Supplementary Methods

Patient recruitment and Quantitative Sensory Testing

High glucosylceramides and low anandamide contribute to sensory loss and pain in Parkinson's Disease

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Recruitment of PD patients and healthy controls, pain and QST analyses and ethical administration were done by:

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Patient recruitment and pain assessment

Israeli PD patients versus young healthy controls

The study population in Israel consisted of Israeli Jewish patients attending the outpatient Movement Disorders Unit of Rabin Medical Center, a tertiary medical facility in Israel. Patients who fulfilled the diagnostic criteria of PD of the United Kingdom PD Society Brain Bank and gave informed consent were included. Patients were interviewed using a structured questionnaire by a specialist for movement disorders. Items included demographic and clinical data including age, gender, ethnicity, disease duration and course, and medical treatment including analgesics. Patients were asked if they experienced pain, and if yes, to describe the location, distribution, intensity (on a scale of 0-10, where zero is no pain and 10 is extreme pain) during rest and during movement, quality, duration (hours per day) and time of onset in relation to the motor symptoms and first diagnosis of PD. The patients also filled the short Mc'Gill questionnaire for evaluation of pain intensity. According to interview and questionnaire, pain was categorized as musculoskeletal, central or radicular/neuropathic pain and

dystonic/dyskinetic pain. Participants with one or more of these types were considered positive for PD-related pain [1-3]. Parkinson's disease severity was assessed in the same interview using the Parkinson Disease Rating Scale (UPDRS). For patients with response fluctuations, the UPDRS was assessed during the "ON" period.

The first cohort of young healthy control subjects (72 male, 152 female) was recruited from students and staff members of the University Hospital Frankfurt, who routinely reported at the institutional occupational health service. Inclusion criteria were age ≥ 18 years, no current medical condition queried by medical interview, and no drug intake for at least one week except contraceptives, vitamins and L-thyroxin. The study was approved by the respective institutional ethics committees (FFM 458/16), and all patients signed an informed consent form to participate in the study. Demographic data in Suppl. Table 1D.

PD patients versus age-matched healthy controls from Germany

PD patients in Frankfurt were consecutively recruited from the Movement Disorders Department of Neurology of the University Hospital in Frankfurt am Main, Germany and diagnosed using ICD10 diagnostic criteria for Parkinson's Disease. Aged healthy control subjects were consecutively recruited from spouse and the circle of friends of PD patients and from the outpatient stroke unit of the University Hospital in Frankfurt am Main, who got a routine check-up one year after a transient ischemic event without any other health conditions. The inclusion criterium for patients was a verified clinical diagnosis of PD [4]. Exclusion criteria were zoster neuralgia, diabetic polyneuropathy or other neurological and metabolic disorders, which affect pain perception or sensory functions. For controls, the inclusion criteria were age above 50 years, no current relevant medical condition, and no regular drug intake, except vitamins, L-thyroxin, acetylsalicylic acid 100 mg or antihypertensive medication.

Pain was assessed by the same investigator, basically as described above, using a structured interview, and the Brief Pain Inventory (BPI) and the Pain Detect questionnaire, the latter for assessment of neuropathic pain [5, 6]. The interview addressed the medical history, comorbidities and medication, and it was followed by a neurological examination. PD disease severity was assessed according to the Hoehn& Yahr rating scale, in "ON" periods for patients who experienced substantial disease fluctuations (16 out of 50).

Informed written consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee of the University Hospital of Frankfurt. Data acquisition and blood sample collection adhered to the Declaration of Helsinki. Demographic data are summarized in Suppl. Tables 1A-C.

Quantitative Sensory Testing (QST)

QST [7, 8] was performed to assess sensory manifestations of PD, which are not readily revealed in standard neurological examinations or electroneurography. In particular, QST is also able to detect small fiber neuropathies not resulting in axonal dysfunctions [9]. Subjects were instructed in a standardized manner, and they were tested according to the standardized protocol in a comfortable position in a quiet room. During QST sessions, the skin of the dorsum of the hand or foot of the right or left side was tested. Because the first study did not reveal significant side differences, subsequent QST studies in the Frankfurt population were done on the hand and foot dorsum on the most affected side. If there was no side dominance, QST was randomly performed on right or left sides. In the control group, the body side was randomly chosen.

The standardized QST battery according to the German Research Network on Neuropathic Pain (DFNS) protocol [6] begins with the determination of thermal thresholds for perception and for pain, followed by mechanical thresholds and vibration detection. The QST procedures can be chronologically listed as follows: Cold and warm detection thresholds (CDT, WDT), number of paradoxical heat sensations (PHS) during testing of thermal sensory limen (TSL), cold and heat pain thresholds (CPT, HPT), mechanical

detection and pain thresholds (MDT, MPT), vibration detection threshold (VDT) and pressure pain threshold (PPT) [6, 10]. Owing to the age and condition of our patients, testing of the dynamic mechanical allodynia (DMA) for stroking light touch, and wind-up ratio (WUR) during pain summation to repetitive pinprick stimuli were omitted. Hence, the set consisted in each nine parameters for hand (h) and foot (f). Descriptive statistics of sensory thresholds are shown in Suppl. Table 2.

Tests for **thermal sensations** mainly address A-delta and C-fiber functions and were performed by means of a Peltier-based computerized thermal stimulator (NeuroSensory Analyzer, Medoc Advanced Medical Systems Ltd. Israel 2003) with a 3x3-cm contact probe. Starting from a baseline temperature of 32°C a ramping cooling or heating stimulus was applied with a ramp of 1°C/s until the subject pressed a stop button. The lower and upper cut-off temperatures were set at 0°C and 52°C, respectively. The **cold detection threshold** (CDT) was determined first, followed by determination of the warm detection threshold (WDT). During assessment of the thermal sensory limen (TSL) of alternating warm and cold stimuli, the subjects were asked about **paradoxical heat sensations** (PHS) upon cold stimulation, which were scored according to a 4-point scale (0-4, meaning 0=no warm feeling, 1 = warm, 2 = hot, 3 = burning pain). Subsequently, **cold and heat pain thresholds** (CPT and HPT) were determined. Each test was repeated 3-times and the mean was used as the respective threshold.

The **mechanical detection threshold** (MDT) is designed to assess A-beta fiber function and was measured with a set of calibrated von Frey filaments forming a geometric series of 12 filaments. The filaments have a rounded tip and contact area of 0.5 mm, and the forces increment by a factor of 2 from 0.25 mN to 512 mN (Optihair$_2$-Set Marstock Nervtest, Germany). The final threshold was the geometric mean of five series of ascending and descending stimulus intensities.

The **mechanical pain threshold** (MPT) was assessed using a pinprick stimulation (MRC System, Germany). Seven weighted pinprick stimulators of ascending pressure (incrementing by a factor of 2 from 8 mN to 512 mN), 0.25 mm diameter and controlled edge curvature were applied. The mechanical pain threshold was determined as the geometric mean of threshold estimates of five series of ascending and descending stimulus intensities. The MPT is designed to assess A-delta fiber function.

The **vibration detection threshold** (VDT) assesses A-beta fiber function and was determined by means of a standard Rydel-Seiffer tuning fork (128 Hz) graded on an 8/8 scale. The tuning fork was placed on the processus styloideus ulnae or malleolus medialis and the subject was asked to tell when the feeling of vibration disappeared. The arithmetic average of three repetitive measurements was the final threshold.

For assessment of the **pressure pain threshold** (PPT), we used a gauge device, which has a contact area of 1 cm² and exerts forces of up to 2000 kPa with an intensity ramp of 50 kPa/s (FDN200, Wagner Pain Test, USA). The algometer was mounted into a drill frame to allow for precisely increasing force [11] and was placed onto the interdigital muscle between thumb and index finger. The subject was asked to respond verbally as soon as the pressure starts to be painful. The final threshold was calculated as the average of htree ascending stimulus intensities. PPT allows for assessment of deep muscle pain sensitivity, likely mediated by muscle A-delta and C fibers.

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