




Reduction of Skin Innervation Is Associated with a Severe Fibromyalgia Phenotype

Dimitar Evdokimov, MD,¹ Johanna Frank,¹ Alexander Klitsch,¹ Stefan Unterecker, MD,² Bodo Warrings, MD,² Jordi Serra, MD,³ Aikaterini Papagianni, MD,¹ Nadine Saffer,¹ Caren Meyer zu Altenschildesche,¹ Daniel Kampik, MD, PhD,⁴ Rayaz A. Malik, MD,⁵ Claudia Sommer, MD,¹ and Nurcan Üçeyler, MD ¹

Objective: To assess patterns and impact of small nerve fiber dysfunction and pathology in patients with fibromyalgia syndrome (FMS).

Methods: One hundred seventeen women with FMS underwent neurological examination, questionnaire assessment, neurophysiology assessment, and small fiber tests: skin punch biopsy, corneal confocal microscopy, microneurography, quantitative sensory testing including C-tactile afferents, and pain-related evoked potentials. Data were compared with those of women with major depressive disorder and chronic widespread pain (MD-P) and healthy women.

Results: Intraepidermal nerve fiber density (IENFD) was reduced at different biopsy sites in 63% of FMS patients (MD-P: 10%, controls: 18%; $p < 0.001$ for each). We found 4 patterns of skin innervation in FMS: normal, distally reduced, proximally reduced, and both distally and proximally reduced ($p < 0.01$ for each compared to controls). Microneurography revealed initial activity-dependent acceleration of conduction velocity upon low frequencies of stimulation in 1A fibers, besides 1B fiber spontaneous activity and mechanical sensitization in FMS patients. FMS patients had elevated warm detection thresholds ($p < 0.01$), impaired C-tactile afferents ($p < 0.05$), and reduced amplitudes ($p < 0.001$) of pain-related evoked potentials compared to controls. Compared to FMS patients with normal skin innervation, those with generalized IENFD reduction had higher pain intensity and impairment due to pain, higher disease burden, more stabbing pain and paresthesias, and more anxiety ($p < 0.05$ for each). FMS patients with generalized IENFD reduction also had lower corneal nerve fiber density ($p < 0.01$) and length ($p < 0.05$).

Interpretation: The extent of small fiber pathology is related to symptom severity in FMS. This knowledge may have implications for the diagnostic classification and treatment of patients with FMS.

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The original observation that small nerve fiber dysfunction and pathology occur in patients with fibromyalgia syndrome (FMS) has profoundly changed the view of physicians, scientists, and patients on this chronic pain condition.¹ Since then, several studies have confirmed that small fiber

impairment is present in 30 to 70% of patients with FMS and may contribute to fibromyalgia pain.²

Thinly myelinated and unmyelinated nerve fibers (ie, A-delta and C fibers) have been investigated functionally,^{1,3–6} electrophysiologically,^{1,7,8} and morphologically

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Address correspondence to Prof. Dr Üçeyler, Department of Neurology, University of Würzburg, Josef-Schneider-Str 11, 97080 Würzburg, Germany.
E-mail: ueceyler_n@ukw.de

From the ¹Department of Neurology, University of Würzburg, Germany; ²Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg, Germany; ³Neuroscience Technologies, Barcelona, Spain; ⁴Department of Ophthalmology, University of Würzburg, Germany; and ⁵Weill Cornell Medicine-Qatar, Qatar Foundation, Education City, Doha, Qatar

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in the skin^{1,3,4,6,9–12} and cornea.^{5,13} Differing patterns of small fiber impairment may characterize distinct subtypes of FMS, and it has been hypothesized that patients with more extensive small fiber involvement may respond differently to treatment. However, because all previous studies assessed small numbers of patients with FMS, individual subgroup analysis was not possible. Furthermore, nerve structure and function were investigated using different methods, limiting comparison and pooling of data. To better understand the complex underlying pathomechanisms of small nerve fiber damage and its consequences, we performed a comprehensive characterization of small nerve fiber function and structure in a large cohort of patients with FMS. We hypothesized that we would identify patient subgroups in relation to the extent and pattern of small nerve fiber damage.

Here we describe the results of detailed phenotyping in a large group of women with FMS at a single center by applying a comprehensive set of 5 small fiber tests for multidimensional characterization: morphometry and microneurography of the peripheral nerve fiber endings and psychophysical and electrophysiological assessment of the small nerve fiber pathways. We report on distinct patterns of skin denervation in patients with FMS and show a spectrum from normal to a generalized reduction of intraepidermal nerve fibers, which reflects fibromyalgia severity.

Patients and Methods

Recruitment of Patients and Controls

From September 2014 to December 2017, we screened 382 potentially eligible patients and enrolled 117 women with FMS (median age = 52 years, range = 22–75 years) at the Department of Neurology, University of Würzburg, Germany. Patients contacted us directly and via patient organizations from all over Germany. Inclusion criteria were age ≥ 18 years and a diagnosis of FMS according to current criteria (Supplementary Table 1).^{14–16} Exclusion criteria were as follows: pain of other origin indistinguishable from FMS, polyneuropathy, epilepsy, current infection, relevant rheumatic or autoimmune disease as assessed before study inclusion by a rheumatologist, malignancy within the last 5 years, severe psychiatric disorder currently requiring treatment, pending compensation claims, drug or alcohol misuse, eye diseases or operations, regular use of hard contact lenses, cardiac pacemaker, diabetes mellitus, renal insufficiency, and untreated thyroid dysfunction. From August 2018 to May 2019, we enrolled an additional 11 female patients with major depressive disorder (MD) according to the Diagnostic and Statistical Manual of Mental Disorders-IV diagnosed by 2 psychiatrists (S.U., B.W.) and chronic widespread pain (MD-P), that is, pain for ≥ 3 months in multiple body regions (median age = 52 years, range = 43–58 years) from the Department of

Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg, Germany, as disease controls.

Data from skin and corneal innervation, sensory profiling, and electrophysiological assessment were compared with the normative reference values of our laboratory (see description in a later section). Microneurography data were compared with those of healthy controls recruited at our department and historical records from healthy women by the same examiner (J.S.). The study was approved by the Ethics Committee of the University of Würzburg Medical Faculty (#121/14), and all study participants gave written informed consent.

Clinical Examination, Laboratory Tests, and Nerve Conduction Studies

All patients underwent a detailed medical interview and complete neurological examination. All patients were also assessed with the German version of the Neuropathic Pain Symptom Inventory (NPSI; 24 hours recall),^{17,18} Graded Chronic Pain Scale (GCPS; 6 months recall),¹⁹ and the Pain Catastrophizing Scale (PCS).²⁰ To investigate FMS symptoms, the Fibromyalgia Impact Questionnaire (FIQ)²¹ was applied. All patients were interviewed with the “Allgemeine Depressionsskala” (ADS) for depressive symptoms.²² Patients with FMS additionally completed the State-Trait Anxiety Inventory (STAI-S, STAI-T).²³ Pain intensity was reported on an 11-point numeric rating scale with 0 = no pain and 10 = worst pain. Laboratory tests (including full blood count, renal and liver function tests, thyroid stimulating hormone [TSH], vitamin B₁₂, HbA_{1c}, and oral glucose tolerance test [OGTT]) were performed to exclude alternative etiologies of small nerve fiber pathology in FMS patients. To exclude large fiber neuropathy, all patients underwent electrophysiological assessment of the right sural and tibial nerve.

Skin Punch Biopsy

To determine the intraepidermal nerve fiber density (IENFD),²⁴ 6mm skin punch biopsies were obtained from the right lateral lower leg and upper thigh of all FMS patients,²⁵ and 3mm skin punch biopsies were obtained in the MD-P disease control group. To visualize intraepidermal nerve fibers, we immunoreacted 40 μ m skin sections with antibodies against the pan-axonal marker protein-gene product (PGP) 9.5 (516-3344, 1:1,000; Zytomed, Berlin, Germany); Cy3 was used as fluorescent secondary antibody (1:100; Dianova, Hamburg, Germany). IENFD was determined as part of the routine evaluation of skin biopsies in our department by the same investigator blinded to subject group allocation. A fluorescence microscope (Axiophot 2, Zeiss, Oberkochen, Germany) equipped with an AxioCam MRm camera (Zeiss) and SPOT software (Diagnostic Instruments, Sterling Heights, MI) were used. Data were compared with normative values in our laboratory from 120 healthy female

controls (median age = 50 years, range = 20–84 years) in skin biopsies obtained from the lower leg (n = 106) and upper thigh (n = 98).

Corneal Confocal Microscopy

Corneal confocal microscopy (CCM) was performed according to an established protocol in all patients.²⁶ Patients first underwent a slit lamp examination to exclude corneal pathologies. A Schirmer test (Haag-Streit UK, Harlow, Essex, UK) was done to assess xerophthalmia. Both eyes were anesthetized using Conjuncain EDO eye drops (Bausch & Lomb, Berlin, Germany) containing 0.4% oxybuprocaine hydrochloride. One drop of Corneregel EDO (Bausch & Lomb) was applied on the lens tip and one on each eye, and a sterile TomoCap (Heidelberg Engineering, Heidelberg, Germany) was mounted over the lens tip. The Heidelberg Retina Tomograph Rostock Cornea Module (Heidelberg Engineering) was used to obtain 6 central images of the sub-basal plexus per subject, and 3 images per eye were selected by an investigator blinded to group allocation. Corneal nerve fiber density (NFD; no/mm²), nerve fiber length (NFL; mm/mm²), and nerve branch density (NBD; no/mm²) were analyzed using ACCMetrics (NFD, NFL) and CCMetrics (NBD) software (M. A. Dabbah, Imaging Science, Manchester, UK). Corneal sensitivity was tested using a Cochet-Bonnet esthesiometer (Luneau Ophthalmologie, Chartres Cedex, France). The control group consisted of 54 healthy women (median age = 50 years, range = 23–65 years).

Quantitative Sensory Testing

Quantitative sensory testing (QST; Somedic, Hörby, Sweden) was performed on the dorsum of the right foot in all patients¹ following the standardized procedure of the German Research Network of Neuropathic Pain.²⁷ We compared QST results with a normative data bank from our laboratory consisting of 178 female controls (median age = 50 years, range = 20–89-years) and calculated *z* scores as (value of the subject − mean value of controls)/(standard deviation of controls). Negative *z* scores indicate a loss of function and positive *z* scores a gain of function. We determined cold and heat detection thresholds (CDT and HDT, respectively), the ability to perceive temperature changes (thermal sensory limen [TSL]), mechanical detection and pain thresholds (MDT, MPT), mechanical pain sensitivity (MPS), pressure pain threshold (PPT), paradoxical heat sensation (PHS), and vibration detection threshold (VDT).

C-tactile mechanosensitive fiber function was assessed using a standardized pleasant touch stimulus as described previously.²⁸ A calibrated brush (Brush-05; Somedic) was applied 3 times to the dominant dorsal forearm (3cm/s, distance of 12cm). The subject rated the pleasantness of the stimulation

on a scale from -10 (most unpleasant) to +10 (most pleasant), and an average value of 3 brush strokes was calculated. Patient data were compared with normative values obtained in our laboratory from 52 healthy female controls (median age = 53 years, range = 21–73 years).

Pain-Related Evoked Potentials

Pain-related evoked potentials (PREP) were recorded in FMS patients using concentric superficial planar electrodes (Inomed Medizintechnik, Lübeck, Germany) and a DS7A stimulator (Digitimer; Welwyn Garden City, UK)¹ for A-delta fiber stimulation. We bilaterally elicited PREP by consecutive stimulation of the skin above the eyebrow and dorsum of the feet, applying 20 triple pulses. Using Signal Software (v2-16; Cambridge Electronic Design, Cambridge, UK) potentials were recorded from above Cz by a subcutaneous needle electrode referred to linked earlobes (A1–A2) of the international 10–20 system. All records were assessed manually on coded files by an investigator blinded to subject allocation; the N1 and P1 latencies and the peak-to-peak amplitude (PPA) were determined. Data were compared with normative values from our laboratory's data bank of 90 healthy female controls (median age = 53 years, range = 22–82 years).

Microneurography

Microneurography was performed in FMS patients and healthy controls.²⁹ An isolated constant-current stimulator (DS7; Digitimer) was used to stimulate the cutaneous receptive fields with a pair of nonisolated needle electrodes resting on the surface of the skin exerting very gentle pressure just to break the stratum corneum. Stimulus duration was set at 0.3 milliseconds. Action potentials from C fibers were recorded using tungsten microelectrodes (200µm diameter, impedance 1MΩ) placed intraneurally into the superficial peroneal nerve at ankle level. Signals were amplified (gain 10,000, bandwidth 100Hz to 2kHz) with an isolated high-input impedance amplifier (NeuroAmpEx; ADInstruments, Bella Vista, Australia) and digitized (NI DAQCARD-6062E; National Instruments Europe, Debrecen, Hungary) at a sampling rate of 20kHz. Stimulation and recording were controlled by QTRAC software (UCL Institute of Neurology, London, UK). Responses were clamped to baseline and the largest peaks displayed as a latency profile or raster plot.⁸ Skin temperature close to the superficial peroneal nerve was recorded continuously with an infrared thermometer (PCE-IR10; PCE Iberica, Albacete, Spain).

Subclasses of peripheral C fibers were identified in the raster plots by their characteristic profile of activity-dependent slowing of conduction velocity (CV) when stimulation rate was increased from 0.25 to 2Hz.^{29,30} Responsiveness to mechanical stimuli was tested using a calibrated set of von Frey filaments

(Optihair2; Marstock Nervtest, Schriesheim, Germany). To identify subtypes of C fibers, CV was measured after a pause, while stimulating at 0.25Hz, followed by a 2-minute train of stimulation at 2Hz, as done previously.^{8,30} Nociceptive C fibers (type-1 C fibers) were identified by showing either constantly progressive slowing of CV or conduction blocks at 2Hz stimulation.²⁹ These fibers were then further classified as mechanosensitive (type 1A), showing <1% slowing of CV at 0.25Hz stimulation, or mechanoinsensitive (type 1B), showing a slowing of CV of at least 2% during stimulation at 0.25Hz.^{30,31} The following parameters were determined in type 1A (mechanosensitive) and 1B nerve (mechanoinsensitive) fibers as previously described:⁸ (1) percentage of CV slowing from rest to 0.25Hz stimulation; (2) percentage of CV slowing after a 3-minute pause following a 0.25Hz baseline stimulation; (3) percentage of CV slowing from 0.25Hz baseline stimulation to the end of a 3-minute period of 2Hz stimulation; and (4) recovery of CV after stopping a 2Hz stimulation train by recording the time until reversal of 50% of the activity-induced latency change and the percentage of recovery at 30 seconds.^{29,32} In type 1B nociceptors, spontaneous activity and mechanical sensitization were also assessed. The investigator (J.S.) was blinded with regard to subject allocation. Data were compared with those from 13 healthy female controls (median age = 42 years, range = 24–61 years).

Statistical Analysis

For statistical analysis, SPSS Statistics 24 software (IBM, Ehningen, Germany) was used. The nonparametric Mann–Whitney *U* test was applied when data were not normally distributed. A *t* test was performed for comparison of the normally distributed *z* scores of QST data. Correlations were calculated using the bivariate Spearman correlation coefficient. Microneurography data were analyzed applying the Welch unequal variance *t* test to the ranked data; microneurography data were visualized using Prism 8 (GraphPad Software, San Diego, CA). Categorical data between groups were compared using the χ^2 test. Statistical significance was assumed at $p < 0.05$.

Results

Clinical and Laboratory Findings

Supplementary Table 1 provides baseline data, and the Table summarizes data on pain and psychological characteristics of the patient cohort. Individual data on analgesic intake of the study population is provided in Supplementary Table 2. FMS pain distribution is illustrated in Figure 1. None of the FMS patients had been diagnosed with a relevant rheumatic or autoimmune disease as assessed by a rheumatologist prior to study inclusion.

Neurological examination and nerve conduction studies were normal in all patients. Cell count and routine biochemical analysis were normal in all FMS patients. Two FMS

patients had a HbA_{1c} of >6.1%, and 15 had a 2-hour glucose value of >140mg/dl on the OGTT. The small nerve fiber test results of these FMS patients did not differ from FMS patients with normal glucose metabolism (data not shown).

The 117 FMS patients mostly reported symmetric (n = 88, 75%) and permanent pain with intermittent increases in pain intensity (n = 91, 78%). Pain character was described as pressing (n = 47, 40%), burning (n = 45, 38%), stabbing, and like muscle soreness (n = 29, 25% for each). Ninety-five (81%) patients were taking analgesics, using 1 (n = 52, 44%), 2 (n = 34, 29%), or 3 or more drugs (n = 9, 8%).

Forty-one (35%) of 117 FMS patients reported depression and 11 (9%) reported anxiety in their medical history, 79 (68%) patients had received or were currently receiving psychological therapy, 57 (49%) patients reported a life event, and 49 (42%) patients also reported that family members had chronic pain. We defined life event as a “very positive” or “very negative” experience that the patient subjectively regarded as causatively linked with the first occurrence of fibromyalgia symptoms.

MD-P patients had been diagnosed with MD for a median of 10 years (1 month to 50 years) and reported the presence of additional chronic widespread pain for a median of 5 years (1–44 years). They described multilocal, symmetric (9 of 11 patients, 82%), and permanent pain with intermittent increases in pain intensity (9 of 11 patients, 82%). Pain character was pressing (8 of 11 patients, 73%), burning (6 of 11 patients, 55%), and stabbing (4 of 11 patients, 36%). Ten of 11 (91%) patients were on analgesics using 1 (7 patients, 64%) or 2 (3 patients, 27%) drugs. All patients with MD-P had received and were currently receiving psychological and/or psychiatric therapy.

Distinct Patterns of Skin Denervation in Patients with FMS

Distal ($p < 0.01$) and proximal ($p < 0.001$) IENFD was lower in patients with FMS compared to healthy controls. In controls, distal IENFD decreased with age (Spearman coefficient = 0.414; $p < 0.001$), but no age-dependency was found for proximal innervation (Fig 2). In FMS patients, distal and proximal IENFD were independent of age. We defined <5.4 fibers/mm (ie, 8.2 minus 2.8 fibers/mm) as pathological at the lower leg and <8.5 fibers/mm (ie, 11.8 minus 3.3 fibers/mm) as pathological at the upper thigh, as compared to normative values obtained from 120 healthy women (mean \pm standard deviation IENFD lower leg = 8.2 ± 2.8 fibers/mm; upper thigh = 11.8 ± 3.3 fibers/mm). Applying these cutoff values, we found 4 distinct FMS subgroups: patients with normal skin innervation (FMS: 37%, controls: 82%), reduced distal IENFD (FMS: 17%, controls: 13%), reduced proximal IENFD (FMS: 31%, controls: 2%), and proximal and distal reduction in IENFD (FMS: 15%,

TABLE. Pain and Psychological Characteristics of Study Cohort

Characteristic	Fibromyalgia Syndrome, n = 117	Major Depressive Disorder and Chronic Widespread Pain, n = 11
Pain duration, yr	12 (0.8–56)	5 (1–44)
Current pain intensity on NRS	5 (0–9)	5 (2–8)
Pain distribution		
Generalized	70/117 (60%)	9/11 (82%)
Proximal	32/117 (27%)	2/11 (18%)
Distal	4/117 (3%)	None
Categorization not possible	11/117 (9%)	None
Pain symmetry		
Bilateral pain	88/117 (75%)	9/11 (82%)
Unilateral pain	29/117 (25%)	2/11 (18%)
Pain dynamics		
Permanent pain with intermittent increases in pain intensity	91/117 (78%)	9/11 (82%)
Permanent	15/117 (13%)	1/11 (9%)
Attacks	9/117 (8%)	1/11 (9%)
Top 3 pain descriptors		
Pressing	49/117 (42%)	Pressing: 8/11 (73%)
Burning	43/117 (37%)	Burning: 6/11 (55%)
Like muscle soreness	29/117 (25%)	Stabbing: 4/11 (36%)
Paresthesias in painful area	29/117 (25%)	6/11 (55%)
NPSI		
Burning score	5 (0–10)	5 (0–10)
Pressure score	6 (0–10)	3 (0–7)
Attacks	4 (0–14)	3 (0–7)
Evoked pain score	4 (0–9)	1 (0–6)
Par-/dysesthesia score	4 (0–10)	1 (0–10)
Sum score	4 (1–9)	3 (0–7)
GCPS		
Current pain intensity	6 (0–9)	6 (3–10)
Maximum pain intensity	9 (5–10)	8 (6–10)
Mean pain intensity	6 (3–10)	7 (4–10)
Days without regular activity	30 (0–365)	1 (0–150)
Impairment everyday life	5 (0–8)	5 (2–9)
Impairment leisure	6 (1–10)	7 (3–9)
Impairment work	7 (0–10)	6 (3–10)
Disability due to pain	58 (10–87)	68 (47–90)
GCPS pain grade	2 (1–4)	3 (2–4)
PCS sum score	23 (0–49)	25 (16–48)

TABLE. Continued

Characteristic	Fibromyalgia Syndrome, n = 117	Major Depressive Disorder and Chronic Widespread Pain, n = 11
Analgesic medication		
None	22/117 (19%)	1/11 (1%)
Monotherapy	51/117 (44%)	7/11 (64%)
Combination of ≥ 2	37/117 (32%)	1/11 (1%)
Relevant pain relief, ≥ 2 NRS	63/88 (72%)	6/11 (55%)
ADS sum score	22 (3–51)	37 (22–46)
STAI-S sum score	46 (27–73)	Not assessed
STAI-T sum score	45 (28–72)	Not assessed
Psychiatric or psychological treatment		
Currently ongoing	19/117 (16%)	11/11 (100%)
Never had	38/117 (32%)	None

Data are given as median with range in parentheses.

ADS = Allgemeine Depressionsskala (German version of the Center for Epidemiological Studies Depression scale questionnaire); GCPS = Graded Chronic Pain Scale; NPSI = Neuropathic Pain Symptom Inventory; NRS = numeric rating scale; PCS = Pain Catastrophizing Scale; STAI-S = State-Trait Anxiety Inventory–State; STAI-T = State-Trait Anxiety Inventory–Trait.

controls: 2%; χ^2 : $p < 0.001$ each for comparison between FMS patients and controls). Distal and proximal skin innervation was normal in all but 1 patient with MD-P (median IENFD lower leg = 6.6 ± 2.0 fibers/mm; upper thigh = 10.3 ± 3.4 fibers/mm).

Corneal Innervation Is Reduced in Patients with FMS

Corneal sub-basal NFD ($p < 0.01$) and NFL ($p < 0.05$; see Fig 2G, H) but not NBD were reduced in patients with FMS compared to healthy controls. Corneal innervation of patients with MD-P did not differ from healthy controls.

Small and Large Fiber Sensory Dysfunction in Patients with FMS

There was hyposensitivity to warm (warm detection threshold [WDT]; $p < 0.01$), tactile (MDT, $p < 0.001$), and painful punctate mechanical stimuli (MPT, $p < 0.001$) in patients with FMS compared to controls. In contrast, FMS patients were more sensitive to painful cold (CPT, $p < 0.01$), mechanical stimulation (MPS, $p < 0.001$), and blunt pressure (PPT, $p < 0.001$; Fig 3A). Brush stimulation of C-tactile afferents on the dominant forearm was perceived as neutral to unpleasant (ie, a reported score of < 1) in 26 of 117 (22%) FMS patients compared to 4 of 52 (7%) healthy controls (χ^2 : $p < 0.05$). Sensory profiles of patients with MD-P did not differ from

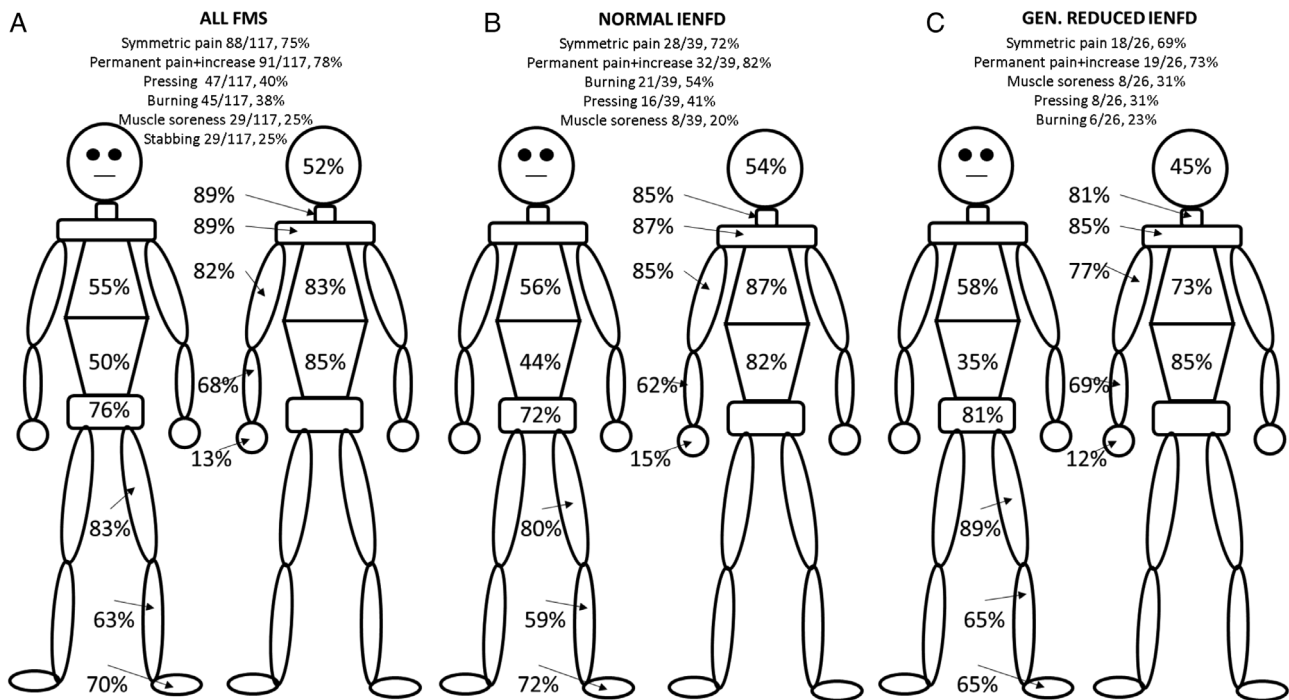


FIGURE 1: Pain distribution in patients with fibromyalgia syndrome (FMS). The schemes illustrate the frequency of pain reported in different body areas in (A) the entire group of FMS patients, (B) in patients with normal skin innervation, and (C) in patients with a generalized (GEN) reduction of intraepidermal nerve fiber density (IENFD). Additionally, pain characteristics are summarized for each subgroup.

controls. Corneal sensitivity, as measured by esthesiometry, did not differ between patient and control groups (data not shown).

PREP Amplitudes Are Reduced in Patients with FMS

PPA was reduced after stimulation at the face and the feet of FMS patients compared to controls ($p < 0.001$ for each; see Fig 3B, C). Although N1 and P1 latencies did not differ between groups after stimulation at the face, patients with FMS showed shorter N1 ($p < 0.01$) and P1 latencies ($p < 0.001$) after stimulation at the feet (see Fig 3D, E).

Activity-Dependent Acceleration, Spontaneous Activity, and Mechanical Sensitization of C Nociceptors in Patients with FMS

Microneurography recordings were obtained from 27 of 29 FMS patients and 13 of 14 healthy controls. Controls were investigated in Barcelona ($n = 9$) and Würzburg (recordings obtained in 4 of 5 cases). Because no site differences were found, data were pooled. Supplementary Table 3 summarizes the electrophysiological properties of the investigated C nociceptors.

In FMS patients, 334 C fibers were recorded, of which 177 were analyzed: 96 (54%) were mechanosensitive type 1A nociceptors and 81 (46%) were mechanoinsensitive type 1B nociceptors. In healthy controls, 278 C fibers were recorded,

of which 97 were analyzed: 44 (45%) were mechanosensitive type 1A nociceptors and 53 (55%) mechanoinsensitive type 1B nociceptors.

CV was higher for type 1A nociceptors in the FMS group compared to controls ($p < 0.05$; Fig 4A). On stimulation at 0.25Hz, activity-dependent acceleration was recorded in patients with FMS compared to controls ($p < 0.01$; see Fig 4B); no differences were detected in the percentage of conduction slowing after stimulation at 2Hz (see Supplementary Table 3). Also, no intergroup differences were found for the time until reversal of 50% of the activity-induced latency change and the percentage of recovery at 30 seconds in type 1A fibers.

When investigating type 1B nociceptors, CV was higher in the FMS group compared to controls ($p < 0.01$; see Fig 4C). Nociceptors of patients with FMS showed greater slowing of CV at 0.25Hz stimulation than controls ($p < 0.001$; see Fig 4D); no differences were detected in the percentage of slowing at 2Hz stimulation when compared to healthy controls (see Supplementary Table 3). We found no intergroup differences for the time to reversal of 50% of the activity-induced latency change and the percentage of recovery at 30 seconds in type 1B fibers.

Spontaneous activity was present in 11 of 27 (40%) FMS patients and in 20 of 81 (25%) type 1B fibers recorded in patients compared to 1 of 14 (7%) healthy controls and 1 of 53 (2%) type 1B fibers

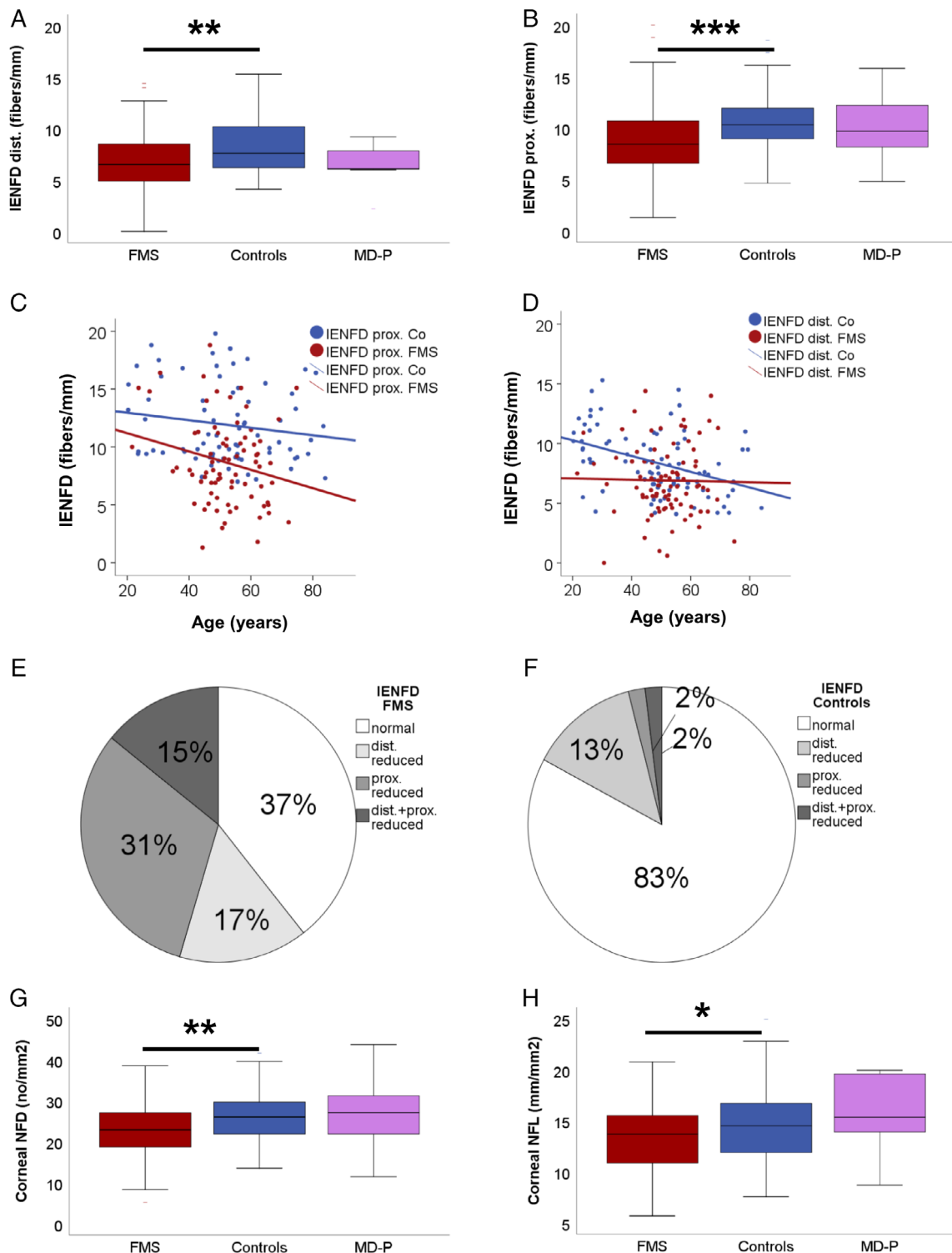


FIGURE 2: Skin and corneal innervation of patients with fibromyalgia syndrome (FMS) compared to healthy controls and patients with major depressive disorder and chronic widespread pain (MD-P). Intraepidermal nerve fiber density (IENFD) was lower (A) at the distal (dist) leg ($p < 0.01$) and (B) at the proximal (prox) thigh ($p < 0.001$) of FMS patients compared to healthy controls (Co); skin innervation did not differ between patients with MD-P and healthy controls. (C) Distal IENFD was independent of age in FMS, but (D) age dependently decreased in healthy controls (Spearman coefficient = 0.414; $p < 0.001$). (E) A total of 63% of patients with FMS showed a reduction of IENFD either at the distal leg or at the proximal thigh or at both sites; 37% of patients with FMS had a normal skin innervation at both biopsy sites. (F) Skin innervation was reduced in 18% of healthy controls either at the distal leg or at the proximal thigh or at both sites; 82% of controls had a normal skin innervation at both biopsy sites. We determined 4 innervation patterns that showed the following distribution for FMS and controls: normal skin innervation (FMS: 37%, controls: 82%), reduced distal IENFD (FMS: 17%, controls: 13%), reduced proximal IENFD (FMS: 31%, controls: 2%), and generally reduced IENFD (FMS: 15%, controls: 2%). When assessing corneal innervation with confocal microscopy, we found (G) lower nerve fiber density ($p < 0.01$) and (H) nerve fiber length (NFL; $p < 0.05$) in FMS patients compared to controls, but no intergroup difference was found when comparing patients with MD-P and healthy controls.

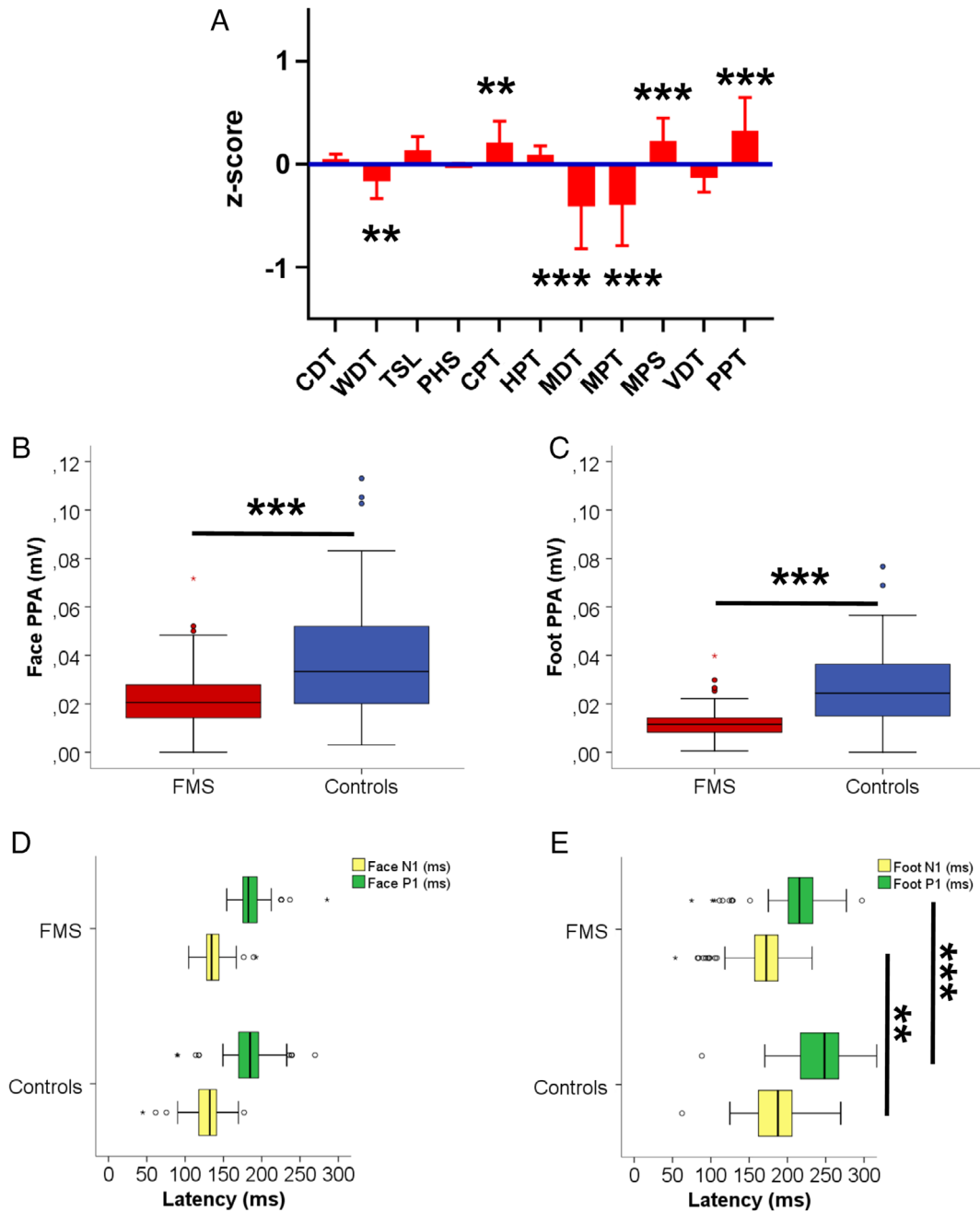


FIGURE 3: Sensory profiles and A-delta pathway analysis in patients with fibromyalgia syndrome (FMS) compared to healthy controls. (A) In quantitative sensory testing (QST), FMS patients (red bars) had higher thresholds for the detection of warm stimuli (WDT; $p < 0.01$), mechanical stimuli (MDT; $p < 0.001$), and pinprick-induced pain (MPT; $p < 0.001$) compared to healthy controls (blue zero line). In contrast, FMS patients had lower cold pain thresholds (CPT; $p < 0.01$), higher mechanical pain sensitivity (MPS; $p < 0.001$), and lower pain pressure threshold (PPT; $p < 0.001$) compared to healthy controls. During recordings of pain-related evoked potentials (PREP), patients with FMS had lower peak-to-peak amplitudes (PPA) after stimulation at face (B) and foot (C) compared to healthy controls ($p < 0.001$ for each). (D) Although PREP latencies did not differ between both groups after stimulation at the face, (E) N1 ($p < 0.01$) and P1 latencies ($p < 0.001$) were shorter in FMS patients after stimulation at the foot. CDT = cold detection threshold; HPT = heat pain threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; PHS = paradoxical heat sensation; TSL = thermal sensory limen; VDT = vibration detection threshold; WDT = warm detection threshold.

recorded in controls (χ^2 : $p < 0.001$; see Fig 4E). Mechanical sensitization was detected in 5 of 27 (19%) patients with FMS and in 14 of 81 (17%) type 1B fibers

recorded in FMS patients compared to 1 of 14 (7%) and 1 of 53 (2%) type 1B fibers recorded in controls (χ^2 : $p < 0.001$; see Fig 4F). Characteristic findings recorded

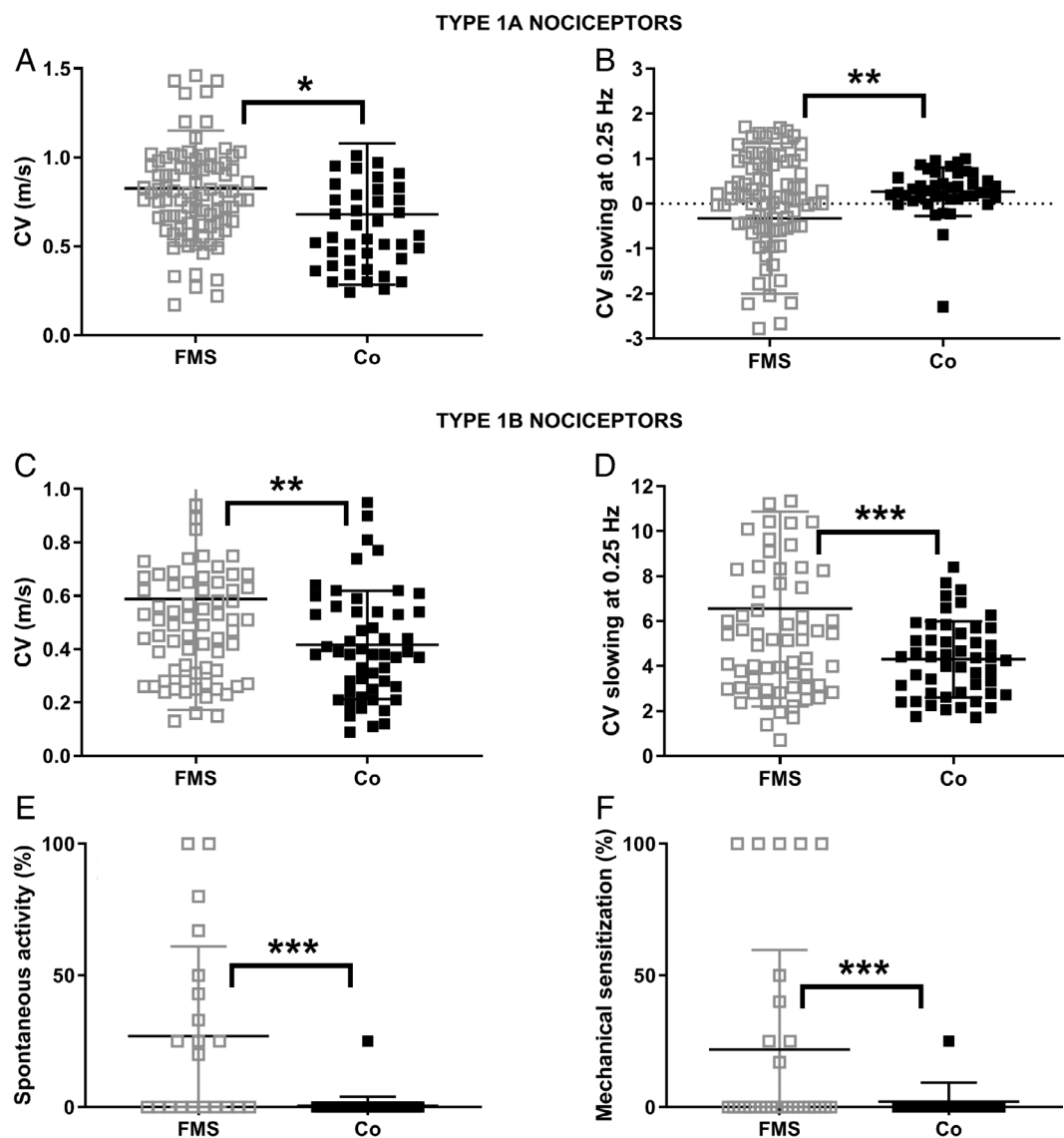


FIGURE 4: Microneurography data of patients with fibromyalgia syndrome (FMS) compared to healthy controls (Co). Patients with FMS had (A) higher conduction velocities (CV; $p < 0.05$) and displayed (B) activity-dependent acceleration ($p < 0.01$) of type 1A nociceptors compared to healthy controls. In type 1B nociceptors, FMS patients also showed a (C) higher CV ($p < 0.01$) than healthy controls and (D) displayed more activity-dependent slowing ($p < 0.001$) compared to controls. Type 1B nociceptors showed (E) more frequent spontaneous activity ($p < 0.001$) and (F) mechanical sensitization ($p < 0.001$) than healthy controls.

in patients with FMS during microneurography are illustrated in Figure 5.

Generalized Skin Denervation Is Associated with a More Severe FMS Phenotype

Next, we investigated whether the pattern of skin denervation is associated with a distinct clinical phenotype in FMS and stratified our patients according to 4 innervation patterns, detailed in Figure 2E (Supplementary Table 4).

Subgroups did not differ for age or disease duration ($p > 0.05$), and no intergroup difference was found between patients with reduced distal or proximal IENFD compared to patients with normal IENFD. Comparison of subgroups only revealed differences between patients with generalized small

fiber reduction and those with normal innervation. FMS patients with both proximal and distal reduction of IENFD had a higher disease burden when compared to FMS patients with normal skin innervation (Supplementary Table 5) with pain on the lower back, hips, and upper thigh and more diffuse pain (see Fig 1B, C). In the NPSI, these patients reported mostly stabbing pain associated with pins and needles dysesthesias ($p < 0.05$ for each) with a higher current ($p < 0.05$), mean ($p < 0.05$), and maximum ($p < 0.01$) pain intensity compared to FMS patients with normal skin innervation. In the GCPS, patients with a proximal and distal reduction of IENFD also reported markedly greater impairment and disability due to pain during everyday living, working, and leisure time ($p < 0.05$), and they had higher FIQ sum scores ($p < 0.05$) compared to patients without skin

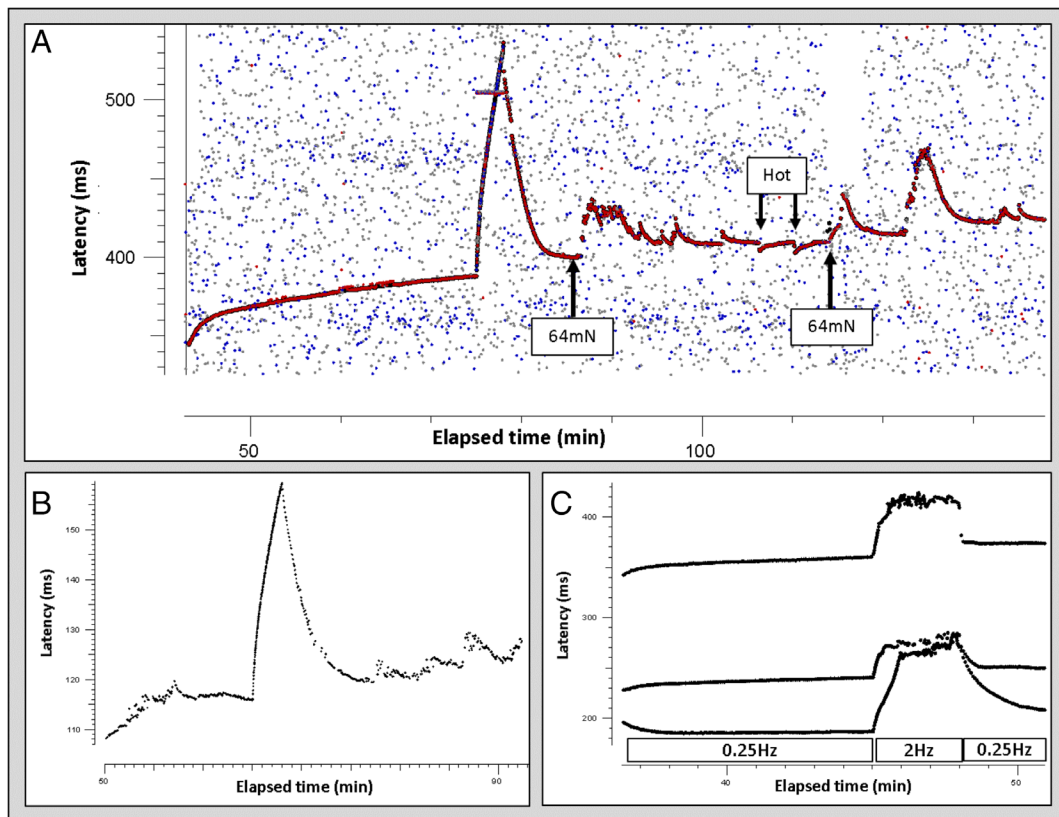


FIGURE 5: Microneurographic raster plots illustrating 3 characteristic findings in patients with fibromyalgia syndrome (FMS). (A) Exclusive mechanical sensitization: C-fiber unit with a profile of activity-dependent slowing of conduction velocity compatible with a type1B mechanoinsensitive C nociceptor according to its behavior during 0.25 and 2Hz stimulation. However, this unit responds to mechanical stimulation with a von Frey filament of 64mN (arrow), producing intense, long-lasting bursts of activity that clearly persist beyond the actual mechanical stimulus for tens of minutes. On the other hand, the unit is insensitive to heat stimulation (arrows) with a hot rod at 55°C, which only induces momentarily conduction velocity “accelerations” due to local warming up of the receptive field. The unit was not spontaneously active for more than 30 minutes before mechanical stimulation (arrow). (B) Spontaneous activity: C-fiber unit with a profile of activity-dependent slowing of conduction velocity compatible with a type1B mechanoinsensitive C nociceptor showing a continuous “saw-tooth” profile indicative of spontaneous ongoing activity. (C) Activity-dependent “acceleration” of conduction velocity: Raster plot showing 3 C fibers with activity-dependent profiles during 2Hz stimulation indicative of a C nociceptor (all 3 fibers block during the 2Hz stimulation period). The 2 units at a longer latency show a slowing of conduction velocity greater than 3% at the beginning of a low-rate 0.25Hz stimulation period and are classified as type 1B mechanoinsensitive C nociceptors. However, the unit at a shorter latency displays a surprising activity-dependent “acceleration” of conduction velocity at 0.25Hz (arrow). All 3 C nociceptors were mechanoinsensitive to forces up to 256mN (not shown).

denervation. The STAI-T score was higher in the FMS group with proximal and distal reduction in IENFD, reflecting anxiety as a trait ($p < 0.05$). The body mass index was higher in the group with both distal and proximal reduction in skin IENFD compared to patients with a normal IENFD ($p < 0.01$).

Corneal Denervation Parallels Skin Denervation

CCM demonstrated a stepwise reduction in corneal innervation in parallel with skin denervation. FMS patients with both proximal and distal reduction of IENFD had a lower NFD ($p < 0.01$) and NFL ($p < 0.05$) compared to patients with normal skin innervation (Fig 6). QST sensory profiles including the perception of pleasant touch and subgroup analysis of microneurography and PREP data did not show differences

between FMS patients with proximal and distal reduction in IENFD compared to those with normal IENFD (see Supplementary Table 4).

Discussion

This is the first study to comprehensively investigate a large cohort of patients with FMS recruited at a single center applying 5 tests for small nerve fiber morphology, function, and electrophysiological properties. We show widespread small nerve fiber dysfunction and damage in FMS patients and provide first evidence that a more severe phenotype is associated with more extensive skin denervation; disease controls with MD-P did not differ from healthy controls. Our findings underscore the importance of the peripheral nervous system for FMS symptoms.

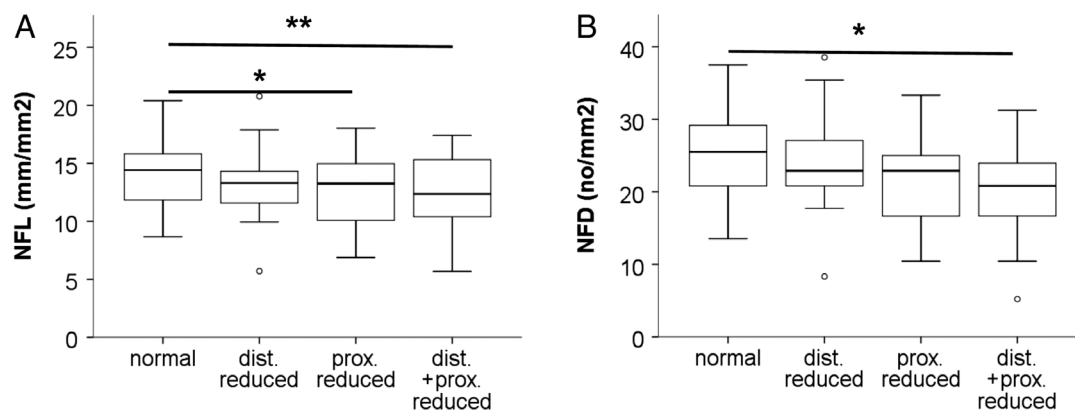


FIGURE 6: Corneal innervation in subpopulations of patients with fibromyalgia syndrome (FMS). The boxplots illustrate the corneal nerve Fiber length (NFL; A) and nerve fiber density (NFD; B) of FMS patients with normal skin innervation, reduced intraepidermal nerve fiber density (IENFD) at the lower leg, the upper thigh, and at both sites. Corneal innervation diminished stepwise and was lower in patients with a generalized reduction of Skin innervation compared to patients with normal skin innervation (NFL: $**p < 0.01$; NFD: $*p < 0.05$). NFL was also lower in patients with a reduction of IENFD at the thigh ($*p < 0.05$, A). Dist = distal; prox = proximal.

That FMS patients with generalized reduction of skin innervation also had a greater reduction in corneal innervation indicates widespread neurodegeneration. However, it remains elusive why these proximal nerve fibers originating from the cranial trigeminal nerve should show early degeneration. The corneal sub-basal nerve plexus anatomically corresponds to the subepidermal plexus, and the profiles identified as nerve fibers with CCM represent unmyelinated nerve fiber bundles.³³

Elevated thermal perception thresholds have been reported in small subgroups of FMS patients and controls,^{1,4-6,13} and the present study confirms warm hyposensitivity. As in our previous study,¹ we also show elevated mechanical detection thresholds, despite lack of other indicators for large nerve fiber impairment detected by history, neurological examination, and nerve conduction studies. The observed increase in mechanical thresholds may be due to impaired C-tactile afferents.³⁴ A higher number of FMS patients perceived a standardized brush stroke stimulus as “neutral” to “unpleasant” compared to control subjects,²⁸ indicating dysfunction of C-tactile afferents. There was no difference in corneal mechano-perception between groups, which may reflect limited tool sensitivity.³⁵

As previously described,¹ we found a generalized reduction of PREP PPA indicative of reduced excitability of A-delta nerve fibers. We and others have reported a reduction in PREP PPA in disorders with small nerve fiber impairment.^{36,37,39} Thus, our PREP results further support that small nerve fiber pathology in subgroups of patients with FMS is not restricted to morphological alterations but has a functional impact. We also found shorter N1 and P1 latencies when electrically stimulating FMS patients at the feet compared to controls, the cause of which remains elusive. So far N1 and P1 latencies have mostly been reported to be

unchanged^{37,38} or prolonged^{36,39} in patients with small fiber pathology.

Here we confirm and extend previous microneurography data⁸ on activity-dependent changes in C-nociceptor CV and C-nociceptor hyperactivity in terms of spontaneous activity and mechanical sensitization in patients with FMS. We also provide evidence for alterations in type 1A mechanosensitive nerve fibers of FMS patients compared to a previous study.⁸ Several C-nociceptor fibers of FMS patients displayed an initial acceleration of CV upon low frequencies of stimulation. This has been described in type 1B nerve fibers of experimental animals and interpreted as a direct consequence of a mild membrane potential depolarization.⁴⁰ Although the pathophysiological mechanisms remain unclear, these findings underscore the impact of the peripheral nervous system on pain in patients with FMS. Of note, we confirmed the finding from previous studies⁸ that peripheral sensitization in FMS patients seems restricted to mechanical stimuli while these pathological C-nociceptors remain insensitive to heat stimuli, a finding in sharp contrast with the situation in patients with small fiber neuropathies. However, it remains to be elucidated how microneurography findings are related to the clinical phenotype and pain of FMS patients.

Two recent studies have provided evidence for FMS subgroups based on small fiber pathology.^{5,41} In a cohort which is 3-fold larger, we confirm that a greater reduction in corneal innervation is related to the occurrence of pins and needles paresthesias and a globally reduced skin innervation independent of age or disease duration. We thus suggest that the subgroup with a reduction in both skin and corneal innervation is a special FMS subgroup that deserves attention.

One possible explanation for skin denervation and pain in FMS might be that the missing fibers physiologically alleviate pain. As previously shown,⁴² decreased A-delta amplitudes are

associated with higher pain ratings in neuropathic pain patients. It is possible that in FMS patients, distinct C-fiber subpopulations are more prone to degeneration contributing to pain. The C-tactile afferents might be one such subpopulation that code for the pleasantness of touch, which was reduced in FMS patients compared to controls. Another possibility is increased susceptibility of pathologically impaired remaining peripheral nociceptors to the influence of local pain mediators.²⁵ As for FMS patients with normal skin innervation and pain, we can only speculate that nerve fiber hyperexcitability may precede nerve fiber degeneration and that some nerve fibers in patients with normal IENFD may be hyperexcitable.

Disturbance of glucose metabolism leading to impaired glucose tolerance or diabetes mellitus has been associated with small fiber neuropathy.⁴³ Surprisingly, small fiber test results of 17 patients, who had an elevated HbA_{1c} and/or elevated glucose values in the OGTT after study inclusion, did not differ from those with normal HbA_{1c} and OGTT. One reason may be that the perturbation in glucose levels was mild and of recent onset; small fiber pathology changes were previously shown with a change in glucose tolerance status.⁴⁴

Our study has some limitations. Although the FMS group was large, microneurography was performed in a subgroup as we could only investigate a small number of patients using this demanding and time-consuming technique. Also, we cannot exclude the possibility that disease pathology may alter nerve fiber physiology and that using neurophysiological parameters to define fiber subgroups may include a bias. We did not undertake autonomic function tests but limited the assessment of potential autonomic disturbance to patients' subjective reports. Patients with MD-P underwent only the most relevant measures of small fiber pathology including QST, CCM, and IENFD due to the reduced physical and mental endurance of these patients, who were all under current psychiatric treatment. The main reason for the small MD-P group was that these neurologically healthy patients refused invasive skin punch biopsy.

This is the first study to investigate a large cohort of FMS patients with 5 different small fiber tests and provides robust support for the growing evidence of small nerve fiber impairment in subgroups of FMS patients. We define 4 different innervation patterns and show that advanced skin denervation is associated with a more severe FMS phenotype and symptom load paralleled by a generalized neurodegenerative process also reflected by accompanying corneal denervation. This knowledge substantially impacts diagnostic classification of FMS and may open new avenues for targeted treatment of FMS.

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Author Contributions

N.Ü. and C.S. contributed to study concept and design; N. Ü., D.E., J.F., S.U., B.W., D.K., A.P., N.S., A.K., J.S., and R.A.M. contributed to data acquisition and analysis; and N. Ü., D.E., and C.S. drafted a major portion of the text or figures.

Potential Conflicts of Interest

Nothing to report.

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