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Pathophysiology of Small-Fiber Sensory System in Parkinson's Disease

Skin Innervation and Contact Heat Evoked Potential

Chin-Hsien Lin, MD, PhD, Chi-Chao Chao, MD, PhD, Shao-Wei Wu, MD, MS, Paul-Chen Hsieh, MD, Fang-Ping Feng, MS, Yea-Huey Lin, BS, Ya-Mei Chen, PhD, Ruey-Meei Wu, MD, PhD, and Sung-Tsang Hsieh, MD, PhD

Abstract: Sensory symptoms are frequent nonmotor complaints in patients with Parkinson's disease (PD). However, few investigations integrally explored the physiology and pathology of the thermonociceptive pathway in PD. We aim to investigate the involvement of the thermonociceptive pathway in PD.

Twenty-eight PD patients (16 men, with a mean age and standard deviation of 65.6 ± 10.7 years) free of neuropathic symptoms and systemic disorders were recruited for the study and compared to 23 age- and gender-matched control subjects (12 men, with a mean age and standard deviation of 65.1 ± 9.9 years). We performed skin biopsy, contact heat-evoked potential (CHEP), and quantitative sensory tests (QST) to study the involvement of the thermonociceptive pathway in PD.

The duration of PD was 7.1 ± 3.2 (range 2–17 years) years and the UPDRS part III score was 25.6 ± 9.7 (range 10–48) during the off period. Compared to control subjects, PD patients had reduced intra-epidermal nerve fiber (IENF) density (2.48 ± 1.65 vs 6.36 ± 3.19 fibers/mm, $P < 0.001$) and CHEP amplitude (18.02 ± 10.23 vs 33.28 ± 10.48 μV, $P < 0.001$). Twenty-three patients (82.1%) had abnormal IENF densities and 18 (64.3%) had abnormal CHEP. Nine patients (32.1%) had abnormal thermal thresholds in the feet. In total 27 patients (96.4%) had at least 1 abnormality in IENF, CHEP, or thermal thresholds of the foot, indicating dysfunctions in the small-fiber nerve system. In control subjects, CHEP amplitude linearly correlated with IENF density ($P < 0.001$). In contrast, this relationship disappeared in PD ($P = 0.312$) and CHEP amplitude was negatively correlated with motor severity of PD independent of age, gender, and anti-PD medication dose ($P = 0.036$), suggesting the influences of central components on thermonociceptive systems in addition to peripheral small-fiber nerves in PD.

The present study suggested impairment of small-fiber sensory system at both peripheral and central levels is an intrinsic feature of PD, and skin biopsy, CHEP, and QST provided an integral approach for assessing such dysfunctions.

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Abbreviations: CHEP = contact heat-evoked potential, CI = confidence interval, CMAP = compound motor action potential, IENF = intra-epidermal nerve fiber, NCS = nerve conduction studies, PD = Parkinson's disease, QST = quantitative sensory tests, SAP = sensory action potential, UPDRS = Unified Parkinson's Disease Rating Scale, VRS = verbal rating scale.

INTRODUCTION

Parkinson's disease (PD) is a multidimensional neurodegenerative disorder with both motor and nonmotor symptoms.[1] Sensory complaints, especially pain, are one of the most common nonmotor symptoms, and more than one-quarter of PD patients experience ''primary pain'' related to dysfunction of the nociceptive system in the early stages of PD when motor symptoms are not prominent.[2,3] However, nerve conduction studies (NCS) are usually normal in PD patients, except for large-fiber neuropathy related to long-term exposure to levodopa with increased homocysteine levels and vitamin B12 deficiency.[4] Taken together, the symptoms of pain at early or premotor stage of PD raise the possibility of small-fiber sensory dysfunctions in either peripheral or central compartments of the nociceptive pathways during the early course of PD.[5]

Previous studies have suggested the involvement of small-fiber pathology in the neurodegenerative process of PD. Skin biopsies from PD patients have revealed peripheral deafferentation and deposition of phosphorylated α-synuclein in cutaneous sensory and autonomic nerves.[6–9] Heat pain thresholds and the amplitudes of laser-evoked potentials are reduced in PD patients compared to control subjects,[10–13] implying that central nociceptive processing is altered. However, a thorough and comprehensive analysis of the involvement of nociceptive system dysregulation in PD using integrated strategies that encompass psychophysical, pathological, and physiological examinations is lacking. Furthermore, the clinical significance of these alterations in relation to the motor progression of PD remains unknown.

Contact heat-evoked potential[11] mediated by Aδ fibers is a recent straightforward noninvasive approach to examine

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From the Department of Neurology (C-HL, C-CC, S-WW, F-PF, Y-HL, S-TH), National Taiwan University Hospital; the Department of Pathology (P-CH), Far Eastern Memorial Hospital; Institute of Health Policy and Management (Y-MC), National Taiwan University, Taipei, Taiwan; and Department of Anatomy and Cell Biology (S-TH), Graduate Institute of Brain and Mind Sciences, and Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan.
Correspondence: Sung-Tsang Hsieh, Department of Neurology, National Taiwan University Hospital, Taipei City, Taiwan (e-mail: shsieh@ntu.edu.tw).
C-HL and C-CC have contributed equally to this study.
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nociceptive dysfunction as a physiological counterpart of skin biopsy.[14,15] In small-fiber neuropathy, characteristic CHEP signatures, including the prolongation of latencies and reduction of amplitudes, parallel the degree of skin nerve degeneration.[16,17] In addition, CHEP parameters also reflect maladaptive plastic changes in the brain of patients with chronic pain.[18] We hypothesized that components of the thermonociceptive system are involved in the degenerative process of PD. We applied 3 different methods, including skin biopsy, CHEP, and quantitative sensory testing (QST) to investigate the physiology and pathology of thermonociceptive dysfunction and their clinical significance in PD.

PATIENTS AND METHODS

Participants

Twenty-eight patients with idiopathic PD who received regular follow-up at the movement disorder clinic of National Taiwan University Hospital (NTUH) were enrolled in the study. The inclusion criteria were fulfillment of the diagnosis of PD based on the United Kingdom PD Society Brain Bank clinical diagnostic criteria[19] and an absence of sustained sensory symptoms, neuropathic pain, or weakness in the limbs. We excluded patients who had systemic medical illnesses, such as diabetes mellitus, chronic liver, and kidney diseases, malignancy, endocrine diseases, autoimmune diseases, alcoholism, toxic exposure, or a family history of neuropathy. None of the patients had clinically relevant signs of autonomic dysfunction and mutations in *Parkin*, *PINK1*, *LRRK2*, *SCA2*, and *SCA3* were previously excluded.[20,21] All patients were treated with L-dopa or a combination with dopamine agonists and had good clinical responses. L-dopa equivalents were calculated according to Möller et al.[22] We defined the daily L-dopa dose as the average daily dosage of L-dopa in the 6 months before entering the study. We also collected the total cumulative dose of L-dopa equivalents in the year prior to enrollment. Each patient was examined using the motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS part III) and Hoehn-and-Yahr staging. Patients maintained their regular anti-PD medications and were examined while in the on status. Twenty-three age- and gender-matched healthy subjects were enrolled for direct comparisons of the results of skin biopsy and CHEP.

Our study was approved by the Ethics Committee of NTUH (201106076RB) and followed the Helsinki Declaration regarding international clinical research involving humans. Written informed consent was obtained from each participant before enrollment in the study.

Skin Biopsy, Immunohistochemical Staining, and Quantification of Epidermal Innervation

A 3-mm skin punch biopsy was taken at the leg 10 cm proximal to the lateral malleolus in the side with more severe motor symptoms. The sampled skin tissue was fixed with 2% paraformaldehyde-lysine-periodate in 0.1 M phosphate buffer overnight. The skin sample was cut perpendicularly into 50 μm thick sections. Skin sections were immunostained with rabbit antibody to protein gene product 9.5 (PGP 9.5, 1: 1000; UltraClone, Isle of Wight, UK) for 16 to 24 hours and were further incubated with biotinylated goat antirabbit antibody (Vector, Burlingame, CA) for 1 hour. The avidin–biotin complex was sequentially applied for another hour and the reaction product was demonstrated using chromogen SG (Vector Laboratories).

Epidermal innervation was counted through the depth of the entire skin section following an established rule by a clinically blinded trained examiner using an Olympus BX40 microscope (Tokyo, Japan) at 40× magnification. The epidermal length along the upper margin of the stratum corneum in each skin section was measured with ImageJ version 1.43 (Image Processing and Analysis in Java, National Institutes of Health, Bethesda, MD). The density of IENF was thereby calculated and expressed as the number of nerve fibers per millimeter of epidermal length. In our laboratory, the normative values (mean ± SD and 5th percentile) of IENF densities at the distal leg are 11.2 ± 3.7 and 5.9 fibers/mm for subjects aged $<$ 60 years and 7.6 ± 3.1 and 2.5 fibers/mm for subjects aged ≥ 60 years. The IENF density lower than 5.9 and 2.5 fibers/mm was classified as abnormal in these 2 age groups, respectively.[23]

Records of Contact Heat Evoked Potential

A contact heat evoked potential stimulator (Medoc, Ramat Yishai, Israel) was used to deliver the heat stimuli.[14] The diameter of the circular thermode is 27 mm; the heating rate is 70 °C/s; and the cooling rate is 40 °C/s. Cooling begins immediately after the thermode reaches its target stimulus temperature based on the default algorithms. CHEP was recorded while subjects sat on a chair, with their eyes closed and muscle relaxed, in a semi-dark room with the room temperature controlled at 25 °C. The heat stimulus was applied to the hairy skin area of the lateral leg around 10 cm proximal to the lateral malleolus. The skin area was divided into 6 adjacent nonoverlapping districts. The thermode was moved clockwise or counter-clockwise across these sites. The heat pulse was delivered from the 32 to 51 °C. The interstimulus interval was randomly set to around 20 to 22 s. CHEP was recorded using a NeuroScan SynAmps 64-channel amplifier and Quik-Caps system (NeuroScan, El Paso, TX). The recording electrode was set at Cz. The references were set as bilateral mastoids. The ground electrode was positioned between FPz and Fz. To control artifacts, we monitored the electrooculogram from supra- and infra-orbital electrodes. The impendence of all electrodes was kept below 5 kΩ and the online EEG was sampled at 1000 Hz with a bandpass filter at 0 to 300 Hz. Before the formal recording of CHEP, we delivered several heat stimuli to subjects to avoid startle responses. During the study, subjects were asked to pay consistent attention to the stimulus and verbally rated the intensity of pain perception for 3 s after each stimulus using a verbal rating scale (VRS; 0–10), in which "0" means no sensation, "4" represents the pain threshold, and "10" corresponds to the intolerable pain. We recorded CHEP from each leg separately and the order of sides was balanced among the subjects.

Analysis of CHEP

The offline processing of CHEP waveforms was based on Scan 4.5 software (NeuroScan, El Paso, TX). The cleaned EEG signals were cut into epochs from 500 ms before to 1500 ms after the stimulus onset (0 ms). Each epoch was baseline corrected from −500 to 0 ms and filtered with a bandpass filter at 0.1 to 30 Hz. Epochs with major artifacts were excluded. CHEP waveforms were analyzed based on an average of the first 16 artifact-free epochs from the leg of the more affected side. In normal subjects, CHEP at Cz consisted of an initial negative-wave (N2-wave) followed by a positive-wave (P2-wave) as described previously.[17] We measured the N2-peak latency as the CHEP latency and the amplitude between the N2-peak and

P2-peak as the CHEP amplitude. If both N2-wave and P2-wave were absent, the CHEP amplitude was defined as the mean amplitude of the average tracing in the time window between 300 and 800 ms. If only N2-wave or P2-wave was absent, the CHEP amplitude was the existent P2-wave or N2-wave amplitude. In our laboratory, the normative values from 72 healthy controls $\geqq$40 years of age were 471.1 ± 42.2 and 534.5 ms for CHEP latency and 38.1 ± 9.1 and 21.4 μV for CHEP amplitude (mean ± SD and 5th percentile).

Quantitative Sensory Testing

QST was performed according to the algorithm of level in the lower limb of more severely affected side using a Thermal Sensory Analyzer (Medoc, Ramat Yishai, Israel) as described before.[24] Thermal thresholds according to the algorithm of level recorded on the foot dorsum were expressed as the warm threshold temperature and cold threshold temperature. Vibratory thresholds were measured with similar algorithms, and expressed in micrometers. These values were compared with normative values for the age, which had previously been documented.[24]

Nerve Conduction Study

NCS was performed in bilateral sural, peroneal, and tibial nerves with a Viking Select Electromyographer (Nicolet, MD) following established methods.[25] Bilateral sural, peroneal, and tibial nerves were studied. Abnormal results in NCS were defined as having reduced amplitude of compound motor action potential (CMAP) or sensory action potential (SAP), prolonged distal latencies, slowing of the nerve conduction velocity.[25]

Statistical Analyses

Data were interpreted according to our previously described normative databases. The results of skin biopsy and CHEP for the PD patients were further compared to those of age- and gender-matched subjects. Numerical variables were expressed as the mean ± standard deviation of the mean. For variables following a Gaussian distribution, data was compared using 2-tailed *t* test. Regression analysis was performed to evaluate the correlations between variables and the correlation was expressed graphically with the slope of regression line and its 95% confidence interval (CI). The correlation was further explored with multivariate analysis, and the covariance of the model (R^2) and the standardized correlation coefficient were presented. We performed all the analysis by Stata (StataCorp LP, College Station) and Prism (GraphPad Software, San Diego) software. A P value < 0.05 was considered significant.

RESULTS

Clinical Characteristics

The mean age of the 28 PD patients was 65.6 ± 10.7 (range 44–88) years and the mean disease duration was 7.1 ± 3.2 (2–17) years. Sixteen of the patients were men. The detailed clinical features of the PD patients enrolled in this study are summarized in Table 1. The mean UPDRS part III score was 25.6 ± 9.7 (10–48) during the off period. The mean L-dopa equivalent dose was 601.8 ± 274.7 (150–1200) mg/day and the total cumulative L-dopa equivalent dose the year before enrollment was 189.2 ± 82.5 (54–360) g/year. The results of neurological examinations were unremarkable in 21 patients (75.0%), and a mild but symmetrical decrease in the deep tendon reflex of the lower limbs was observed in 7 patients (25.0%). All of these patients had full muscle power and did not reveal subjective sensory impairment.

The independent control group for skin biopsy and CHEP comparisons comprised 23 age- and gender-matched healthy subjects, including 12 men. The mean age was 65.1 ± 9.9 years ($P = 0.73$ for gender and $P = 0.86$ for age).

Nerve Conduction Study and Quantitative Sensory Testing

For large-fiber nerve functions according to NCS of the lower limbs, the results were within normal limits in 24 patients (85.7%). The other 4 patients had normal sural nerve studies but mildly reduced amplitudes of compound muscle action potentials in peroneal or tibial nerves. For the psychophysical parameters of both small-fiber and large-fiber nerves assessed by QST, 9 patients (32.1%) had abnormal thermal thresholds in the foot (all 9 with elevated warm thresholds and 1 with elevated cold thresholds) and 16 patients (57.1%) had elevated vibratory thresholds in the lateral malleolus.

Skin Biopsy

Skin biopsy was performed to investigate the pathology of small-diameter sensory nerves. In control subjects, intra-epidermal nerve fibers (IENFs) with a typical varicose appearance arose from the subepidermal nerve plexuses, and dermal nerve fibers exhibited dense and linear immunoreactivities (Figure 1A). In contrast, IENFs were markedly reduced and dermal nerve fibers fragmented in PD patients (Figure 1B), which is consistent with nerve degeneration. IENF densities were significantly lower in PD patients compared to control subjects (2.48 ± 1.65 vs 6.36 ± 3.19 fibers/mm, $P < 0.001$, Figure 1C). Among the 28 PD patients, 23 (82.1%, Table 1) had IENF densities lower than the 5th percentile of the normative data: 6 cases (6/6) with Hoehn-and-Yahr stage 1 in the off state, 10 (10/11) with stage 2, 6 (6/8) with stage 3, 1 (1/2) with stage 4, and none (0/1) with stage 5.

Contact Heat Evoked Potentials

Figure 2A shows the grand average of CHEPs from PD patients and age- and gender-matched control subjects. In controls, CHEPs had well-defined biphasic waveforms with an initial negative peak (N2-wave) followed by a positive peak (P2-wave) (Figure 2A). The CHEP amplitude was attenuated in PD patients (Figure 2B). Furthermore, the CHEP amplitude was significantly reduced in PD patients compared to control subjects (18.02 ± 10.23 vs 33.28 ± 10.48 μV, $P < 0.001$, Figure 2C), and 18 patients (64.3%) had abnormal CHEP parameters. There was no difference in the N2-wave latencies between PD cases and controls ($P = 0.41$) or any difference in CHEP amplitude between the side with more severe motor symptoms and the side with less involvement (18.02 ± 10.23 vs 16.36 ± 11.31 μV, $P = 0.16$). Upon contact heat stimulation, the mean intensity of pain perception on the verbal rating scale (VRS) was 4.4 ± 1.7, which is in the range of mild to moderate pain.

Correlation Between Clinical and Thermonociceptive Parameters

All patients except 1 (96.4%) had at least 1 abnormality in IENF density, CHEP amplitude, or thermal thresholds in the foot, providing quantitative evidence for small-fiber physiology

TABLE 1. Clinical Data, Skin Innervation, and Contract Heat Evoked Potentials of Patients with Parkinson's Disease

Patient	Age,	Sex	Disease Duration, (Y)	UPDRS III (On)	UPDRS III (Off)	H-Y Stage (On)	H-Y Stage (Off)	Current L-Dopa Dose (mg/day)	Cumulative L-Dopa Dose (g/Y)	IENF Density (Fibers/mm)	N2-P2 Amplitude of CHEP (μV)
1	44	M	6	10	18	1	2	600	216	3.38	29.08
2	51	M	8	8	28	1	2	400	126	4.79	31.39
3	52	F	4	6	15	1	2	400	101.25	6.26	20.21
4	53	F	3	8	14	1	1	300	108	2.93	40.44
5	54	F	8	25	32	3	4	1200	360	5.87	26.16
6	57	F	5	5	18	1	2	150	54	0.71	12.22
7	57	M	7	18	25	2	2	450	121.5	4.61	15.55
8	58	F	5	12	20	2	2	600	216	1.73	12.01
9	59	F	7	6	12	1	1	150	54	3.42	16.69
10	59	M	3	20	30	2	3	600	195.75	2.32	3.43
11	60	M	17	15	32	2	3	800	288	3.30	7.09
12	63	M	5	7	12	1	1	600	135	1.27	17.47
13	65	F	8	20	34	2	3	700	234	1.58	17.72
14	65	F	13	28	32	3	3	800	288	3.67	26.11
15	65	M	9	20	32	1	2	600	216	1.76	23.12
16	66	M	4	6	13	1	1	300	90	1.18	21.22
17	69	M	6	17	28	2	2	800	252	1.23	24.25
18	69	F	6	7	15	1	1	600	189	1.48	27.02
19	71	F	12	20	30	2	3	600	216	1.34	28.45
20	71	M	9	20	30	2	3	800	252	3.51	12.62
21	74	M	5	36	42	3	4	800	180	0.44	11.85
22	74	F	2	6	10	1	1	300	108	2.15	36.46
23	75	M	8	17	33	2	3	1000	270	0.89	2.52
24	77	M	8	20	26	2	2	800	234	1.51	10.38
25	78	M	6	18	24	2	2	300	108	1.86	4.05
26	81	M	6	20	28	2	2	600	189	0.62	14.90
27	82	F	8	40	48	4	5	1200	351	4.92	3.37
28	88	M	10	28	36	3	3	400	144	0.74	8.84

CHEP = contact heat evoked potential, F = female, H-Y stage = Hoehn-and-Yahr stage, IENF = intraepidermal nerve fiber, M = male, UPDRS III = Unified Parkinson's Disease Rating Scale part III.

and pathology. First, we investigated the relationship between skin innervation, CHEP amplitude, and the thermal threshold in the foot, and analyzed the effects of age, gender, disease duration, and anti-PD drugs on these parameters using univariate linear regression. In PD, there was no relationship between CHEP amplitude and IENF density ($P = 0.312$, Figure 3A) although CHEP amplitude and IENF density both negatively correlated with age ($r = -0.414$, $P = 0.021$ and $r = -0.079$, $P = 0.005$, respectively). The warm threshold in the foot positively correlated with the daily dose and yearly cumulative dose of L-dopa equivalent ($r = 0.004$ and 0.012, $P = 0.019$ and 0.030, respectively). These findings contrasted with the normal control group in which the CHEP amplitude positively correlated with IENF density ($r = 2.698$, $P < 0.001$, Figure 3B) in addition to the correlation of CHEP amplitude and IENF density with age ($r = -0.749$, $P < 0.001$ and $r = -0.233$, $P < 0.001$, respectively). When simultaneously considering the effects of age and gender in the multivariate linear regression model, CHEP amplitude only significantly correlated with IENF density in the control group ($r = 1.637$, $P = 0.011$ for IENF density; $R^2 = 0.76$ and $P < 0.001$ for the model).

Next, we examined the relationship between the small-fiber sensory dysfunction and the motor symptoms of PD. The CHEP amplitude negatively correlated with the Hoehn-and-Yahr stages and UPDRS part III scores in both the on and off state (Figure 4A–D), whereas the warm threshold in the foot positively correlated with Hoehn-and-Yahr stages and UPDRS part III scores in both the on and off state. There was no correlation between IENF density and PD motor severity. In multivariate linear regression analysis incorporating age, gender, daily L-dopa equivalent dose, disease duration of PD, and CHEP amplitude or warm threshold in the foot as independent variables, CHEP amplitude still negatively correlated with Hoehn-and-Yahr stages in the off state ($r = -0.032$, $P = 0.036$ and $R^2 = 0.69$, $P < 0.001$ for the model).

To further explore whether peripheral or central parameters were associated CHEP in PD, the multivariate regression model was applied with CHEP amplitude as dependent variable, and age, gender, IENF density and motor scores, that is, Hoehn-and-Yahr stage as independent variables. In this model, IENF density represented the peripheral deafferentation and Hoehn-and-Yahr stage served as a surrogate marker of central effects of PD. Hoehn-and-Yahr stages in the off state was the only factor correlated with CHEP amplitude ($r = -4.26$, $P = 0.031$ and $R^2 = 0.41$, $P = 0.013$ for the model) (Table 2).

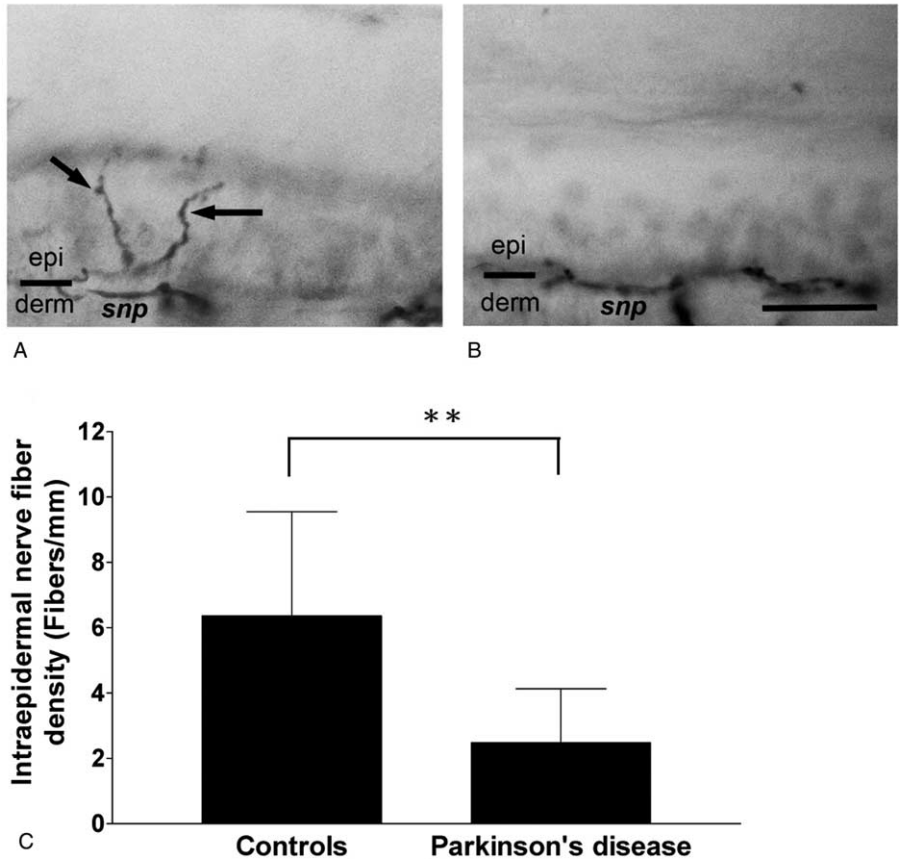

FIGURE 1. Skin innervation in Parkinson's disease. Skin sections from the distal leg of an age- and gender-matched control subject (A) and a representative patient with Parkinson's disease (B) were immunostained with antiprotein gene product 9.5 (PGP 9.5). (A) In the epidermis (epi) of the control subject, intraepidermal nerve fibers (arrows) ascended from the subepidermal nerve plexus (snp) in the dermis (derm). (B) In the skin of the Parkinson's disease patient, intraepidermal nerve fibers were reduced and the subepidermal nerve plexus fragmented, suggesting nerve degeneration. (C) The intraepidermal nerve fiber density was markedly reduced in the legs of Parkinson's disease patients compared to age- and gender-matched control subjects. Scale bar = 25 μm for (A) and (B). **: $P < 0.001$. PGP 9.5 = antiprotein gene product 9.5, snp = subepidermal nerve plexus.

DISCUSSION

In the present study, we reported the integrated data of CHEP, skin biopsy, and QST in PD patients who were free of neuropathic sensory symptoms and systemic diseases. Almost all enrolled PD patients (96.4%) had quantitative evidence of dysfunction in the thermonociceptive system based on reduced IENF density, reduced CHEP amplitude, and elevated warm threshold in the foot. Furthermore, CHEP amplitude and warm threshold in the foot correlated with the severity of PD motor dysfunction, and CHEP amplitude reflects the motor severity independent of age, gender, and the dose of anti-PD medications.

We observed a high prevalence of small-fiber sensory dysfunction in PD based on combined neurophysiological, pathological, and psychophysical assessments. This finding provides objective evidence and explanations for previous epidemiological studies indicating a high prevalence of pain in PD patients.[26,27] The exclusion of systemic diseases, toxin exposure, and organic brain lesions in the present study ascertained that the small-fiber physiology and pathology was intrinsic to the disease course of PD. Although recent studies have emphasized the relationship between the use of levodopa and peripheral neuropathy,[4,28] the absence of a correlation between L-dopa equivalent doses and the IENF density or CHEP parameters, as well as the preservation of large fiber physiology in our PD patients, suggest that the small-fiber nerve degeneration and thermonociceptive pathophysiology were independent of the use of anti-PD medications and an integral part of the neurodegeneration that occurs in PD. Postmortem pathological studies have revealed progressive pathological changes in the thermonociceptive pathway at multiple levels in PD.[26] Progressive involvement of Lewy body deposition in PD brains could affect the structures of the medial and lateral pain processing systems, that is, the locus coeruleus, nucleus raphe magnus, and gigantocellular reticular nucleus, which are key areas in the descending regulation of pain,[29] as well as the anterior cingulated cortex, amygdala, and prefrontal cortex, which are associated with motivational and emotional aspects of pain.[27] In addition to the cutaneous denervation being pathological evidence of primary thermonociceptive nerves,[8,9] recent studies in PD have demonstrated the involvement of medium-sized multipolar projecting neurons at lamina I of the spinal cord, the initiating component of the spinothalamic tract.[30,31] According to Braak's pathology staging, many of

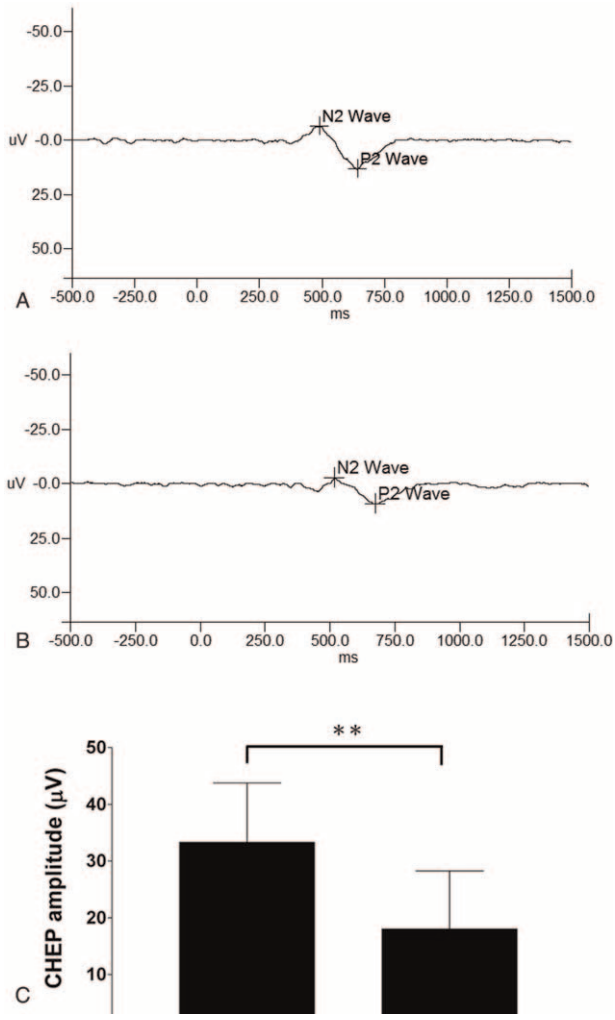

FIGURE 2. Contact heat-evoked potentials in Parkinson's disease. Grand average of CHEP in control subjects (A) and patients with Parkinson's disease (B). (A) In the control subjects, the CHEP evoked by stimulation of the leg revealed a well-defined negative-positive (N2-P2) biphasic waveform. (B) In patients with Parkinson's disease, the amplitude of the N2-P2 wave was reduced and the peak-latency of the N2 wave prolonged compared to controls. (C) The N2-P2 amplitude of CHEP was significantly smaller in patients with Parkinson's disease compared to the age- and gender-control subjects. **: $P < 0.001$; CHEP = contact contact heat evoked potential.

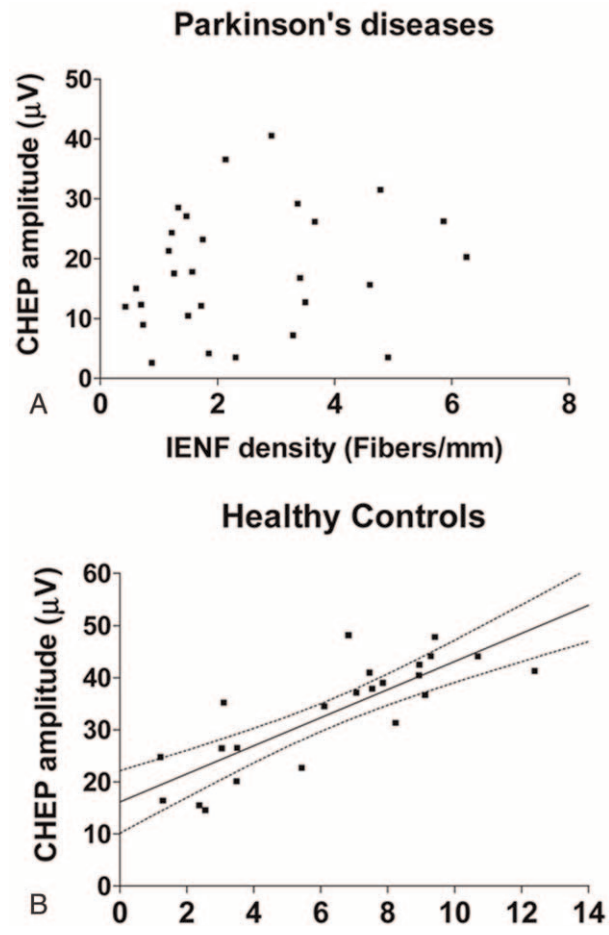

FIGURE 3. Loss of correlation between the amplitude of contact heat evoked potentials and skin innervation in Parkinson's disease. No relationship was found between the N2-P2 amplitude of CHEP evoked by stimulation of the leg and the intraepidermal nerve fiber density in the leg of patients with Parkinson's disease. (B) In the age- and gender-matched controls, the N2-P2 amplitude of CHEP evoked by stimulation of the leg highly correlated with the intraepidermal nerve fiber density of the leg ($P < 0.001$). CHEP = contact heat evoked potential.

these structures are affected in the early course of PD, even before the obvious involvement of motor function.[29,31] All of these pathological changes may contribute to abnormalities in skin innervation, CHEP amplitude, and thermal thresholds in PD patients.

This study demonstrates the feasibility of the CHEP as a noninvasive clinical examination for assessing thermonociceptive dysfunctions in PD. The CHEP amplitude was reduced in PD patients compared to controls, and 64.3% patients had reduced CHEP amplitudes compared to normative values from healthy subjects of similar age and gender. We found no difference in CHEPs between the sides regarding the severity of motor dysfunction. These findings extended a previous study that reported reduced N2-P2 amplitude of laser-evoked potentials in PD regardless of the clinically affected side.[12] Given that CHEP records brain activities after heat pain stimuli on skin as conveyed by the thermonociceptive pathway, CHEP could detect dysfunctions in this pathway at either the peripheral and central level. Previous studies have showed a correlation between reduced CHEP amplitude and decreased IENF density in diseases of pure peripheral nerves, that is small-fiber neuropathy of different etiologies.[17,32] In the present study, CHEP amplitude was only correlated with the IENF density in the control group. Despite significant reduction of IENF density and CHEP amplitude in PD patients, the relationship between CHEP amplitude and IENF density altered, that is, CHEP amplitude was associated with Hoehn-and-Yahr stage in PD. These observations suggest the change in CHEP amplitude was affected not only by peripheral deafferentation but also central components in PD, that is, the striatum.[29,30,33] Recently, imaging studies have demonstrated striatal activations by heat pain

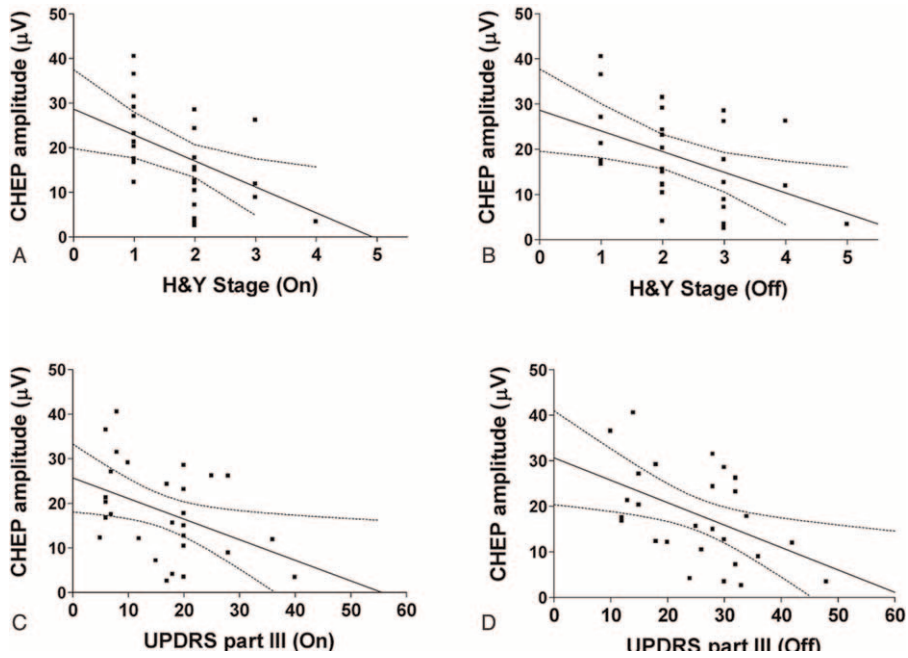

FIGURE 4. The relationship between contact heat evoked potential and motor dysfunction in patients with Parkinson's disease. The N2-P2 amplitude of CHEP evoked by stimulation of the leg negatively correlated with the on- and off-time Hoehn-and-Yahr scales ($r = -5.83$, $P = 0.012$ and $r = -4.57$, $P = 0.015$) (A and B), and the on- and off-time of UPDRS part III ($r = -0.46$, $P = 0.026$ and $r = -0.49$, $P = 0.012$) (C and D). CHEP = contact heat evoked potential, H&Y stage = Hoehn-and-Yahr scales, UPDRS = Unified Parkinson's Disease Rating Scale.

stimuli in healthy subjects and neuropathic patients, implying an unexplored role of the striatum in nociceptive processing.[33,34] Taken together, the present report might provide evidence of the central effects of PD contributing to the alterations of CHEP amplitude. In this study, we also observed a correlation between CHEP amplitudes and the warm threshold in the foot and motor severity in PD as measured by Hoehn-and-Yahr stages and UPDRS part III scores. Even after considering the confounding effects of age, gender, anti-PD medication dose, and disease duration of PD, the CHEP amplitude still highly correlated with Hoehn-and-Yahr stages. Compatible with previous literatures, our study showed no correlation between the cutaneous sensory innervations and motor dysfunction in PD patients,[9] suggesting that separate pathological processes underlie the central motor system and peripheral thermonociceptive nerves in PD.[35] Unlike IENF density, CHEP represents integrated brain responses to heat-pain stimuli, including the activation of somatosensory areas, insula, and cingulated cortex, which are further influenced by deep structures of the thalamus, basal ganglion, amygdala, and brainstem.[33,36,37] Many of these structures become progressively involved in PD; thus, the changes in CHEP amplitude might potentially reflect the motor progression of PD.[29,38] A recent study investigating laser-evoked potentials in PD patients with shoulder muscular pain did not show a relationship between N2-P2 amplitude and motor symptom severity.[10] Possible reasons for the discrepancy between this study and ours include differences in motor status on recordings, sensory symptoms, heat pain stimuli used, the location at which the stimulus was applied, and disease duration. These issues await further studies

TABLE 2. Multiple Linear Regression Models for Contact Heat Evoked Potential (CHEP) Amplitude

		t, *P*			
Group	**CHEP Amplitude R, *P***	**Age**	**Gender**	**IENF Density**	**Hoehn-and-Yahr stage, Off**
Controls	0.76, <0.001*	−0.39, 0.048*	−5.29, 0.050	1.64, 0.011*	–
Parkinson's disease	0.41, 0.013*	−0.20, 0.339	−6.07, 0.086	0.64, 0.631	−4.26, 0.031*

In this model, the CHEP amplitude of controls or patients with Parkinson's disease was set as the dependent variable, and age, gender, IENF density, and Hoehn-and-Yahr stages in the off state (Parkinson's disease only) were set as independent variables.

CHEP = contact heat evoked potential, IENF = intra-epidermal nerve fiber.

P = for *R* or *t*, R = correlation coefficient based on the model of multiple linear regression, *t* = *t* value for the coefficient of each parameter in the model.

* = statistically significant. $P < 0.05$.

for clarification. In addition to CHEP, the warm threshold in the foot also correlated with motor severity in our PD patients. Similar to CHEP, the thermal thresholds assess an integral outcome of thermal sensations. Taken together with recent studies showing an improvement in temperature sensation after deep brain stimulation of the subthalamic nucleus in PD,[39,40] these observations suggest that the dysfunction in the thermonociceptive networks might be associated with impairment of the cortical-striatal-nigral dopaminergic circuits.

There are several limitations in this study. For the studied PD patients, we excluded patients with sustained sensory symptoms, neuropathic pain, or weakness in the limbs to avoid confounding etiologies causing neuropathic disorders or pain. The study therefore did not address the relationships between the clinical sensory symptoms and the assessed thermonociceptive dysfunctions. Another aspect is the small sample size of PD patients, which hinders us from further stratification of patients with different motor severity. Finally, we did not check the serum level of vitamin B12 in these PD patients, which might underestimate the effects of levodopa use on the risk of neuropathy. However this limitation would not significantly impact our findings since most reported neuropathy related to levodopa use was mainly large-fiber neuropathy,[4] and we had adjusted the cumulative dosage of levodopa in our analysis. Nevertheless, the present study documents that the small-fiber physiology and pathology of the thermonociceptive pathway at both the peripheral and central levels are intrinsic features of PD. Skin biopsy, CHEP, and QST provide an integral and sensitive approach for assessing such dysfunctions.

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