doi:10.1093/brain/awn102 *Brain* (2008), **131**, 1903–1911

Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation

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Sensory disturbances are part of the clinical picture of Parkinson's disease. Abnormalities in sensory processing, through a basal ganglia involvement, are thought to be responsible for the sensory dysfunction since sensory nerve conduction velocity (NCV) is usually normal. However, NCV does not examine small fibres or terminal endings of large sensory fibres, whereas skin biopsy is more suitable for these purposes. To evaluate peripheral sensory nerves in Parkinson's disease, we studied cutaneous free and encapsulated sensory nerve endings in 18 patients and 30 healthy controls using 3-mm punch biopsies from glabrous and hairy skin. Ten patients had additional skin biopsies from the contralateral side. Further evaluation included NCV and Quantitative Sensory Testing. Parkinson's disease patients showed a significant increase in tactile and thermal thresholds ($P < 0.01$), a significant reduction in mechanical pain perception ($P < 0.01$) and significant loss of epidermal nerve fibres (ENFs) and Meissner corpuscles (MCs) ($P < 0.01$). In patients with bilateral biopsies, loss of pain perception and ENFs was higher on the more affected side ($P < 0.01$). We found evidence suggesting attempts at counteracting degenerative processes as increased branching, sprouting of nerves and enlargement of the vascular bed. Morphological and functional findings did not correlate with age or disease duration. Disease severity correlated with loss of MCs and reduction in cold perception and pain perception. We demonstrated a peripheral deafferentation in Parkinson's disease that could play a major role in the pathogenesis of the sensory dysfunction.

Keywords: Parkinson's disease; pain; skin biopsy; Meissner's corpuscles; peripheral nervous system

Abbreviations: ENFs = epidermal nerve fibres; IMEs = intrapapillary myelinated endings; MCs = Meissner's corpuscles; NCV = nerve conduction velocity; QST = quantitative sensory testing; SNAP = sensory nerve action potential

Received December 3, 2007. Revised March 5, 2008. Accepted May 1, 2008. Advance Access publication May 31, 2008

Introduction

Disability in Parkinson's disease is mostly due to motor impairment (Gelb *et al.*, 1999; Litvan *et al.*, 2003) but sensory symptoms are frequent complaints and many patients experience pain during the course of their illness (Snider *et al.*, 1976; Goetz *et al.*, 1986; Quinn *et al.*, 1986). Different causes have been recognized as the sources of pain (Ford, 1998; Serratrice and Michel, 1999; Lee MA *et al.*, 2006) but a primary 'central' pain has also been described (Snider *et al.*, 1976; Schott, 1985; Chudler and Dong, 1995; Waseem and Gwinn-Hardy, 2001). However, little is known about the pathogenesis of this primary parkinsonian pain (Scherder *et al.*, 2005).

Abnormalities in nociceptive (Guieu *et al.*, 1992; Djaldetti *et al.*, 2004) and mechanical (Prätorius *et al.*, 2003; Zia *et al.*, 2003) thresholds have been described in Parkinson's disease. They have been considered to be due to anomalies of central nociceptive processing and sensorimotor integration through the affection of basal ganglia and dopaminergic pathways (Lewis and Byblow, 2002; Zia *et al.*, 2003; Tinazzi *et al.*, 2007). Another hypothesis is a reduction in the inhibition on the ascending nociceptive pathway through a diencephalospinal dysfunction (Buzas and Max, 2004).

The idea of a peripheral sensory defect in Parkinson's disease has not been considered as nerve conduction velocity (NCV) studies are normal. However, NCV does not evaluate large sensory endings and small fibres. In a post-mortem study of Parkinson's disease patients, researchers reported a loss

of unmyelinated nerve fibres in the sural nerve (Kanda *et al.*, 1996), but a sural nerve biopsy, although useful in studying small fibres, cannot differentiate autonomic nerve fibres from sensory ones.

Studying cutaneous innervation allows for the identification of autonomic and sensory myelinated and unmyelinated nerve fibres through the recognition of their target. We evaluated peripheral sensory nerve involvement by studying the function and morphology of cutaneous free and encapsulated sensory nerve endings in a group of Parkinson's disease patients.

Subjects and Methods

Patients

Eighteen idiopathic Parkinson's disease patients (mean age 62.7 ± 8.0; nine females and nine males) were enrolled after screening to rule out diabetes, glucose intolerance, dysendocrinopathies, vitamin E, B12 and folic acid deficiency, hepatic and renal failure, HIV or connective tissue disorders. Parkinson's disease was diagnosed according to UKPDSBB criteria (Hughes *et al.*, 1992) and classified according to the Hoehn and Yahr stage (Table 1). Motor impairment was assessed by means of UPDRS-M. Three of the patients had disease onset before the age of 45, therefore mutations for PARK 2 and PARK 6 genes were tested and ruled out before enrolment.

Controls

Thirty age- and sex-matched healthy subjects were enrolled for morphological data comparison.

Quantitative Sensory Testing (QST) findings were compared to 54 age- and sex- matched controls (patient: control ratio = 1: 3, age matching tolerance = ±2 years, mean age 61.4 ± 8.0), extracted from our database containing results from 100 healthy volunteers.

The Institutional Ethics Committee approved the study and all participants gave written informed consent.

Methods

Electrophysiology

Electrophysiological evaluation included recordings, via surface electrodes, of antidromic sensory NCV along median, ulnar and sural nerves and motor NCV along median, ulnar and peroneal nerves.

QST

Testing was performed on the dorsum of the hand and foot of the less affected side in Parkinson's disease patients. Ten had the tests done bilaterally. A single investigator (blinded to the inclusion of a patient in our research study at time of testing) performed all QST and all patients were tested during ON condition.

'Tactile threshold' was assessed using a series of 18 calibrated nylon monofilaments (Semmes–Weinstein), moving stepwise from the thicker towards the thinner filament, in order to detect the thinnest one perceivable five times out of 10. Null stimuli were randomly applied during the test to evaluate subject reliability.

'Mechanical pain perception' was evaluated using a calibrated monofilament, with a bending force of 95 mN, connected to a sharp non-penetrating probe (50 µm tip). This was applied 10 times for 1–2 s. The percent of stimuli perceived as painful and the pain magnitude using a visual analogue scale was recorded. Three null stimuli were randomly applied during testing to evaluate subject reliability.

'Thermal thresholds' (cold, warm, cold pain and heat pain) were evaluated using a thermal sensory analyser (Medoc, TSA 2001, Israel) with a Peltier probe measuring 3 × 3 cm and the method of limits (Yarnitsky and Sprecher, 1994). Four stimuli were delivered for each modality.

Table 1 Clinical characteristics of Parkinson's disease patients

Patient	Sex	Age	Disease duration (years)	Hoehn and Yahr Stage	UPDRS	More affected site	Sensory symptoms	Pharmacological treatment
P1	F	67	1	1	12	Left	Paraesthesia, RLS	None
P2	M	72	5	3	33	Left		LD, CBG, PPX
P3	F	61	10	4	30	Right		LD, PPX
P4	M	63	1	1	10	Left		None
P5	M	67	11	4	33	Right		LD, CBG, PPX
P6	M	54	2	1	15	Left	Paraesthesia, RLS	None
P7	M	56	16	3	20	Right		LD, CBG, PPX
P8	M	68	11	5	43	Right	Burning feet	LD, CBG, AMN
P9	F	61	3	2.5	32	Right	Paraesthesia	LD, PPX
P10	M	62	7	2	17	Left		LD, CBG
P11	F	58	5	3	27	Right		LD, PPX
P12	M	46	6	3	28	Right		LD, CBG, PPX
P13	F	69	7	2.5	26	Left		LD, PPX
P14	F	75	2	1.5	21	Right	Burning feet	LD, PPX
P15	F	67	17	3	29	Right		LD, CBG, RPN
P16	M	70	15	3	50	Left		LD, PPX
P17	F	47	2	2	22	Left		None
P18	F	65	15	3	30	Left	Burning feet	LD, AMN
Mean ± SD		62.7 ± 8.0	7.6 ± 5.5	2.6 ± 1.1	26.6 ± 10.2			

RLS = Restless leg syndrome; LD = L-Dopa; CBG = Cabergoline; PPX = Pramipexole; AMN = Amantadine; RPN = Ropinirole.

Morphological study

All subjects underwent 3-mm punch skin biopsies, under local anaesthesia, from the fingertip, thigh and distal leg. In Parkinson's disesase patients, biopsies were taken from the less affected side and in 10 subjects biopsies were taken bilaterally from thigh and leg. One Parkinson's disesase patient refused biopsy of the fingertip.

Samples were cut at 50 µm using a sliding freezing microtome and sections were processed for indirect immunofluorescence using previously described techniques (Kennedy and Wendelschafer-Crabb, 1993). A panel of primary antibodies was used to mark vascular structures and nerves (Table 2). Digital images were acquired using a CARV confocal system (ATTO Biosciences, Rockville, MD, USA) connected to an Axioskop 2 Mot Zeiss microscope (Jena, Germany) using 10×, 20× and 100× Plan Apochromat and 40 × F-Fluar objectives. The density of epidermal nerve fibres (ENFs), Meissner's corpuscles (MCs) and intrapapillary myelinated endings (IMEs) was calculated following previously described procedures (Kennedy *et al.*, 1996; Nolano *et al.*, 2003) using the software Neurolucida (MicroBrightFieldBioscience, Williston, VT, USA) and ScionImage (Scion Corporation, Frederick, MD, USA). Tracing was performed by the same operator (blinded to diagnosis) who routinely performs nerve quantification at our laboratory.

Table 2 Primary antibodies used in immunohistochemistry

Primary antibodies	Abbreviation	Source	Dilution
Rabbit anti-protein gene product 9.5	r-PGP	Biogenesis	1 : 1000
Rabbit anti-substance p	r-Sub P	Incstar	1 : 1000
Rabbit anti-calcitonin gene related peptide	r-CGRP	Amersham	1 : 1000
Rabbit anti-s 100	r-s100	Maxim Biotech	1 : 10
Rabbit anti-vasoactive intestinal peptide	r-VIP	Incstar	1 : 1000
Mouse anti-vasoactive intestinal peptide	m-VIP	Santa Cruz	1 : 1500
Mouse anti-protein gene product 9.5	m-PGP	Biogenesis	1 : 800
Mouse anti-collagen type IV	m-Col IV	Chemicon	1 : 800
Mouse anti-myelin basic protein	m-MBP	Ultraclone	1 : 800

Statistical analysis

We used Student's *t*-test for paired data to compare patient findings from more affected and less affected sides, *t*-test for unpaired data and Mann–Whitney test (when analysing non-parametric data) to compare morphological and functional findings from patients and controls, linear correlation (Pearson) to correlate patient data with age, severity and disease duration.

Results

Functional findings

Clinical features for all Parkinson's disease patients are summarized in Table 1. Sensory disturbances unrelated to the OFF phase, muscular, or osteoarticular conditions were reported in six out of 18 patients. In all Parkinson's disease patients sensory and motor NCV studies were normal (Table 3). QST showed a significant increase in tactile and thermal thresholds ($P<0.01$) and a significant reduction in mechanical pain perception both at the hands and feet ($P<0.01$) (Fig. 1). There was no correlation between functional findings and disease duration or UPDRS-M scale, while disease severity correlated with an increase in cold threshold ($r=0.65$; $P=0.020$) and impairment of

Table 3 Sensory nerve conduction findings

Patient	Sex	Age	Median NCV	Median SNAP amplitude	Ulnar NCV	Ulnar SNAP amplitude	Sural NCV	Sural SNAP amplitude
P1	F	67	52.9	26.4	51.4	33.9	57.0	15.0
P2	M	72	51.0	16.8	49.1	14.3	43.0	7.0
P3	F	61	54.0	18.2	47.0	17.3	46.0	12.0
P4	M	63	53.0	15.4	46.0	17.2	46.0	10.0
P5	M	67	52.0	13.0	51.4	5.8	48.0	26.0
P6	M	54	54.0	17.5	50.2	13.4	49.3	7.0
P7	M	56	55.2	16.0	51.9	16.0	52.0	8.2
P8	M	68	53.0	12.0	56.0	11.0	44.0	3.9
P9	F	61	53.7	13.4	50.0	15.5	48.0	21.0
P10	M	62	55.2	11.3	51.0	13.0	47.8	6.1
P11	F	58	61.5	19.0	50.0	21.0	50.0	17.0
P12	M	46	64.0	18.4	47.0	22.3	56.0	14.0
P13	F	69	50.0	21.7	47.0	18.4	46.0	8.0
P14	F	75	51.3	16.9	48.0	19.7	46.0	5.5
P15	F	67	51.6	19.7	49.0	11.5	57.0	6.5
P16	M	70	53.4	18.1	48.4	18.9	47.0	7.2
P17	F	47	56.7	59.0	49.3	15.0	54.0	14.0
P18	F	65	51.7	50.4	49.0	43.0	56.6	30.0
Mean ± SD Patients		62.7 ± 8.0	54.1 ± 3.6	21.3 ± 12.8	49.5 ± 2.3	18.2 ± 8.5	49.7 ± 4.6	12.1 ± 7.4
Mean ± SD Controls		61.2 ± 11.6	53.5 ± 5.2	29.3 ± 11.0	51.4 ± 3.2	26.3 ± 9.3	49.7 ± 4.3	13.8 ± 4.9

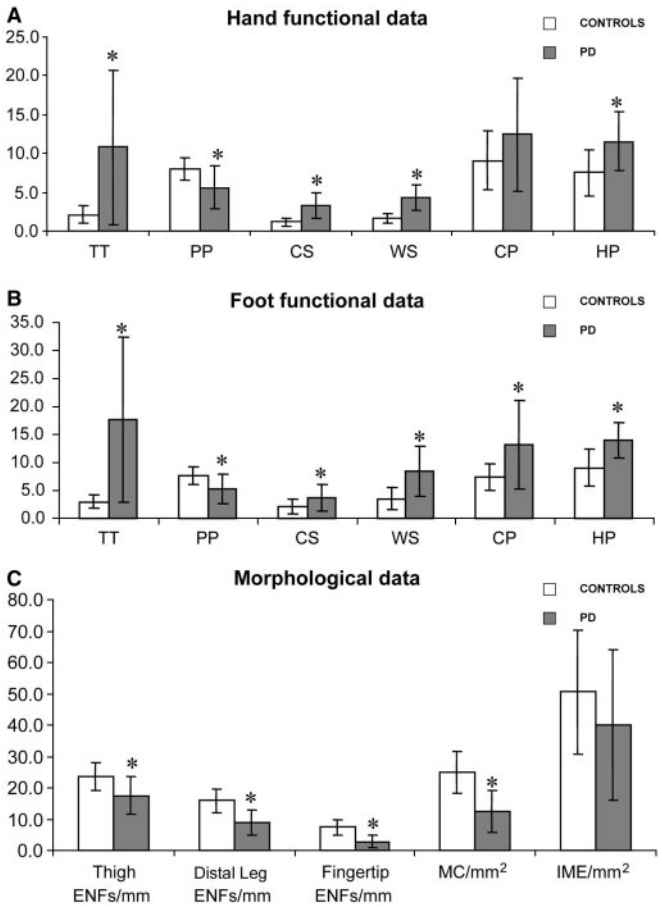

Fig. 1 QST and morphological findings in Parkinson patients (PD) compared to controls. CS = cold threshold; WS = warm threshold; CP = cold pain; HP = heat pain; TT = tactile threshold; PP = mechanical pain perception. Thermal threshold values are expressed in degree celsius; tactile threshold values are expressed in milliNewtons; pinprick values are expressed as number of stimuli perceived as painful out of 10. ENF density is expressed as number of fibres per linear mm; MC and IME density is expressed as number of structures per square mm. $^{*}P < 0.01$ compared to controls.

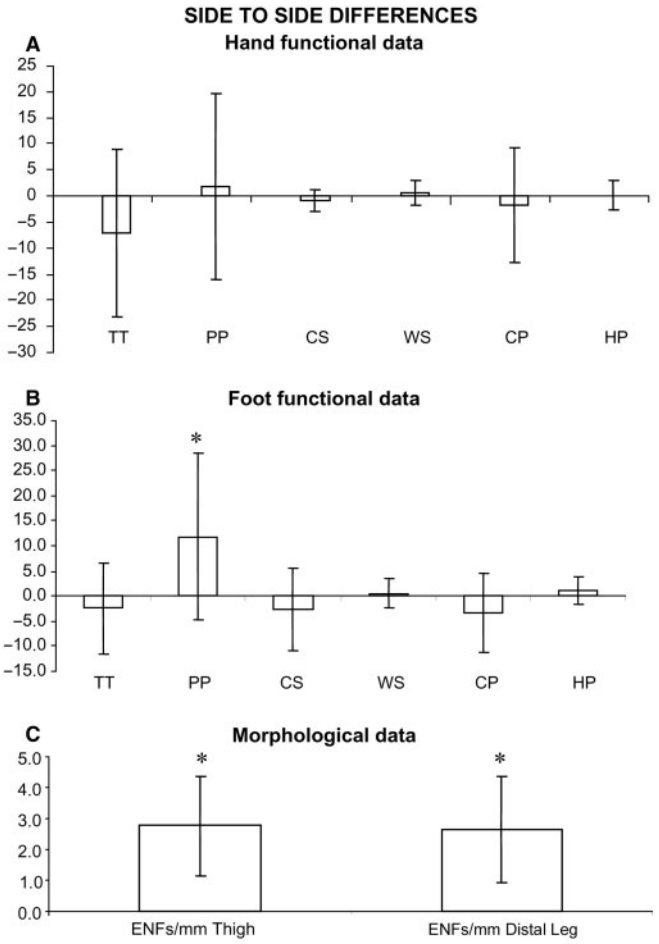

Fig. 2 Differences between less affected and more affected side in QST and morphological findings of Parkinson patients. CS = cold threshold; WS = warm threshold; CP = cold pain; HP = heat pain; TT = tactile threshold; PP = mechanical pain perception. Thermal threshold values are expressed in degree celsius; tactile threshold values are expressed in milliNewtons; pinprick values are expressed as number of stimuli perceived as painful out of 10. ENF density is expressed as number of fibres per linear mm; MC and IME density is expressed as number of structures per square mm. $^{*}P < 0.01$ comparing the two sides.

mechanical pain perception ($r = 0.54$; $P = 0.022$) at the dorsum of the foot. In the 10 subjects tested bilaterally, there were no differences in QST between sides except for mechanical pain which was more impaired at the foot of the more affected side ($P < 0.01$) (Fig. 2).

Morphological findings

Unmyelinated sensory fibres

In controls, as previously described (Kennedy and Wendelschafer-Crabb, 1993), single ENFs arise from subepidermal neural plexus bundles and penetrate the basement membrane, losing their Schwann cell sheath. Then, as naked axons, they cross the entire epidermis reaching the stratum corneum with an even distribution in hairy skin (Fig. 3A), and cluster at the apex of dermal papillae in glabrous skin. In the dermis, some of the papillary nerve fibres are immunoreactive (-ir) to neuropeptides CGRP (Fig. 4C) and Sub P (Fig. 4E) but only a few reach the epidermis. No VIP-ir fibres are present at the level of the papillary dermis or in the epidermis (Fig. 4A).

In Parkinson's disease patients there was ENF loss (Table 4 and Fig. 3B) with fibre swellings (Fig. 3D) together with frequent aspects of nerve remodelling such as irregular distribution along the epidermis (evidence of clusters), and an increase in nerve branching (Fig. 3C). An abnormal innervation pattern was also evident in the papillary dermis which appeared rich in sprouts attempting to reach the epidermis but failing to penetrate the basement membrane (Fig. 5E). Several of these fibres appeared to be CGRP-ir (Fig. 4D). VIP-ir fibres were abnormally present in the papillary dermis. This finding was particularly evident in the glabrous skin where VIP-ir fibres occasionally appeared

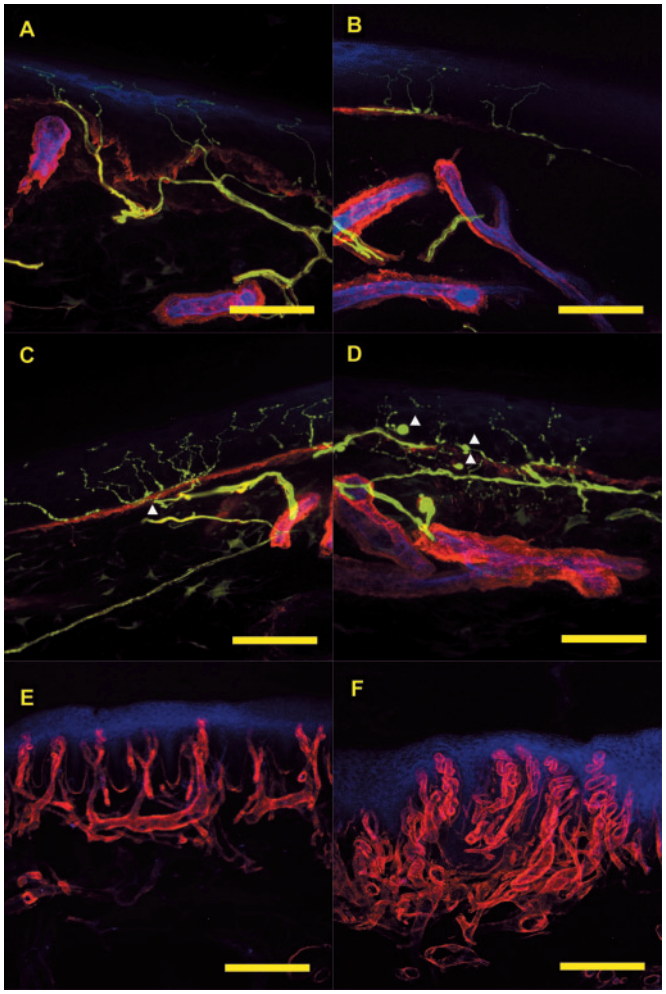

Fig. 3 Confocal digital images showing abnormalities in Parkinson's disease patients of ENFs (**B–D**) and of capillaries and dermal vessels (**F**) compared to control (**A, E**). Nerve fibres are in green (PGP), vessels and basement membrane in red (Col IV), endothelium and epidermis in blue (Ulex europaeus). The loss of ENFs in Parkinson's disease patients (**B**) compared to control (**A**) is counteracted by regenerative attempts as the evidence of clusters suggests (arrowhead in **C**). In **D**, abnormal swellings (arrowheads) along the course of nerve fibres. Capillary bed appears abnormally complex and enlarged in Parkinson's disease patients (**F**) compared to normal (**E**). Scale Bar = 50 μm in **A–D**, and 200 μm in **E** and **F**.

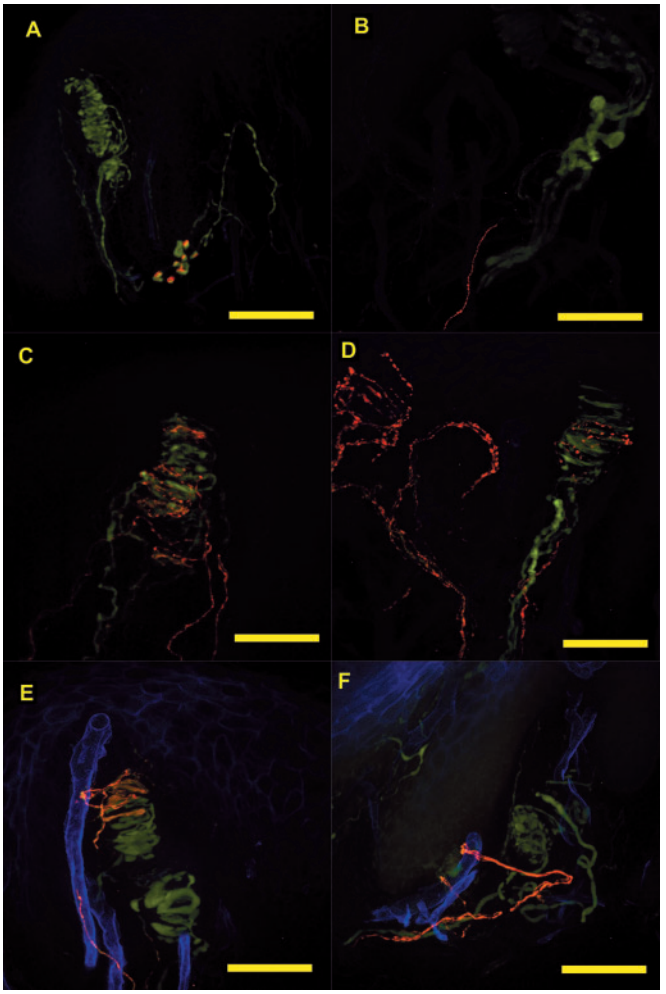

Fig. 4 Confocal digital images showing abnormalities of peptidergic innervation in the skin of Parkinson's disease patients (**B, D** and **F**) compared to control (**A, C** and **E**). In green PGP (**A–F**), VIP in red (**A** and **B**), CGRP in red (**C** and **D**), Sub P in red (**E** and **F**). A VIP-ir fibre appears in an area usually devoid of it, attempting to get inside a MC (**B**) compared to control (**A**). CGRP-ir fibres appear particularly abundant in the papillary dermis of Parkinson's disease patients (**D**) compared to control (**C**) and even more expressed than PGP-ir fibres. Sub-P-ir fibres are less represented inside MCs in Parkinson's disease patients (**F**) compared to control (**E**). Scale Bar = 50 μm.

to move towards a MC (Fig. 4B). Sub P-ir fibres were rather sparse compared to control skin (Fig. 4F).

The reduction in ENFs was significant ($P<0.01$) (Table 4), but did not correlate with age, duration or severity of the disease, or pharmacological treatment. In the 10 patients with bilateral skin biopsies, the ENF density was significantly lower on the more affected side ($P<0.01$) (Fig. 2).

Myelinated endings

In controls, as previously described (Nolano *et al.*, 2003), glabrous skin is particularly rich in myelinated endings of A-beta type, that arise from the subepidermal neural plexus, coursing a long and regular route reaching the apex of dermal papillae. IMEs (Fig. 6A) innervate MCs but they lose their myelin sheath before getting into the corpuscle capsule. In hairy skin, myelinated fibres appear scattered with a higher concentration around hair follicles (Provitera *et al.*, 2007).

In Parkinson's disease patients, glabrous skin IME density was lower than in controls but the difference did not reach significance (Table 4). In PGP/MBP double-stained sections, in both glabrous and hairy skin, we observed frequent aspects of axonal swelling and myelin abnormalities (Fig. 6), such as paranodal (Fig. 6B) and distal demyelination, profile segmentation (Fig. 6C) and sometimes internodal demyelination (Dyck *et al.*, 2005).

Table 4 Morphological data

Patient	Thigh ENFs/mm	Leg ENFs/mm	Fingertip ENFs/mm	MCs/mm^2	IMEs/mm^2
P1	13.2	11.5	1.2	17.5	23.2
P2	13.2	8.9	1.2	7.3	46.9
P3	11.6	6.8	n.a.	n.a.	n.a.
P4	15.2	13.8	5.9	21.0	32.7
P5	21.7	13.4	3.3	2.1	12.5
P6	11.9	5.9	1.6	14.2	16.1
P7	14.8	4.2	2.1	11.4	91.7
P8	10.7	3.6	2.3	2.3	25.7
P9	20.6	11.0	2.7	9.7	56.0
P10	14.1	3.8	2.7	8.5	48.8
P11	25.2	12.1	4.9	10.9	27.6
P12	30.0	11.5	1.2	17.0	74.8
P13	6.9	1.4	2.2	21.3	56.1
P14	25.1	4.7	0.6	5.9	10.2
P15	18.0	11.1	2.8	15.4	40.0
P16	19.5	9.1	7.8	17.4	6.7
P17	18.9	15.0	4.5	23.9	45.0
P18	21.8	9.3	0.0	5.7	63.5
Mean values ± SD Patients	17.4 ± 6.0*	8.7 ± 4.0*	2.8 ± 2.0*	12.4 ± 6.7*	39.9 ± 23.9
Mean values ± SD Controls	23.75 ± 4.46	15.87 ± 3.85	7.35 ± 2.37	24.95 ± 6.82	50.53 ± 19.68

ENF density is expressed as number of fibres per linear mm; MC and IME density is expressed as number of structures per square mm. n.a. = not available. * = $P < 0.01$ compared to controls.

Moreover, several nerve fibres, seen especially with the PGP staining, appeared intensely fluorescent, with a stronger signal. When measured, the fibres were double or triple in diameter (Fig. 7) compared to controls (Nolano *et al.*, 2003). MCs were significantly reduced in number ($P < 0.01$) (Table 4) and presented, as did Merkel complexes, a wide range of anomalies suggesting the coexistence of degenerative and regenerative processes (Fig. 5C–F). The loss of MCs correlated with disease severity ($r = 0.54$; $P = 0.012$) but not with duration.

Vascular anomalies

In patients, dermal papillae were elongated both in hairy and glabrous skin with a large vascular bed. Capillary loops appeared enlarged and complex (Fig. 3F) compared to control skin (Fig. 3E).

Correlation between functional and morphological findings

ENF loss at the distal leg and fingertip correlated with the impairment in mechanical pain ($r = 0.44$; $P = 0.045$ and $r = 0.63$; $P = 0.007$, respectively) but not with the other sensory modalities.

Loss of myelinated fibres correlated with the increase in tactile thresholds ($r = -0.052$; $P = 0.03$).

Discussion

Sensory disturbances are part of the clinical picture of Parkinson's disease. Sensory dysfunction has been demonstrated in this disease by means of QST (Djaldetti *et al.*, 2004), quantitative nociceptive thresholding (Guieu *et al.*, 1992; Gerdelat-Mas *et al.*, 2007) and laser evoked potentials (Tinazzi *et al.*, 2007). Abnormalities of mechanical sensitivity (Prätorius *et al.*, 2003; Zia *et al.*, 2003) and of nociception (Guieu *et al.*, 1992; Gerdelat-Mas *et al.*, 2007) have been taken into account to explain the impaired balance control in Parkinson's disease or the primary parkinsonian pain. Clinical and electrophysiological evaluation have failed to show peripheral abnormalities in Parkinson's disease, consequently, the involvement of basal ganglia and thalamocortical-basal ganglia circuits are considered the basis for this sensory defect (Brown *et al.*, 1997; Tinazzi *et al.*, 2007). Our Parkinson's disease patients showed sensory impairment with increased thresholds for all of the tested modalities (cold, warm, cold pain, heat pain, mechanical pain, touch) consistent with previous reports of impairment in touch, vibration and tactile discrimination (Schneider *et al.*, 1987; Sathian *et al.*, 1997; Prätorius *et al.*, 2003; Zia *et al.*, 2003) but conflicting with recent studies reporting on a decreased pain threshold (Djaldetti *et al.*, 2004; Brefel-Courbon *et al.*, 2005). Disparity in results on nociception can be due to differences in experimental conditions such as ON and OFF phases, presence of spontaneous pain, stage of the disease, or levodopa consumption (Brefel-Courbon *et al.*, 2005). In addition to the sensory impairment, we demonstrated, a loss in free and encapsulated nerve endings, which could explain, at least in part, the sensory deficit in Parkinson's disease. To date, this report is the first to describe these latter findings of loss in ENFs, IMEs and MCs.

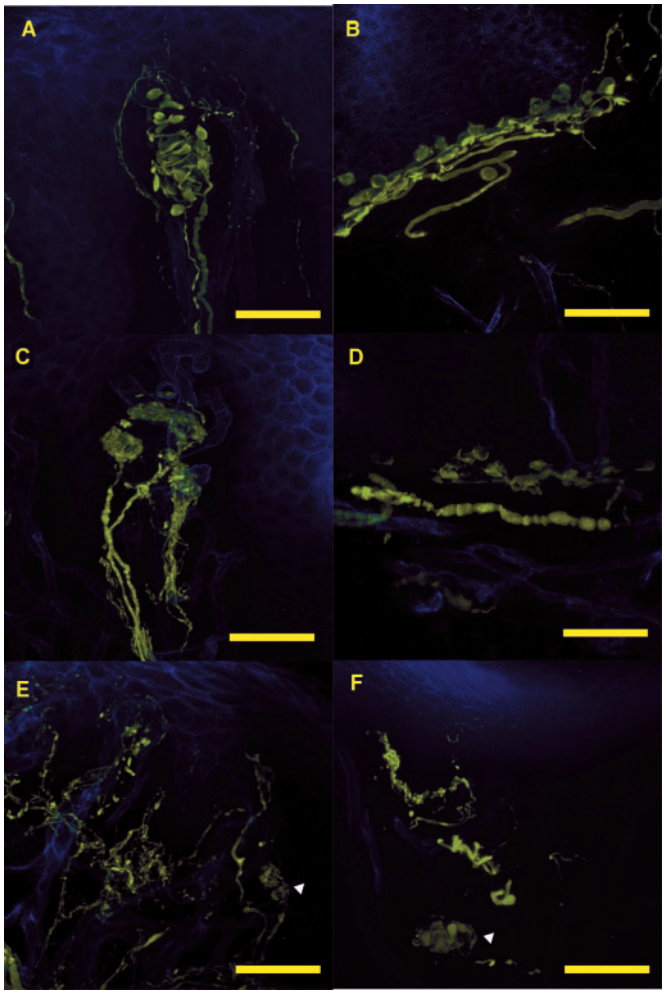

Fig. 5 Confocal digital images showing abnormalities of mechanoreceptors in Parkinson's disease patients (**C–F**) compared to normal subjects (**A** and **B**). In green axons and receptors (PGP), in blu endothelium and epidermis (Ulex europaeus). Moderate (**C** and **D**) to severe (**E** and **F**) degenerative aspects of MCs and Merkel complexes. The presence of MCs at a lower position (**E** and **F**; arrowheads) suggests the coexistence of regenerative processes. Scale Bar = 50 μm.

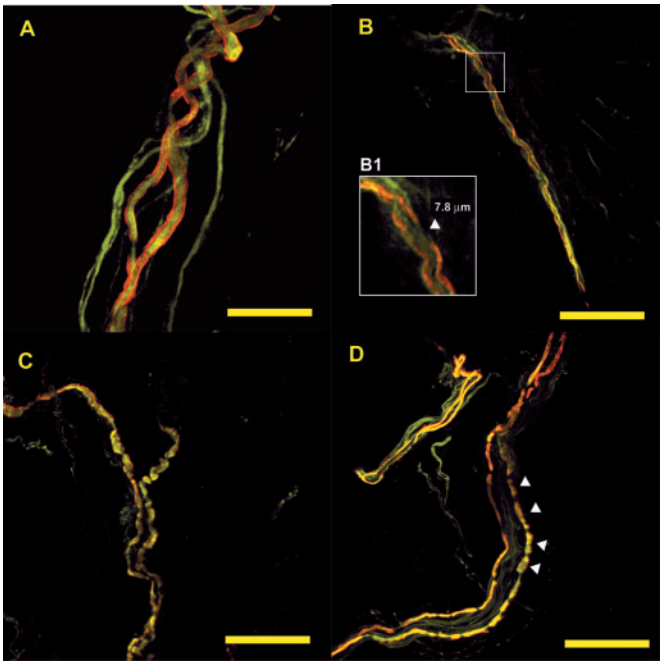

Fig. 6 Digital confocal images of double stained PGP/MBP myelinated fibres (axons in green and myelin in red). In **A** cutaneous myelinated fibres in controls. In **B, BI, C** and **D:** abnormalities of myelinated fibres in Parkinson's disease patients as nodal lengthening (**B** and **BI**), moderate (**C**) to severe (**D**) aspects of myelin wrinkling and segmentation into large ovoids (**D**; arrowheads). Scale Bar = 20 μm in **A**, 200 μm in **B** and 100 μm in **C** and **D**.

The cutaneous denervation and the sensory impairment were evident in all of the examined sites, and were unrelated to age, disease duration or pharmacological treatment. It has been suggested that pharmacological treatment may cause nerve degeneration (Shulman *et al.*, 1999; Muller *et al.*, 2004). However, four of our patients, untreated at the time of enrolment, showed loss of cutaneous nerves and QST abnormalities ruling out that possibility.

In our 18 patients, ENF loss occurred regardless of disease severity. However, in those tested bilaterally, the loss was more evident on the more affected side where mechanical pain perception was also more impaired. Our findings suggest that epidermal denervation may be an early feature of Parkinson's disease and may reflect to some extent the asymmetry of the disease. The denervation process must progress quite slowly, counteracted by regenerative processes suggested by the presence of increased nerve branching, sprouting and clustering. Neuropeptide anomalies such as the presence of VIP-ir fibres in areas usually devoid of VIP, and the increased CGRP-ir fibres in the subepidermal plexus are further proof of nerve regeneration attempts (Ramien *et al.*, 2004). Predegenerative aspects, as large axonal swellings, could be due to an impaired axonal transport (Lee *et al.*, 2006) through a microtubule system dysfunction or due to intra-cytoplasmic accumulation of mitochondria (Martin, 2007).

In our patient group disease severity correlated with the loss of MCs but not with ENF loss. A greater structural stability and a reduced need for energy protect large myelinated fibres compared to unmyelinated or poorly myelinated ones that are preferentially involved in Parkinson's disease (Braak *et al.*, 2006). Therefore, the involvement of MCs, encapsulated endings of large myelinated fibres, would reflect the severity of the disease.

Recently, Braak *et al.* (2007) examined post-mortem Parkinson's disease patients and found an involvement of medium-sized multipolar neurons of lamina I of the spinal cord. The main projection on these neurons comes from unmyelinated and sparsely myelinated primary afferent A-delta and C fibres, the same fibre populations we found

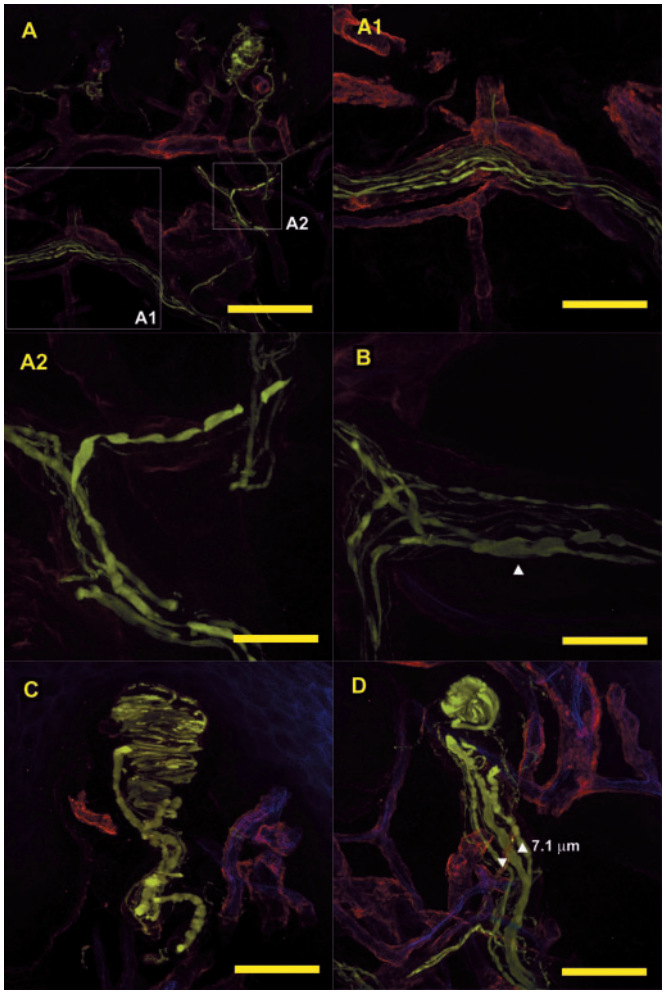

Fig. 7 Confocal digital images showing abnormalities of cutaneous innervation in the glabrous skin of Parkinson's disease patients. Nerves are in green (PGP), basement membranes and vessels in red (Col IV) and endothelium and epidermis in blue (Ulex europaeus). In **A** degenerative aspects of nerve fibres in the dermis. In **AI** (40×), **A2** (100×): details at higher magnification showing the marked irregularity of the axonal caliber with large swellings alternating with severe thinning; in some tracts axons appeared segmented. In **B** evidence of a large fusiform axonal swelling (arrowhead). In **C** and **D** calibre irregularity and marked enlargement of axons directed to MCs. The diameter of these axons is three to four times larger than normal. Scale Bar = 100 μm in **A**, 50 μm in **AI, C** and **D**, 20 μm in **A2** and **B**.

involved in our patients. If or how the two findings might be related is yet to be clarified. The loss of ENFs, IMEs and MCs seen in our patients as well as the reported autonomic cardiac (Goldstein *et al.*, 2000; Li *et al.*, 2002) and cutaneous (Dabby *et al.*, 2006) denervation, could be an expression of a generalized distal axonopathy within the context of this multi-system disease (Braak *et al.*, 2006). In the skin of our patients, in addition to aspects of nerve regeneration, we observed vascular abnormalities with complex, tortuous and enlarged capillary loops. Similar findings have been described in the substantia nigra of Parkinson's disease patients (Faucheux *et al.*, 1999) and attributed to an increased expression of vascular endothelial growth factor (VEGF) (Wada *et al.*, 2006). Therefore, we can speculate that a VEGF-dependent enhancement of neurogenesis and angiogenesis could also be occurring in the skin.

Peripheral neuropathy has been described in patients with early onset Parkinson's disease due to *parkin* mutation (Okuma *et al.*, 2003; Ohsawa *et al.*, 2005). Experiments (RT-PCR) revealed the expression of *parkin* gene in peripheral nerves but its role in the pathogenesis of peripheral neuropathy remains unknown (Abbruzzese *et al.*, 2004). Our patients were diagnosed with idiopathic Parkinson's disease and genetic causes were ruled out. However, the occurrence of peripheral neuropathy in a genetic type of Parkinson's disease suggests that a link may be present between central and peripheral neuronal degeneration. Future work is warranted to clarify pathophysiological mechanisms inducing the peripheral nerve damage seen in idiopathic Parkinson's disease trying, in particular, to discern why the degenerative process seems to be confined to nerve endings; or why it seems to mirror the asymmetry of the motor impairment.

In conclusion, this work demonstrates for the first time a peripheral deafferentation in Parkinson's disease that could account (at least in part) for the impairment in sensory function. Further studies are warranted and the skin biopsy, a minimally invasive tool, may prove helpful in providing more answers.

Acknowledgements

We gratefully acknowledge Dr. Dino F. Vitale for his valuable contribution to data analysis.

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