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Somatotopic Heat Pain Thresholds and Intraepidermal Nerve Fibers in Health

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Abstract

Introduction—For sequential and somatotopic assessment of small fiber neuropathy (SFN), heat pain (HP) tests of hypoalgesia might be used instead of decreased counts of epidermal nerve fibers (ENFs), but then healthy subject reference values of HP thresholds are needed.

Methods—Using the CASE IVc system, HP thresholds of hypoalgesia were estimated for ten unilateral sites and counts of ENFs for four of them in healthy subjects.

Results—In healthy subjects, small but statistically significant differences of both HP thresholds of hypoalgesia and counts of ENFs were observed among tested sites. Significant correlations between HP thresholds and counts of ENFs were not found.

Discussion—For the studied somatotopic sites, we provide 95th and 99th percentile reference limits for HP 0.5 and 5 of 1-10 HP thresholds of hypoalgesia and decreased counts of ENFs at 5th and 1st percentile levels.

Keywords

Heat pain thresholds; Epidermal nerve fibers; Heat pain tests of hypoalgesia; Somatotopic sites; Distal polyneuropathy; Small fiber neuropathy

INTRODUCTION

Small diameter sensory nerve fibers of physiologic classes A δ (small myelinated) and dorsal root C (unmyelinated) are frequently involved in distal sensorimotor polyneuropathies.^{1–3} In

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Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of conflicts of interest

None of the authors report a conflict of interest.

small fiber sensory polyneuropathies, feet and legs are usually selectively and symmetrically involved, but other patterns occur.^{2,4} Symptoms of pain are a frequent but not invariable occurrence in these small fiber sensory polyneuropathies.^{5,6} Quantitation of heat pain (HP) thresholds and counts of epidermal nerve fibers (ENFs) have been introduced to aid in detection, characterization, and quantitation of small fiber sensory polyneuropathies.^{7–14} Other physiologic approaches (near nerve sensory recordings, single sensory nerve fiber recordings, and recording of the compound action potential in vitro of excised fascicles of cutaneous nerves) have and are being used to study small fiber sensory nerve involvement in health and in peripheral nerve diseases.^{15,16,4,17–19} Tests of peripheral autonomic dysfunction are also used to quantify peripheral small nerve fiber disease.^{3,20}

The present studies provide healthy subject reference values of HP thresholds and ENF densities at somatotopic sites chosen to measure abnormality of small sensory nerve fibers in distal peripheral polyneuropathies (DPNs).^{9,21,22} These reference values are made available for use in medical practice and therapeutic trials. Also assessed is the somatotopic variability of HP sensation and ENF density and the correlation between HP threshold and ENF densities in health.

The two assessments, quantitative sensation tests (QSTs) of HP and estimates of ENFs, measure somewhat different aspects of small fiber DPNs. QSTing of HP assesses function and dysfunction, and for the latter, both hyper- and hypoalgesia. ENF counts, by contrast, are not a measure of function or dysfunction, but are a pathologic measure of small nerve fiber density from developmental or disease increase or decrease.^{14,23,12,24–26} As compared to counts of ENFs, QSTs of HP provide immediate results (assuming availability of reference values), can be assessed at the same site repeatedly, cost less, and can be assessed at body sites not usually biopsied, e.g., the face.

Quantitative assessment of ENFs has emerged as a widely used objective and referenced measure of small fiber sensory polyneuropathies.^{24,25,8,27–29} Typically, counts of ENFs are assessed on 3 mm skin punch biopsy specimens obtained at standard somatotopic sites for which reference values are needed and increasingly have become available.^{24,25,12,27,30,31,29,32,33}

It is usually assumed that increased thresholds of thermal pain sensation are correlated with decreased ENF counts in small fiber sensory DPNs. However, whether these two measures are correlated in health is not known.^{34,15}

METHODS

Choice of community healthy subjects

Healthy subjects were mainly recruited from Olmsted County, MN, USA by a systematic electronic search of Mayo Clinic's diagnostic disease registries identifying persons 18 years and older with an Olmsted County, MN, USA address, and without coded neurologic, metabolic, or neoplastic diseases. Only subjects who had previously given consent to allow their medical records to be reviewed for research purposes were included in this search. These persons were then sent letters with the protocols of studies (reviewed by Mayo

Clinic's Institutional Review Board) inviting them to participate in healthy subject sensation studies.

The QSTs of HP at different somatotopic sites were performed over a span of almost 20 years and under three protocols of study reviewed by Mayo Clinic's Institutional Review Board. Healthy subject counts of ENFs were obtained over a span of ~4 years under the purview of a fourth Institutional Review Board protocol. For counts of ENFs, a broad range of ages and both men and women were assessed for lower abdomen and dorsal foot sites. To reduce labor and cost, counts of ENFs at lateral leg and thigh were performed only in a young adult and an aged group. The methodology for HP QSTs remained unchanged over the span of the studies. Before their acceptance as healthy subjects, consenting persons were examined by us to exclude persons with neurologic signs of neurologic disease or nerve conduction abnormalities (5th or 95th percentile) of attributes of nerve conduction of ulnar, fibular, tibial, and sural nerves. Also, patients with elevated levels of creatinine or glycated hemoglobin (A1c), performed at this research visit, were excluded from further study.

The consenting healthy subjects so identified were then studied using CASE IVc assessment of touch-pressure and other modalities of large nerve fiber sensation (not further reported on here) and of HP sensation thresholds at 10 standard sites of the left (or occasionally as needed of the right) side of the body. A subset of consenting healthy subjects had 3 mm skin punch biopsies of lateral leg and anterior thigh or dorsal foot and lower abdomen by approaches previously described.³⁵

Choice of somatotopic sites

The somatotopic sites were chosen to represent the body's surface distribution of sensation loss in length dependent sensory or sensorimotor polyneuropathy.⁹ The sites were: 1 (dorsal foot), 2 (lateral leg), 3 (anterior thigh), 4 (mid anterior lower abdomen), 5 (mid anterior upper abdomen), 6 (subclavian region), 7 (dorsal hand), 8 (volar forearm), 9 (deltoid region), and 10 (maxillary region of face).⁹ Skin biopsy assessments of ENFs were performed at sites 1-4. In a given healthy subject, we obtained only two punch biopsy specimens.

HP assessment

In previous reports, we have described the design and performance of the thermode and the CASE IVc system originally developed by Mayo Clinic's Section of Engineering and one of us (PJD) and then developed further and fabricated to industry standards by WR Medical Electronics, Maplewood, MN, USA.^{36,13} The nature and quantitation of the thermal heat stimuli, the algorithm of testing, and the approach to defining 3 HP thresholds, HP0.5 (threshold), HP5 (an intermediate degree of pain of 1-10 severity), and the stimulus response slope (HP 5-0.5) have previously been described and illustrated.^{37,13} The thermoelectric element in contact with the skin has an area of 3 × 3 cm. In CASE IVc system testing, all aspects of testing, except for patient instruction, keyboard entry of patient biographic, disease, and demographic information, test responses, and clinical management of the patient and test, are managed by computer software. HP 0.5 and HP 5 are determined by fitting a quadratic equation to the numeric response at different stimulus step levels tested.

Synthetic stimulus step values of 26, 27, 28, 29, and 30 are assigned when the HP response is 4, 3, 2, 1, or 0 at maximal stimulus strength, i.e., step 25. For explanatory purposes, we show the HP test responses of 2 patients in Supplementary Figure 1.

The CASE IVc system is fabricated and calibrated by WR Medical Electronics, Maplewood, MN, USA, according to International Electrotechnical Commission standards. It employs redundant trip circuits and temperature controls and other safety design features. In several thousand HP tests at our medical center, we have experienced no skin burns from use of the system.

The clinometric performance of the CASE IVc system was evaluated in a previously conducted prospective proficiency trial involving the University of Maryland, University of Michigan, and the Mayo Clinic (Clinical vs Neurophysiology Trial 5). Using 3 identical and calibrated CASE IVc systems and evaluating the same 12 patients with and without diabetic polyneuropathy independently on two occasions by each of the technologists, no statistically significant intra- or inter-observer differences of QST results were identified.³⁸

Assessment of epidermal nerve fibers

The methodology of obtaining 3 mm skin punch biopsy specimens, reacting nerve fibers with PGP 9.5, counting their number and estimating ENFs/0.05 mm² (epidermal area) and reporting results with confidence intervals was described in our earlier publication.³⁵ We followed the methodology of earlier investigators.^{39,40,8} In brief, after ascertaining that nerve fibers were adequately visualized, we estimated their number and confidence intervals using methodologies previously described.^{35,41}

Assuming that on average the epidermal/dermal interface of each of the 6 evaluated skip sections is 2.5 mm in length and is 0.05 mm in width, the epidermal surface area evaluated is $2.5 \times 0.05 \times 6 = 0.75 \text{ mm}^2$ (much smaller than the skin area (~900 mm² evaluated by HP testing).

Data analysis

Data were analyzed using standard statistical techniques with a primary intention to compare differences among anatomical sites for HP threshold and counts of ENFs. The data were clustered within participants on account of the multiple anatomical sites studied. To account for this statistical dependency, a random effects modeling framework was used. A base model consisting of only HP5 (step) and ENFs (#/0.05 mm²) regressed on age was used. A more complex model consisting of anatomic site and the interaction of anatomic site and age was used to allow for testing of the relationship of HP5 and ENFs by site and age. Likelihood ratio tests were used to determine the joint tests for the site and site by age interaction. T tests for individual fixed effects were used to simplify the model. Reference lines at the median, 95th and 99th percentile were estimated based on linear interpolation of the ranked residuals. Brown-Forsythe tests were used to assess homogeneity of variances between HP5, HP0.5, and HP5-0.5. P-values less than 0.05 were considered statistically significant. No adjustment for multiple testing has been applied to reported p-values. Statistical analyses were conducted using the SAS System version 9.3 (Cary, NC).

RESULTS

The age and gender of healthy subjects at different somatotopic sites are shown in Table 1. For HP tests, limb sites were more frequently studied than face or trunk sites as described in Materials and Methods. For ENF counts, an even lower number of healthy subjects were evaluated. For most somatotopic sites, HP thresholds had been assessed broadly from ages 18 years to old age and for both men and women (Figure 1).

The regression lines for HP5 threshold on age were remarkably similar among the 10 somatotopic sites studied. All regression lines increased with advancing age. This increase was statistically significant at lateral leg and foot sites for the 50th percentile line (Figure 1).

The regression lines of counts of ENF on age were similar among the tested anatomic sites, although intermediate ages had not been tested for the thigh and leg sites. For all tested sites, there was a decrease in counts of epidermal nerve fibers with age and this was statistically significant for the 50th percentile line for the thigh and leg sites (Figure 2).

In Table 2, we compare the 50th, 95th, and 99th percentile regression line y intercepts at age 18 years for HP5 (of 1-10 pain severity) at the 10 studied somatotopic sites and ENF 50th, 5th, and 1st y intercepts at 3 lower limb and 1 abdominal site. Whereas ENF density regression line intercepts increase from most distal (foot) to most proximal (lower abdomen) sites, this was not the case for HP5 thresholds.

Likelihood ratios and t tests were used to assess HP5 and ENF regression line differences among somatotopic sites (Tables 3 & 4). Statistically significant differences were found, but the magnitude of these differences were small.

Whereas ENF densities decreased progressively from lower abdomen to anterior thigh, lateral leg, and dorsal foot, a monotone increase in HP5 thresholds from abdomen to foot was not observed. In a second analysis, we assessed for the correlation of HP thresholds (HP0.5 or HP5) with counts of ENFs at each of the 4 assessed somatotopic sites using Spearman's Rank Correlation. Of the 8 analyses, none showed a significant correlation (Supplementary Table 1).

DISCUSSION

In the present study, we have found that 50th, 95th, and 99th percentile regression lines of HP 5 thresholds on age have statistically significantly different intercepts and slopes among tested somatotopic sites. However, these differences are small and of unclear clinical significance. This finding is not unlike that of Hardy, Wolff, and Goodell's observations, using a radiant heat lamp source.⁴² They found a higher threshold of pricking pain threshold for the heel which they attributed to thickened skin at that site. In more recent studies, the German Research Network on Neuropathic Pain using either the Medoc or the Samedic systems (both using thermoelectric technology and using linear ramp testing) also found small differences among somatotopic sites.^{43,44}

Punch skin biopsy quantitation of cutaneous sensory nerve endings (Meissner's corpuscles) was introduced to study their density and somatotopic distribution in development and in polyneuropathy.^{45,46} With the availability and use of large fiber sensory nerve conduction tests, enumeration of Meissner's corpuscles was not developed further. By comparison, counts of small sensory ENFs based on visualization of fibers reacted for PGP 9.5 has become widely used in medical practice and research.^{23,47,25,24,12,14,40} Lauria et al writing for the European Federation of Neurologic Sciences proposed guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy.²⁹ An evidence-based review³² concluded that skin biopsy and assessment of ENFs "may be considered for the diagnosis of sensory DPN, particularly small fiber sensory neuropathy." A taskforce from the European Federation of Neurological Societies/Peripheral Nerve Society provided guidelines on the use of skin biopsy in the diagnosis of small fiber sensory neuropathy.³² Healthy subject reference values of ENFs have been generated, especially for the distal leg 10 cm proximal to the lateral malleolus.^{12,27,30,31,48,35,49,50} There are published reference values also for other somatotopic sites.⁵¹ There is limited information for ENF density differences among somatotopic sites. Typically, however, distal sites like lateral leg have lower values than more proximal sites.^{24,27,31,51} For most studied lower limb sites, ENFs decrease with age. This decrease is greater for men than for women.²⁷ ENF reference values are also higher in children.^{52,14}

Although we found that HP 5 thresholds (of 1-10 pain severity) were similar among somatotopic sites, 95th percentile regression line intercepts and slopes were frequently statistically different among sites. However, these differences were usually small and of unclear clinical importance. In health, and for different tested sites, pain thresholds did not correlate significantly with ENF counts. Possible explanations for this lack of a statistical correlation includes: 1) the number of thermal nociceptors in biopsied skin was inadequately estimated because less than 1/1000th of the skin area assessed by thermoelectric testing of HP5 is assessed by ENF enumeration; 2) the number of thermal nociceptors among counted ENFs is unknown; 3) there are differences of the thickness of stratum corneum among somatotopic sites; and 4) there are differences of central nervous system integration of nerve impulses from different somatotopic sites. While it appears likely that several of the listed factors may explain this lack of correlation of HP threshold with ENF counts in health, a significant correlation between raised HP threshold and decreased ENFs will be tested in our subsequent studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CASE IVc	Computer Assisted Sensation Evaluator IVc, manufactured by WR Medical Electronics, Maplewood, MN, USA
DPN	Distal polyneuropathy
ENFs	Epidermal nerve fibers
HP	Heat pain
HP0.5	Heat pain threshold 0.5 of 1-10 severity
HP5	Heat pain stimulus response 5 of 1-10 severity
HP5-0.5	Heat pain stimulus response slope
QSTs	Quantitative sensation tests

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Figure 1.

Plotted HP5 threshold values and regression lines of healthy subjects at three lower limb and an abdominal site. Note that threshold regression lines increase with age and this increase is statistically significant for leg and foot. Not shown are threshold values for six other somatotopic sites.

Figure 2.

Plotted values and regression lines of counted epidermal nerve fiber densities with age. For these sites, regression lines show a decrease with age and this was statistically significant for thigh and leg sites.

Table 1

Age, Gender and Test Information at Somatotopic Sites

	Site #	n	Sex		Age (yrs)		
			Males	Females	Median	Range	
HP5 thresholds (CASE IVc test steps)	Dorsal Foot	1	311	157 (50.5)	154 (49.5)	53.0	24 - 79
	Lateral Leg	2	279	135 (48.4)	144 (51.6)	53.0	24 - 79
	Anterior Thigh	3	278	133 (47.8)	145 (52.2)	53.0	24 - 79
	Lower Abdomen	4	102	47 (46.1)	55 (53.9)	60.0	18 - 91
	Upper Abdomen	5	97	43 (44.3)	54 (55.7)	59.0	18 - 91
	Subclavicular	6	96	43 (44.8)	53 (55.2)	58.0	18 - 91
	Dorsal Hand	7	273	133 (48.7)	140 (51.3)	53.0	24 - 79
	Volar Forearm	8	278	133 (47.8)	145 (52.2)	53.0	24 - 79
	Deltoid	9	277	132 (47.7)	145 (52.4)	53.0	24 - 79
	Face	10	207	94 (45.4)	113 (54.6)	57.0	18 - 91
ENFs / 0.05 mm ²	Dorsal Foot	1	57	26 (45.6)	31 (54.4)	45.0	22 - 83
	Lateral Leg	2	68	32 (47.1)	36 (52.9)	70.0	18 - 89
	Anterior Thigh	3	67	31 (46.3)	36 (53.7)	70.0	18 - 89
	Lower Abdomen	4	58	26 (44.8)	32 (55.2)	46.5	22 - 83

Table 2

Order of Percentile Line Intercepts

		Intercept at Age 18 years		
		50th	95th/5th	99th/1st
HP:5 (step)	Dorsal Foot	22.25	24.13	26.11
	Lateral Leg	23.03	24.83	27.52
	Anterior Thigh	23.04	25.07	27.01
	Lower Abdomen	22.87	25.76	26.68
	Upper Abdomen	22.57	25.39	27.24
	Subclavicular	22.93	26.16	27.99
	Dorsal Hand	23.58	25.99	27.27
	Volar Forearm	22.70	24.43	26.78
	Deltoid	23.37	25.60	26.86
	Face	22.32	25.84	27.35
ENFs 0.05 / mm ²	Dorsal Foot	9.29	3.12	2.57
	Lateral Leg	10.28	5.90	5.27
	Anterior Thigh	12.81	9.42	6.96
	Lower Abdomen	21.82	11.49	10.89

Table 3

Heat Pain 5 Differences Among Somatotopic Sites

Test	Group	Comparison	Line	Slopes		Intercepts	
			Likelihood Ratio Test	t Tests			
				P	t	p	t
Lower Limb & Abdomen							
HP5 thresholds (CASE IVc test steps)	Males and Females	Dorsal Foot vs. Lateral Leg	<0.0001	-3.00	0.0029	-5.10	<0.0001
		Dorsal Foot vs. Anterior Thigh	<0.0001	-3.76	0.0002	-4.84	<0.0001
		Dorsal Foot vs. Lower Abdomen	0.2090	-	-	-	-
		Lateral Leg vs. Anterior Thigh	0.0010	-4.06	<0.0001	-0.50	0.6199
		Lateral Leg vs. Lower Abdomen	0.1492	-	-	-	-
	Males	Anterior Thigh vs. Lower Abdomen	0.9472	-	-	-	-
		Dorsal Foot vs. Lateral Leg	<0.0001	-3.56	0.0005	-5.04	<0.0001
		Dorsal Foot vs. Anterior Thigh	0.0002	-2.31	0.0223	-3.32	0.0012
		Dorsal Foot vs. Lower Abdomen	0.0666	-	-	-	-
		Lateral Leg vs. Anterior Thigh	0.2649	-	-	-	-
	Females	Lateral Leg vs. Lower Abdomen	0.0074	-3.58	0.0023	0.54	0.5979
		Anterior Thigh vs. Lower Abdomen	0.0483	-2.87	0.0112	0.12	0.9026
		Dorsal Foot vs. Lateral Leg	<0.0001	-0.64	0.5200	-5.35	<0.0001
		Dorsal Foot vs. Anterior Thigh	0.0040	-3.06	0.0027	-3.55	0.0005
		Dorsal Foot vs. Lower Abdomen	0.0882	-	-	-	-
Upper Limb & Face							
HP5 thresholds (CASE IVc test steps)	Males and Females	Dorsal Hand vs. Volar Forearm	<0.0001	1.43	0.1552	10.05	<0.0001
		Dorsal Hand vs. Deltoid	0.0427	2.41	0.0168	1.57	0.1187
		Dorsal Hand vs. Face	<0.0001	-0.86	0.3893	10.75	<0.0001
		Volar Forearm vs. Deltoid	<0.0001	1.25	0.2124	-14.72	<0.0001
		Volar Forearm vs. Face	<0.0001	-6.89	<0.0001	0.35	0.7305

Test	Group	Comparison	Line <i>P</i>	t Tests			
				Slopes		Intercepts	
				<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
	Males	Deltoid vs. Face	<0.0001	-12.35	<0.0001	1.66	0.0996
		Dorsal Hand vs. Volar Forearm	<0.0001	1.64	0.1035	7.27	<0.0001
		Dorsal Hand vs. Deltoid	0.2730	–	–	–	–
		Dorsal Hand vs. Face	<0.0001	-1.07	0.2893	9.37	<0.0001
		Volar Forearm vs. Deltoid	<0.0001	0.81	0.4220	-9.96	<0.0001
		Volar Forearm vs. Face	<0.0001	-7.06	<0.0001	-0.07	0.9406
		Deltoid vs. Face	<0.0001	-10.10	<0.0001	0.77	0.4429
		Dorsal Hand vs. Volar Forearm	<0.0001	0.46	0.6493	6.95	<0.0001
	Females	Dorsal Hand vs. Deltoid	0.1106	–	–	–	–
		Dorsal Hand vs. Face	<0.0001	-0.08	0.9386	6.19	<0.0001
		Volar Forearm vs. Deltoid	<0.0001	0.92	0.3582	-10.93	<0.0001
		Volar Forearm vs. Face	0.0197	-0.30	0.7659	3.26	0.0017
		Deltoid vs. Face	<0.0001	-0.75	0.4531	8.00	<0.0001

Table 4

Epidermal Nerve Fiber Differences Among Somatotopic Sites

Test	Group	Comparison	Line	Slopes		Intercepts	
			Likelihood Ratio Test	t Tests			
				p	t	p	t
Lower Limb & Abdomen							
ENFs / 0.05 mm ²	Males and Females	Dorsal Foot vs. Lateral Leg	0.4490	–	–	–	–
		Dorsal Foot vs. Anterior Thigh	0.0001	–1.36	0.1950	–4.64	0.0003
		Dorsal Foot vs. Lower Abdomen	<0.0001	–0.77	0.4464	–12.83	<0.0001
		Lateral Leg vs. Anterior Thigh	<0.0001	0.49	0.6247	–8.24	<0.0001
		Lateral Leg vs. Lower Abdomen	<0.0001	–0.14	0.8872	–14.03	<0.0001
	Males	Anterior Thigh vs. Lower Abdomen	<0.0001	–0.15	0.8840	–9.58	<0.0001
		Dorsal Foot vs. Lateral Leg	0.2126	–	–	–	–
		Dorsal Foot vs. Anterior Thigh	0.0002	–2.37	0.2544	–4.17	0.0530
		Dorsal Foot vs. Lower Abdomen	<0.0001	–1.28	0.2150	–8.89	<0.0001
		Lateral Leg vs. Anterior Thigh	<0.0001	–0.97	0.3381	–8.23	<0.0001
	Females	Lateral Leg vs. Lower Abdomen	<0.0001	–0.60	0.6547	–9.37	0.0112
		Anterior Thigh vs. Lower Abdomen	<0.0001	–0.43	0.7427	–6.72	0.0214
		Dorsal Foot vs. Lateral Leg	0.8362	–	–	–	–
		Dorsal Foot vs. Anterior Thigh	0.0477	–0.28	0.7839	–3.33	0.0068
		Dorsal Foot vs. Lower Abdomen	<0.0001	–0.08	0.9342	–9.30	<0.0001
	Females	Lateral Leg vs. Anterior Thigh	0.0004	4.82	<0.0001	–0.66	0.5136
		Lateral Leg vs. Lower Abdomen	<0.0001	0.61	0.5585	–10.56	<0.0001
		Anterior Thigh vs. Lower Abdomen	<0.0001	0.18	0.8587	–7.01	<0.0001