s for Aδ fibres of both low and high mechanical thresholds. For pain-related somatosensory evoked potentials following laser CO2 stimulation, human Aδ pain primary afferents were shown to have a conduction velocity of 9.0 m/s (Kakigi et al., 1991). In the few QST studies that have been performed, the conduction velocities of Aδ fibres and C fibres were found to be 2.6 and 1.6 m/s, respectively (Yarnitsky and Ochoa, 1991) and 2.1 and 0.2 m/s, respectively (Fowler et al., 1988). The ‘textbook’ conduction velocities are, thus, 5–30 m/s for myelinated Aδ fibres and 0.5–2 m/s for C fibres (Martin and Jessell, 1991). The conduction velocities found here for warm sensation fall well within expected limits. The conduction velocities for cold sensation, however, deviate from those reported by the previous psychophysical reports, probably because of methodological drawbacks in those papers; Fowler et al. (1988) used reaction times to thermal stimuli at distal and proximal sites, without considering the precise threshold at each site. Yarnitsky and Ochoa compared thresholds of reaction time-inclusive and -exclusive methods for a specific site, assuming a certain value for central processing time. In the present method, these two drawbacks are taken care of. The present study found results which are consistent with the neurophysiological literature.

The spinal conduction of both thermal sensations is through the spinothalamic tract (Darian-Smith, 1984; Lindsay et al., 1991). There is considerably less information about the central processing of thermal sensations. It has been shown recently that the pathway starting with peripheral Aδ fibres reaches cold cells in the thalamus, and that the pathway starting with C fibres reaches heat-pinch-cold cells (HPC cells) in the thalamus, via lamina I at the dorsal horn (Craig and Bushnell, 1994). The anatomical location of these cells was suggested to be the posterior part of the ventral medial nucleus for cold cells and the causal part of medial dorsal nucleus for the HPC cells (Craig, 1994). A low-temperature stimulus activates, according to these authors, both the Aδ fibre–cold cell and the C fibre–HPC cell pathways and thus generates a dual message with a cold component and a heat or pain component. Normally, the Aδ fibres inhibit the C fibres peripherally when the two primary afferents meet at the dorsal horn (gate control equivalent (Lindsay et al., 1991)), and centrally at the thalamic level, where the cold cells inhibit the HPC cells so that only the cold component of the sensation is perceived (Craig and Bushnell, 1994) (Fig. 1A).

The phenomenon of PHS raises fundamental issues about the nature of thermal sensation. Principally, it is necessary to elucidate the very nature of the way in which stimuli are encoded. Hypotheses pertaining to the encoding of PHS are presented below.

A pattern code-encoding modality in which PHS is conducted via the Aδ–cold cell pathway but is felt as heat because of an altered pattern of firing of the normally cold-sensing receptor

Firing pattern had been suggested to be a determinant in the generation of thermal sensations (Emmers, 1976; Schingnitz and Werner, 1980). The conduction velocity of PHS would be similar to that of cold sensation in the QST model of conduction velocity measurement (Fig. 1B).

A labelled-line code modality in which PHS is conducted via the C fibre–HPC cell pathway as a result of disinhibition

It is currently accepted that almost all sensory coding is done via the labelled-line code (Lindblom and Ochoa, 1986; Martin, 1991). This would suggest that PHS has to be conducted through a heat-sensing pathway, the C fibre–HPC pathway, since the sensory pathways are committed. Disinhibition could be either peripheral or central.

The peripheral hypothesis

Malfunctioning Aδ fibres disinhibit C fibre nociceptors by a mechanism of gate control in the dorsal horn of the spine, thus facilitating PHS (Yarnitsky and Ochoa, 1990; Lindsay et al., 1991; Ochoa and Yarnitsky, 1994; Yosipovitch et al., 1995) (Fig. 1C). This theory is supported by experiments on selective conduction blockade of the Aδ fibres using a sphygmonanometer cuff. As a result, the subjects felt a hot burning sensation upon further cooling stimuli (Yarnitsky and Ochoa, 1990). The peripheral hypothesis is in accordance with the finding of PHS in patients with uraemic polyneuropathy and in patients with the triple cold syndrome.

The central hypothesis

Malfunctioning cold cells in the thalamus disinhibit the HPC cells in the thalamus, thus facilitating PHS (Craig, 1994; Craig and Bushnell, 1994; Hansen et al., 1996) (Fig. 1C). The central hypothesis is in accordance with the finding of PHS in patients with multiple sclerosis.
In both the peripheral and the central hypothesis, the PHS is ultimately delivered via the C fibre–HPC pathway. Therefore, the indirectly measured peripheral conduction velocity of PHS would be similar to that of warm sensation in both cases.

An additional model in which PHS is conducted peripherally by Aδ cold fibres but is transmitted onto the C fibre–HPC pathway centrally

Findings regarding the phenomenon of secondary hyperalgesia in normal skin surrounding a local injury may be applicable to a separate type of understanding of PHS. It has been shown (although controversy exists regarding these findings) that secondary hyperalgesia is conducted peripherally by Aβ fibres, which normally do not mediate painful sensations. The message conveyed by these fibres is transformed in the dorsal horn onto wide-dynamic range and high-threshold cells in the spinothalamic tract, thus producing a sensation of pain (LaMotte, 1992). Analogously, this model can be suggested for PHS, where the low-temperature stimulus activates Aδ primary afferents, which, in turn, transmit the message abnormally to the heat-sensing pathway in the CNS, although no experimental evidence exists for this model (Fig. 1D). In this case, the peripheral conduction velocity of PHS would be similar to that of cold sensation.

In this study, our aim was to produce PHS in a maximum number of subjects in order to amass as much data as we could in order to calculate the peripheral conduction velocity. As stated in the Introduction, we know that preheating the designated area increases the incidence of PHS to ~35%. Through our preliminary tests we have also come to realize that this percentage can be further increased by the use of a probe with a smaller area, and by raising the rate of change of the stimulus temperature. These conditions are somewhat unexpected, since the higher density of cold than of warm receptors predicts the opposite. It might be that temporal summation properties are different for the two types of fibre, such that the warm message is further augmented under these conditions. Thus, we defined the testing sequence described in the Methods section.

The result was conclusive. The peripheral conduction velocity of PHS was found to be almost equal to that of warm sensation. Thus, we propose that PHS is conducted peripherally by C fibres, and not by Aδ fibres. Consequently, we conclude that PHS is encoded via the C fibre–HPC heat-sensing pathway, in accordance with the labelled-line code theory (Fig. 1C). The present study cannot determine where in the sensory pathway the disinhibition takes place—peripherally or centrally. These two possibilities are not necessarily mutually exclusive and can thus coexist. Thus, peripheral neuropathies may cause higher incidences of PHS through Aδ fibre dysfunction, and multiple sclerosis may cause the same symptom through a completely different mechanism of central thalamic cold-cell dysfunction.
As stated above, the nature of the method used in our study does not enable us to distinguish which one of these mechanisms, peripheral or central, is responsible for PHS in healthy subjects. This is due to the indirect nature of the peripheral conduction velocity measurement used in quantitative sensory testing. Nonetheless, our study suggests that PHS may be a peripheral phenomenon in healthy subjects; the dependence of PHS on probe size and the rate of temperature change corresponds to the relatively small number of Aδ fibres in the peripheral nerve—a smaller probe and a shorter stimulus duration reduce the amount of Aδ evoked activity to less than the minimum required for sensation. Another issue not resolved by this conduction velocity-based study is the type of C fibre conducting the PHS. On the one hand, the low thresholds of PHS suggest non-nociceptive fibres, but on the other hand evidence from the cuff experiments (Yarnitsky and Ochoa 1990), from Simone (1994) and from Craig and Bushnell (1994) suggests that it is the C nociceptors that mediate PHS. An additional consideration is the abundance of nociceptors compared with warm-specific fibres. The appearance of PHS after preheating also favours the latter possibility, via a possible mechanism of nociceptor sensitization.

In summary, the peripheral conduction velocity of PHS was found to be statistically equivalent to that of warm sensation using a QST model of indirect conduction velocity measurement. We thus conclude that PHS is encoded via the C fibre–HPC pathway in accordance with the labelled-line code theory.

Since PHS can be an early expression of the uraemic polyneuropathy (Yosipovitch et al., 1995) and potentially other polyneuropathies, further studies into its mechanism in such patients will be of clinical value.

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