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Disturbed sensory perception of changes in thermoalgesic stimuli in patients with small fiber neuropathies

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ABSTRACT

The assessment of functional deficits in small fibre neuropathies (SFN) requires using ancillary tests other than conventional neurophysiological techniques. One of the tests with most widespread use is thermal threshold determination, as part of quantitative sensory testing. Thermal thresholds typically reflect one point in the whole subjective experience elicited by a thermal stimulus. We reasoned that more information could be obtained by analyzing the subjective description of the ongoing sensation elicited by slow temperature changes (dynamic thermal testing, DTT). Twenty SFN patients and 20 healthy subjects were requested to describe, by using an electronic visual analog scale system, the sensation perceived when the temperature of a thermode was made to slowly change according to a predetermined pattern. The thermode was attached to the left ventral forearm or the distal third of the left leg and the stimulus was either a monophasic heat or cold stimuli that reached 120% of pain threshold and reversed to get back to baseline at a rate of 0.5°C/s. Abnormalities seen in patients in comparison to healthy subjects were: (1) delayed perception of temperature changes, both at onset and at reversal, (2) longer duration of pain perception at peak temperature, and (3) absence of an overshoot sensation after reversal, ie, a transient perception of the opposite sensation before the temperature reached again baseline. The use of DTT increases the yield of thermal testing for clinical and physiological studies. It adds information that can be discriminant between healthy subjects and SFN patients and shows physiological details about the process of activation and inactivation of temperature receptors that may be abnormal in SFN.

1. Introduction

Small fiber neuropathies (SFN) are characterized by sensory disturbances attributable to the involvement of thinly myelinated Aδ-fibers and unmyelinated C-fibers of somatic and autonomic nerves [24,28]. Conventional electrodiagnostic techniques such as nerve conduction studies and electromyography are of little use for assessment of SFN, and therefore, additional techniques should be taken into account [24,29,46]. Probably, the most widely used method to reveal the hypothesized dysfunction of small fibers in patients with suspected SFN is quantitative sensory testing (QST) and, more specifically, quantitative thermal testing (QTT) for determination of thermal thresholds [43,46].

Thermal thresholds are typically examined by applying controlled thermal stimuli. Subjects are expected to detect the stimulus and produce a relatively simple answer [43]. The need for subjects' collaboration and the risk of having equivocal results are still the main drawbacks of the technique. Furthermore, the information obtained with thresholds determination regards typically one point along the whole stimulus-induced experience (the point at which subjects perceive warmth, cold or pain), disregarding the sensations that subjects might experience during the presentation and withdrawal of the stimulus. We considered that analyzing such transient change in sensation could allow for more data points of interesting information and therefore add to the clinical applicability of the test. This requires that the change in temperature is slow enough to allow for the subjects to express the ongoing changes in their sensory perception. A slowly modifying thermal stimulus has been used only scarcely with clinical purposes [23,44,48]. However, we considered that it would reflect the

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processes of activation and inactivation of receptors for heat and cold in the skin under the thermode, albeit filtered through subjective perception and conduction time in the thermoalgesic fibers [5].

In the study presented here, we used relatively long lasting slowly varying wide temperature changes to request our subjects to express their sensation online, using an electronic visual analog scale system. This form of dynamic thermal testing (DTT) has been used so far for the evaluation of refractoriness in the thermoalgesic pathway in healthy subjects [39,40] as well as an additional test for the assessment of patients with syringomyelia [44]. We aimed at expanding our knowledge on psychophysical perception during thermoalgesic stimuli and establishing relevant outcome measures from DTT as well as their normative reference values after exploring a group of healthy volunteers. We also explored the clinical utility of DTT in the assessment of SFN patients through comparison with conventional thermal thresholds determination.

2. Methods

2.1. Subjects

The study was carried out in 20 healthy subjects (HS) and in 20 patients with SFN. HS were 12 men and 8 women with a mean age of 42.4 ± 16.9 years (range 22 to 76 years). None of them reported neurological symptoms or had personal or family history of diseases that could potentially lead to polyneuropathy. They referred no exposure to alcohol or other social, pharmacological or environmental toxins and their basic physical neurological exam of motor and sensory domains was normal. The SFN patients were recruited among those followed in the Neuropathic Pain Unit of the Neurology Department of the Hospital Clinic in Barcelona due to painful polyneuropathy. They were selected according to the diagnostic criteria for SFN on clinical and electrodiagnostic exams [14,23,28]. Patients considered were those that complained of neuropathic pain of length-dependent topographical distribution, with history of a disease known to cause SFN or that, in the absence of an etiological diagnosis, had histological evidence of decreased density of intraepidermal nerve fibers in skin biopsy [9]. Candidate patients were all screened before inclusion using clinical assessment, conventional electrodiagnostic tests, nociceptive evoked potentials recording and thermal threshold determination (see below). Exclusion criteria were: (1) unilateral, asymmetrical or multifocal symptoms or signs; (2) suspicion of radiculopathy or myelopathy; (3) clinical signs of relevant dysfunction in large sensory fibres or signs of demyelinating, predominantly motor or predominantly large fibre polyneuropathies after assessment with conventional nerve conduction studies; (4) patients who were unable to understand the instructions. All participants gave their informed consent for the study, which was approved by the ethics committee of the Hospital Clinic.

2.2. Characterization of patients

2.2.1. Clinical assessment

Patients were presented with questions in regard to their sensory symptoms which included the quality of their spontaneous sensation and the distribution of their symptoms. We assessed muscle strength, patellar and ankle tendon jerks; joint position at the first toe, vibration sensation at the first toe and the internal malleolus; tactile sensation (with a cotton swab) and pricking pain (with a disposable needle) in the dorsum of the foot, mid leg, and thigh.

2.2.2. Conventional electrodiagnostic tests

Nerve conductions studies (NCS) of the lower limbs were performed using either a Mystro5Plus electromyograph (Oxford Instruments, Oxford, UK) or a KeyPoint Net (Alpine Medical Instruments). We calculated nerve conduction velocity and measured compound action potential amplitude of common peroneal (motor) and sural (sensory) nerves, according to standard methods [22]. We determined whether or not the results were within normal limits, according to the reference values of our department [35].

2.2.3. Nociceptive evoked potentials

Contact heat stimuli were applied to the left ventral forearm and to the distal third of the left leg, with a thermofoil thermode stimulator, with a surface area of 572.5 mm^2 (Pathway, Medoc Ltd, Israel). Baseline temperature of the thermode was set at 32°C and increased at a rate of 70°C/s, to reach a peak temperature of 53°C. Contact heat-evoked potentials (CHEPs) were recorded from Cz (vertex potentials) referenced to linked earlobes (A1–A2), which is where CHEPs have their maximal amplitude [5]. Impedance was kept below 5 kΩ. Blinking was monitored for artifact control with surface electrodes attached over the orbicularis oculi muscle. For recording, we used a KeyPoint (Alpine Medical Instruments) electromyograph. Filters were set at 0.1 to 30 Hz and signals were sampled at a rate of 500 Hz.

CHEPs were recorded in a warm and dimly lit room. Stimulus sites were randomized (foot and hand) according to established recommendations [18]. Care was taken to maintain the patient's attention while applying 18 to 20 consecutive stimuli at each region. A random interval of 10 to 20 s was left between 2 consecutive stimuli. In each subject, we obtained a minimum of 12 blink and artifact free traces that were conveniently stored for off-line analysis. We performed an electronic averaging to measure mean N2 latency and N2/P2 amplitude. Results were compared to the reference values of our laboratory for CHEPs latency and amplitude [9]. Results above or below 95th percentile were considered abnormal.

2.3. Psychophysical testing

2.3.1. Thermal thresholds determination

All psychophysical studies were performed in a quiet room at a constant temperature of 24°C. Thermoalgesic stimuli were applied with a Peltier thermode with a surface area of 12.5 cm^2 (MSA, Somedic, Sweden). Stimulation sites were the left ventral forearm and the distal third of the left leg. Baseline thermode temperature was always set at 32°C. The same technician (MM) performed all psychophysical tests.

We used the method of limits [46] to determine warm detection threshold (WDT), heat pain threshold (HPT), cold detection threshold (CDT), and cold pain threshold (CPT). Four stimuli were provided at a pace of one every 20 s, with ramps of 1°C/s. Cutoff temperatures were 10°C and 50°C. Subjects were given a switch button to press at the moment at which they perceived the sensation under examination (either warmth, heat pain, cold, or cold pain). After each stimulus, subjects were asked for the quality of sensation perceived in order to detect possible paradoxical sensations [37,38].

2.3.2. DTT examination

The study of DTT was done in a separate session, the same time of the day, in the same body sites, and by the same examiner (MM) as for thermal threshold determination. We devised a simple stimulus paradigm according to preliminary experiences and previous

data [10,11,39,40,44]. The same paradigm was systematically applied to all subjects. After an initial period of 5 s at 32°C (baseline), the temperature of the thermode was made to either increase or decrease at a rate of 0.5°C/s. For heat stimuli, we programmed the thermode to reach 120% of the subject's heat pain threshold or 50°C. For cold stimuli, the temperature descended up to threshold for cold pain or 10°C. At those points, the thermode was made to return to baseline at the same rate. These monophasic heat and cold stimuli were repeated 3 times for each site and presented in a random order. The thermode was moved gently to a slightly different position along an imaginary line perpendicular to the axis of the limb after each stimulus.

Subjects were instructed to use the electronic visual analog scale (VAS) system, consisting of an 11-cm-long linear potentiometer (SENSELab; Somedic, Sweden). The potentiometer was installed in a metallic box where it was activated by a lever that could be moved without resistance along its course. We marked on the side of the lever a neutral point and 2 continuous scales, one toward warm and heat pain sensations and the other toward cold and cold pain sensation. It was emphasized that the direction of the temperature change from baseline could be toward either cold or heat at any given trial. To assist the subjects in working with the electronic VAS, positions were color graded (white in the neutral position, yellow turning to red in the heat zone, and turquoise turning to blue for the cold zone).

Subjects were not told about the type of stimulus that they were going to receive, nor that the stimuli were going to be monophasic. The specific instructions were that they had to pay attention to the quality and intensity of the thermal sensation, move the lever with their right hand according to the ongoing changes in their sensation, and keep signaling those changes until the experimenter declared the end of the trial. Data from the electronic VAS were recorded together with the temperature signal generated by the thermode during the entire trial. The 2 inputs were digitized at a sampling rate of 200 Hz and fed into a computer equipped with software for off-line analysis (Acknowledge; Biopac Systems, Bionic Iberica, Spain). Correct thermode temperature output was checked at onset and end of every experiment using a precision thermometer (4600, Medoc, Israel).

2.3.3. Psychophysical data analysis

We first analyzed the data gathered from HS and set the reference values for the analysis of data from individual patients. In thermal threshold determination, we calculated the mean ± 2.5 standard deviations in healthy subjects and considered abnormal those values in patients that were beyond those limits. We also measured the difference between initial detection of temperature change and pain perception for heat and cold (respectively, WDT to HPT = ΔH, and CDT to CPT = ΔC). We recorded the number of trials, if any, in which subjects referred to paradoxical sensations during heat or cold stimuli.

In the analysis of DTT data, we considered the results gathered in the form of graphs from the electronic VAS. Shapes and timing of the events were consistent in all HS, in accordance with data partially reported elsewhere by our group [40]. Representative recordings are shown in Fig. 1 for heat and cold stimuli. On heat stimulation, healthy subjects marked the onset of perception, followed by a slow rising slope that paralleled the increase in temperature until reaching a plateau that typically began at a mean temperature of 38.3 ± 1.5°C for the upper extremity [40] and 39.2 ± 2.4°C for the lower extremity. This plateau ended at pain detection, with a steep rising slope up to the highest level of the individual's VAS. The descending phase was steeper than the rate of temperature decrease, stopping at about the neutral temperature level (baseline) or descending beyond baseline, to mark cold sensation. Such an overshoot sensation of cold (OC) was seen in most HS while temperature was still above baseline. In some, it occurred shortly after reaching baseline, as shown in Fig. 1. Because of the consistency in the behavior described, we defined the following 4 parametric outcomes in the domain of time (measured from onset of the temperature increase), as marked in Fig. 1A: (a) warm onset latency, as the time of the first VAS movement, (b) heat pain latency, as the time when subjects began to raise the VAS lever after the plateau, (c) max-VAS onset, as the time at which subjects first reached their maximum VAS score, and (d) duration of max-VAS, as the time at which subjects remained at their maximum VAS score. Obviously, measures (a), (b), and (c) were interchangeable with those of the temperature of the thermode at each time value, which we used for comparison with thermal threshold determination. Also, we measured the following 2 parametric outcomes on the domain of intensity and quality of the sensation: (e) the maximum VAS intensity perceived (max-VAS level), expressed as a percentage of the maximum possible displacement of the lever, and (f) the presence or absence of OC. OC was operationally defined as a decrease in temperature below baseline for at least 100 ms that occurred in the descending phase of the stimulus when the temperature did not yet reach baseline.

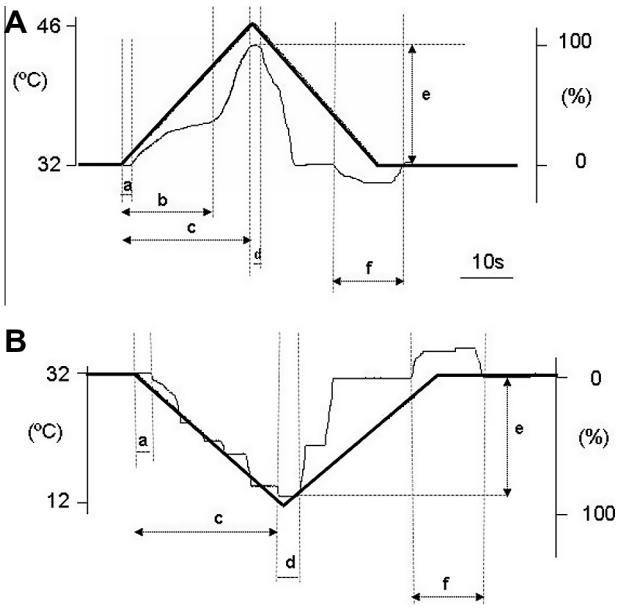

Fig. 1. Examples of DTT for heat (A) and cold (B) stimuli in a healthy subject that perceived overshoot cold and heat sensations. VAS, visual analog scale. In each graph, thermode temperature changes are represented as straight thick lines, according to the temperature scale on the left side, while the electronic VAS score is represented as thin lines, according to the VAS scale on the right side. Timing of the events was measured with respect to onset of temperature increase, considered as time 0, marked with an arrowhead in each graph. (a) Warm/cold onset; (b) heat pain onset; (c) max-VAS onset; (d) max-VAS duration; (e) max-VAS amplitude; (f) presence/absence of overshoot sensation. Note that (b) is absent in (B) because cold pain onset was not marked by our healthy subjects.

During cold stimuli, healthy subjects represented their sensation as cold in a linear progression to the peak of temperature decrease, followed by the recovery of the sensation up to baseline, or beyond, to mark heat sensation (overshoot sensation of heat, OH). Unlike warm stimuli, cold pain threshold could not be established with DTT because no clear plateau was marked by any subject (Fig. 1B). Therefore, the variables analyzed on cold stimuli were: (a) cold onset latency, (c) onset of max-VAS score, and (d) duration of max-VAS score in the time domain. We also measured (e) max-VAS, as the maximum VAS level in percentage of the maximum displacement of the lever, and (f) the presence or absence of OH.

2.4. Statistical analysis

Data for each of the parameters defined above were checked for normality with the Kolmogorov–Smirnoff test and were expressed as mean and standard deviation. Values deviating more than 2.5 standard deviations from the HS mean were considered abnormal.

We used the 1-factor ANOVA for comparison of parametric data between the 2 groups of subjects (HS and SFN). The Bonferroni's post hoc analysis was applied when significant differences were found to identify the parameter that caused the difference. For nonparametric data, we applied Mann–Whitney *U* tests. Statistical analyses were performed by SPSS software, version 18.0 (SPSS Inc, Chicago, IL, USA). A *P* value of <.05 was considered statistically significant. A 2×2 contingency table and Fisher's exact test was used for statistical comparison of the contingency data within each group of subjects.

3. Results

Etiologies of neuropathy were diabetes mellitus and impaired glucose tolerance in 8 patients, familiar amyloidotic polyneuropathy in 4, monoclonal gammopathy in 2, chemotherapy in 1, alcoholism in 1, hepatitis C infection in 1, and multifactorial in 1 (diabetes mellitus, familiar amyloidosis, alcoholism, and hepatitis C virus infection). Etiology was unknown in 2 patients.

3.1. Clinical, NCS, and CHEPs results

Table 1 shows epidemiological and clinical data, as well as results of NCS and CHEPS in patients. As per our exclusion/inclusion criteria, all patients had neuropathic pain and NCS within normal limits. We were unable to identify CHEPs to stimuli in both arm and leg in 2 patients, and to stimuli to the lower limbs only in 5 patients. Compared to reference data from our laboratory [9], patients had a statistically significant decrease of mean CHEPs amplitude and delay in mean CHEPs latency to lower limb stimulation (Table 1).

3.2. Thermal thresholds

Fig. 2 shows WDT, HPT, CDT, and CPT obtained in HS and patients. There were statistically significant differences between groups in data gathered in the upper limb (ANOVA; $F[1,38] = 10.5$; $P < .001$) and in the lower limb (ANOVA; $F[1,38] = 26.6$; $P < .001$). Post hoc analyses showed that patients had a higher WDT and CDT, and lower CPT, ΔW, and ΔC than HS ($P < .02$ for all comparisons). However, there were no significant differences between HS and patients in HPT for the upper limb and CPT for the lower limb. A warm/burning sensation during cold stimuli was reported by 50% of SFN patients ($n = 10$) in the upper limb and 65% of SFN patients in the lower limb ($n = 13$).

3.3. DTT

Table 2 shows a summary of the data in the time domain (mean, SD, and 95% confidence limits) obtained in the HS after analysis of the 11 parameters defined as in Fig. 1 (6 for heat stimuli and 5 for cold stimuli), and Fig. 3 shows the comparative distribution of relevant data between HS and patients for heat and cold stimulation in the temperature domain.

3.3.1. Heat stimuli

There were significant differences between groups for upper limb (ANOVA; $F[1,38] = 9.5$, $P = .004$) and lower limb (ANOVA; $F[1,38] = 21$, $P < .001$). The post hoc analysis indicated that patients had significantly delayed warm onset and max-VAS onset latencies and longer max-VAS duration than HS for both upper and lower limbs. There were no significant differences in max-VAS level for the upper or the lower limbs, but in general, the mean value was lower and the standard deviation was larger in patients than in HS. Heat pain detection threshold, reflected in DTT as the onset of the steep rising slope in VAS (marked in Fig. 1A as b) was consistently present in HS only. Its mean value, given as latency from onset of temperature stimuli in Table 2, corresponded to a mean temperature value of 40.8 ± 1.4°C (95th percentile 41.6°C) for the upper extremity and 42.4 ± 1.5°C (95th percentile 43.3°C) for the lower extremity. This point of inflexion of the VAS signal was not consistent in patients (Fig. 4A) and was therefore not included in the comparative analysis. The max-VAS onset latency in patients did not always occur before the peak of the temperature stimulus. This was the case for 1 HS and 6 patients in the forearm (5% vs 30%) and for 3 HS and 11 patients in the leg (15 vs 55%). These differences were significant in a 2×2 contingency table (Fisher's exact test; $P < .03$ for both comparisons). Although in HS the maximum

Table 1
Patient demographic, clinical, and electrodiagnostic data.[a]

Characteristic	Variable	SFN ($n = 20$)
Epidemiological data	Sex, M/F	8/4
	Age, y, mean (range)	56.7 ± 12.6 (29–78)
	Symptom duration, y, mean (range)	2.9 ± 2.7 (0.25–8)
Clinical data	Burning pain, *n* (%)	8 (66.7)
	Shooting pain, *n* (%)	5 (41.7)
	Allodynia, *n* (%)	1 (8.3)
	Paraesthesia, *n* (%)	2 (16.7)
	Dysesthesia, *n* (%)	4 (33.3)
	Sensory loss, *n* (%)	1 (8.3)
Nerve conduction studies	SNAP amplitude	12.1 ± 2.7
	SCV	48.9 ± 6.3
	CMAP amplitude	5.0 ± 0.7
	MCV	49.4 ± 5.6
CHEPs	Latency to foot stimulation (462 ± 63)	604 ± 60*
	Amplitude to foot stimulation (39 ± 10)	13.9 ± 7.9*
	Latency to hand stimulation (385 ± 69)	521 ± 63
	Amplitude to hand stimulation (40 ± 9)	17.6 ± 9.1

SFN, small fiber neuropathy; SNAP, sensory nerve action potential; SCV, sensory conduction velocity; CMAP, compound muscle action potential; MCV, motor conduction velocity; CHEPS, contact heat evoked potential.

[a] Values are expressed as mean ± SD, in μV for SNAP and CHEPs amplitude, in mV for CMAP amplitude, in ms for latency, and in m/s for conduction velocity. Sensory nerve conduction results correspond to sural nerve and motor nerve conduction to common peroneal nerve.

* Statistically significant delayed latency or reduced amplitude with respect to our own reference values (provided in parentheses), which are reported elsewhere [9].

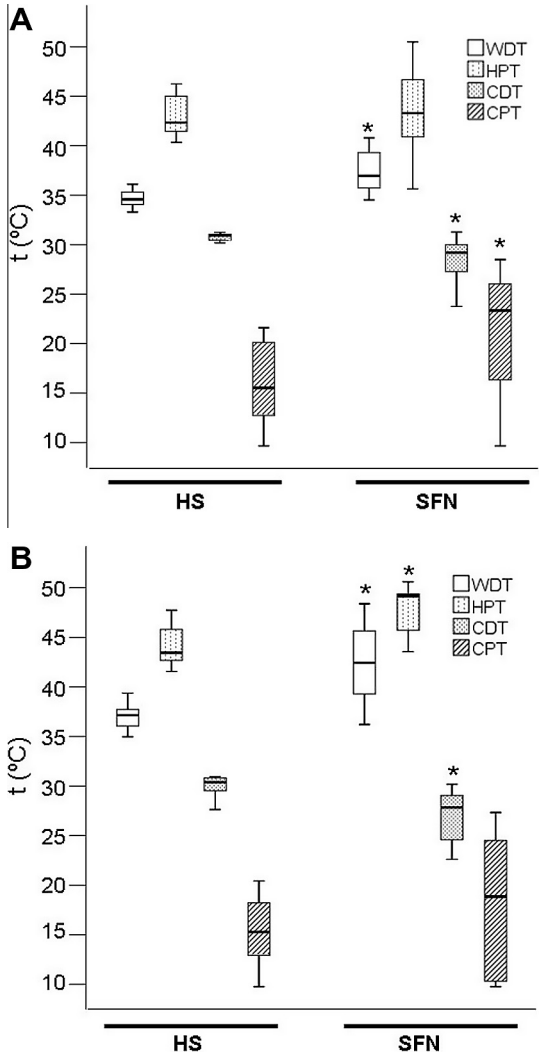

Fig. 2. Temperature thresholds obtained in the upper (A) and lower (B) limbs in HS and SFN patients. Box plots represent the median value with 5th and 95th percentiles. The asterisks indicate thresholds that showed statistically significant difference between HS and patients with SFN ($P < .02$). Note the reduced difference between thresholds for warm and heat pain, as well as between those for cold and cold pain, in patients with respect to HS. WDT, warm detection threshold; HPT, hot pain threshold; CDT, cold detection threshold; CPT, cold pain threshold; HS, healthy subject; SFN, small fiber neuropathy.

delay in max-VAS with respect to temperature peak was 1.5 s, it reached up to 7.5 s in patients to leg stimulation. For cold stimuli, no significant delay was observed in HS, and only 1 patient showed a delay of 2.2 s. These patients were assigned the value of their corresponding peak temperature in the graphs, showing the temperature values for comparison with HS (Fig. 3A,B). Interestingly, the delay in max-VAS did not shorten the time during which the patients reported maximum pain perception (max-VAS duration). On the contrary, there was no statistically significant difference between patients who reached max-VAS before and those that reached it after the temperature peak in regard to the time spent at max-VAS (*t* test; $P = .2$).

OC was observed in the upper and lower limbs of HS and SFN patients. However, there was a statistically significant difference in the percentage of subjects of each group that showed OC (95% of HS vs 40% of SFN patients in the upper limb and 85% of HS vs 25% of SFN patients in the lower limb; Mann–Whitney *U* test; $P = .007$ for the upper extremities and $P = .002$ for the lower extremities). Instead of OC, 7 patients (35%) marked a prolonged warm/hot sensation that continued for a few seconds even after stimuli had stopped. This abnormality was observed in both the arm and leg in 2 patients, in the leg in 4 patients, and in the arm in 1 patient.

3.3.2. Cold stimuli

Results obtained in HS and SFN patients with cold stimuli are also summarized in Table 2 and Fig. 3, and the graph results obtained from a representative patient are shown in Fig. 4B. ANOVA showed significant group differences between HS and patients for upper ($F[1,38] = 20.1$; $P < .001$) and lower limbs (ANOVA; $F[1,39] = 21.9$; $P \leqslant .001$). Post hoc analyses showed that patients had increased threshold (and therefore delayed onset) of cold perception. OH sensation was perceived only in the upper limbs by 2 HS and 1 SFN patients.

4. Discussion

SFN usually presents as a length-dependent painful neuropathy with disabling symptoms such as tingling, shooting, burning, prickling, aching, and other types of painful sensations, which worsen at night [24,28,29]. History taking and physical examination remain the gold standard for the diagnosis of SFN [28], but specific tests may be applied for further characterization of small fibers involvement. These include skin biopsy [12,27], nociceptive evoked potentials [7,42], noninvasive autonomic testing [31,32], microneurography [12,41], and psychophysical testing [38,46]. Among them, the latter is likely the technique most widely used. A standardized thermal threshold determination allows for categorization of SFN according to the patient's subjective experience of

Table 2
Parametric data from dynamic thermal testing.[a]

Parameter	Upper limb		Lower limb	
	HS	SFN	HS	SFN
Warm onset latency, s	3.1 ± 1.3 (2.2–3.9)	7.3 ± 5.4*	7.2 ± 3.2 (5.1–9.2)	20.3 ± 8*
Heat pain latency, s	17.8 ± 2.9 (14.2–20.1)	…	19.3 ± 3.9 (16.8–23.0)	…
Max-VAS onset time, s	25.9 ± 3.8 (23.6–27.8)	27.4 ± 6.4	27.0 ± 4.3 (24.8–29.8)	30.1 ± 6.9*
Max-VAS duration, s	2.1 ± 1.1 (0.8–2.9)	4.3 ± 1.4*	3.7 ± 1.4 (2.3–4.4)	10.3 ± 4.3*
Max-VAS level, %	87.5 ± 9.0 (81.8–93.2)	81.7 ± 21.9	87.8 ± 13.8 (79.1–96.6)	78.9 ± 29.7
OC, %	95	40*	85	25*
Cold onset latency, s	3.1 ± 1.4 (2.2–4.0)	7.7 ± 3.2*	3.4 ± 1.1 (2.7–4.1)	11.3 ± 6.6*
Max-VAS onset time, s	32.4 ± 3.8 (27.8–35.8)	34.1 ± 9.1*	33.1 ± 4.6 (30.5–36.3)	38.6 ± 9.8*
Max-VAS duration, s	2.6 ± 1.3 (0.8–2.1)	3.8 ± 1.3*	3.2 ± 1.3 (1.7–4.9)	5.7 ± 3.9*
Max-VAS level, %	83.9 ± 8.6 (80.1–89.0)	72.9 ± 17.8	85.5 ± 11.9 (79.6–93.1)	66.3 ± 15.8
OH, %	10	5*	0	0

HS, healthy subject; SFN, patients with small fiber neuropathy; MAX-VAS, mean of the individual maximal visual analog scale level; OC, overshoot cold; OH, overshoot heat.
[a] Data are expressed as mean ± 1 standard deviation (95% confidence values).
* Statistically significant differences with respect to data in HS.

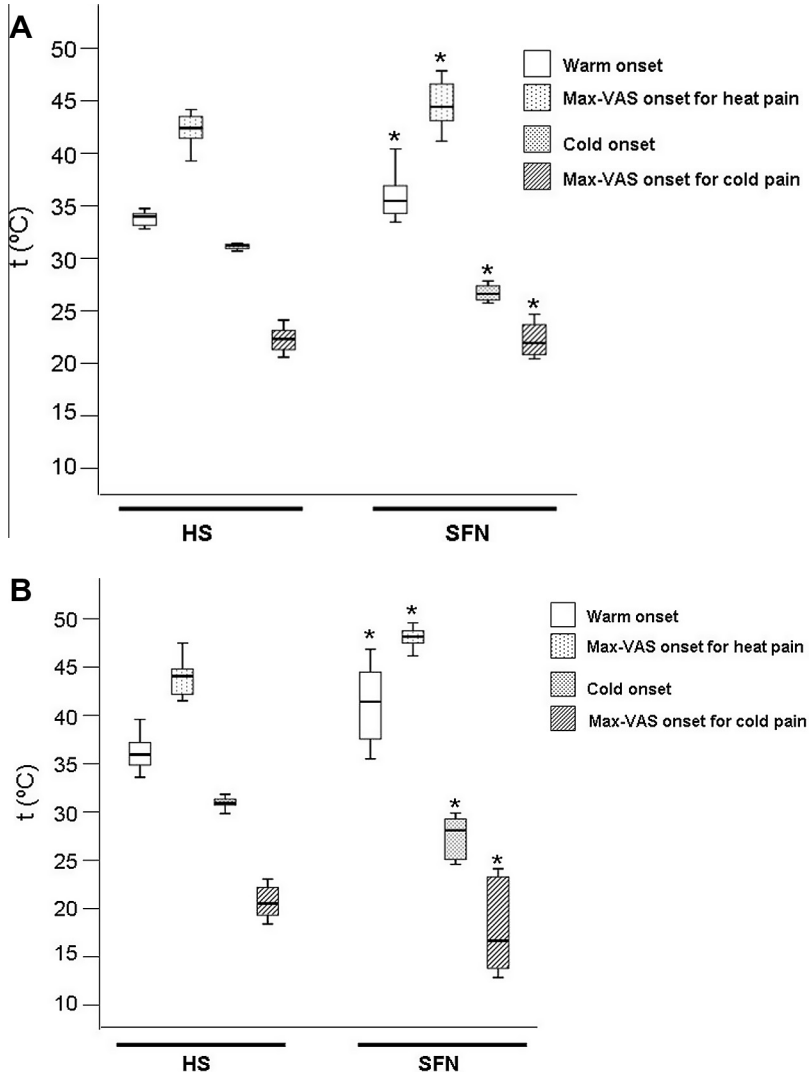

Fig. 3. Distribution of data obtained with DTT from HS and SFN patients on warm onset, cold onset and max-VAS onset latencies for heat and cold stimuli in upper (A) and lower (B) limbs. The median value is represented as a bar. The asterisks mark the statistically significant differences between HS and patients. Max-VAS, maximal visual analog scale; HS, healthy subject; SF, small fiber neuropathy.

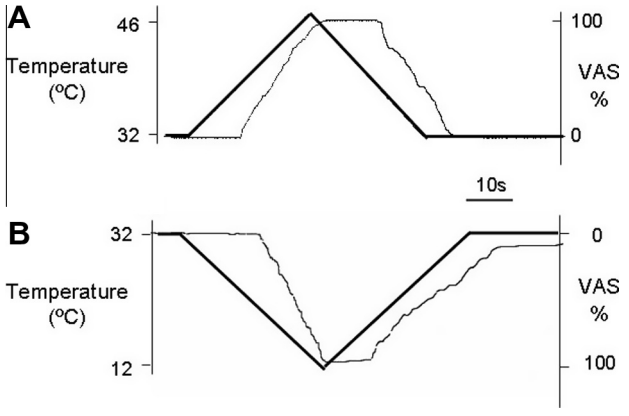

Fig. 4. Example of DTT recordings to 2 SFN patients in the upper limbs. Recordings from (A) heat stimulus and (B) cold stimulus. In both instances, latency of initial detection of temperature change (warm onset or cold onset) was delayed, duration of max-VAS perception was prolonged, and overshoot sensations (OC and OH) were absent. VAS, visual analog scale.

pure hypoesthesia, pure hyperalgesia/allodynia, hypoesthesia associated with hyperalgesia, and others [36,38,45].

In our study, thermal threshold determination revealed mainly warm and cold hypoesthesia in the upper and lower extremities (increased WDT and CDT), heat hypoalgesia in the lower extremities (increased HPT), and cold hyperalgesia in the upper extremities (decreased CPT). A similar type of abnormalities was obtained with the assessment performed with DTT, as summarized in Table 2 and Fig. 3. It is of note, though, that warm and cold detection threshold with DTT were reduced in comparison to, respectively, WDT and CDT. This is likely due to the different rate of temperature increase in both methods (1°C/s in QTT and 0.5°C/s in DTT), which leads to a larger increase in temperature in QTT than in DTT during reaction time [15,47]. In addition, DTT allowed us to obtain novel information on the characteristics of the sensory deficit in SFN patients.

The differences found between healthy subjects and patients can contribute to the diagnosis of SFN. One striking difference was the absence of any differentiation between warm and heat pain sensations when increasing the temperature in patients, in such a way that they had a significantly reduced or completely absent plateau preceding the onset of pain [10,39] (Fig. 1A). This plateau probably relates to the ceasing of warm receptor activation at a temperature in which there is not yet activation of pain receptors [39,40]. Being

delayed in their perception of onset of temperature stimuli, patients run late in their expression of temperature change. Pain sensation may come more sudden in patients than in HS to replace the progressively increasing warm sensation. It is difficult to know if there is a clinical correlate of such disturbance, but we could speculate that it may contribute to hyperalgesia because of reduced warning for the incoming pain. The Aδ fibers conveying pain have a faster conduction velocity and could have been relatively more spared in our patients than the C fibers conveying warm sensation. At low rates of temperature increase, sensation may be due to activation of unmyelinated nerve fibers [49], which are likely damaged in patients with SFN. This may lead to unexpected pain sensation if larger fibers are relatively spared.

After max-VAS, patients kept marking high VAS values for a longer time than healthy subjects. As a striking difference with HS, 7 of the patients with most marked clinical impairment kept expressing a mild sensation of warmth up to the end of the trial and described a descent of VAS only after the temperature again reached baseline. These observations likely document the difficulties that our patients had in the detection of ongoing temperature changes. Although we have not specifically examined attention and reaction time, we believe that the consistency of the observations among patients and the fact that patients with the most severe clinical impairment exhibited the most striking differences with healthy subjects suggest that the delay in detecting changes in temperature is indeed a feature of patients with SFN. One possible reason for such longer perception of pain after the stimulus may be the generation of multiple spikes in C-nociceptors, which has been suggested as a form of hyperalgesia triggered by thermal stimuli [4,42]. Other explanations include central nervous system amplification of signals, sensitization of the primary or secondary afferents, and induction of ectopic activity in nociceptive tracts at spinal cord and brain [2,16,26]. In fact, an abnormally long-lasting frontal lobe activity has been reported after nociceptive stimulation in patients with chronic pain [6].

In our study, HS showed a very fast decrease in their sensation after max-VAS, which, in most instances, reached up to the neutral zone when the temperature of the thermode was still above warm perception threshold. Such fast decrease in perception after heat pain has been described as offset analgesia [20] and is probably due to an active analgesic mechanism that may warn the subject about the release of a painful stimulus in preparation for the escape reaction. In a similar line, Yelle et al. [48] found that the rate of decrease in temperature perception does not depend on the velocity at which thermode temperature drops. Additionally, the abrupt decline in temperature perception can also be related to the fast inactivation rate of warm receptors that takes place with the decrease in skin temperature [17]. In our patients, the decrease in perception was significantly slower than in HS, suggesting an impairment of the mechanisms engaged in offset analgesia. A similar observation has been made recently by Niesters et al. [36], who reported no effect of ketamine or morphine on offset analgesia in spite of reduction of spontaneous pain scores in 10 patients with SFN. The physiological mechanisms of this interesting phenomenon have been extensively studied in healthy subjects by Martucci et al. [33,34].

Once perception returned to the neutral zone after heat pain, healthy subjects showed an overshoot, marking cold even if the thermode temperature was still above warm threshold. Expressing an overshoot sensation to the change from extreme to milder temperatures is a common experience in everyday life—for instance, when getting under shade on a hot summer day or sheltering from the wind in a cold winter day. Overshoot sensation is different from the sensations reported as paradoxical cold and paradoxical heat, which are defined by the presence of such sensations during, respectively, heat pain or cold pain stimuli [7,8,18]. These paradoxical sensations have been ascribed to the activation of polymodal receptors after functional loss of receptors for specific modality. Even though our findings show also a somehow paradoxical sensation (subjects felt cold when the temperature was still above warm threshold or warmth when the thermode temperature was still below cold threshold), they have a clearly different physiological mechanism. We considered that the term *overshoot sensation* fits well with the findings reported because the phenomenon described occurred as a prolongation of the change in sensation caused by the decrease or increase of temperature after reaching their maximum values. We found that compared to HS, SFN patients perceived this sensation in a significantly reduced percentage of trials, as another expression of dysfunction in the combined activation and inactivation of receptors. Cold receptors, like warm receptors, have 2 patterns of discharge. At static skin temperatures warm and cold fibers have an ongoing discharge and they increase or stop their activity in opposite directions depending on temperature changes [1,8,17,18,30]. Cold receptors discharge statically between 10 and 40°C, with maximum activity at 20 to 31°C [3,30]. They are sensitive to skin cooling, increasing their frequency of discharge even with temperature decrease of 0.5°C [8,17,18,30]. Campero et al. [8] have shown that cold receptors start to fire phasically below 35°C. Perception of paradoxical heat has been also described during cold stimulus [19,21]. Harrison and Davis [21] observed 2 types of paradoxical heat: a painful heat/warm perception that was more frequent during the cooling phase, and an innocuous warm perception that was more frequent during the rewarming phase and once stimulus had stopped [13,21]. Although overshoot cold was a consistent finding in our healthy subjects, this was not the case for overshoot heat. We have no clear explanation for this difference, but the fact that there is a different density and a different preferred temperature of receptor static discharge for warmth and cold skin receptors [25,30] should be taken into account. Cold receptors discharge statically between 10 and 40°C, with maximum activity at 20 to 31°C [3,30]. Warm receptors exhibit a static discharge between 30 and 48°C, with maximum activity at 43 to 47°C [1,30]. With the baseline temperature used in our study (32°C), cold receptors should have been activated to a relatively greater extent than warm receptors.

In summary, the use of DTT allowed us to separate between HS and SFN patients at a group level. The results have clinical value because they expand on the data from thermal threshold determination, bringing information on specific aspects of disturbance of thermoalgesic sensation. In comparison to HS, our patients had a shorter time lapse between onset of thermal sensation and peak of the heat pain; a longer duration of their maximum pain sensation; a slower rate of perception decrease after extreme temperature sensations; and a lack of transient overshoot sensation. All these abnormalities are likely consequences of peripheral and central nervous system dysfunctions in patients with neuropathic pain. The study of such dysfunctions with DTT may shed some light onto pathophysiological mechanisms contributing to pain that add to the conventional use of psychophysical testing of threshold determination.

Conflict of interest statement

The authors report no conflict of interest.

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