Appendix S1

SUPPLEMENTARY METHODS

Quantitative sensory testing

Quantitative sensory testing was performed according to the protocol of the German Research Network for Neuropathic Pain (21). Thermal thresholds were assessed, using a 3x3 cm contact thermode (TSA II NeuroSensory Analyzer, Medoc Ltd., Israel). Temperature rose from a baseline of 32°C with a ramp rate of 1.0°C/s (cut-off: 0ºC and 50ºC). Study participants were asked to press a button when the sensation induced by the thermode changed from neutral to a cold or warmth (CDT: cold detection threshold; WDT: warmth detection threshold) and to cold or heat pain (CPT: cold pain threshold; HPT: heat pain threshold). Additionally, the difference limen for alternating warmth and cold stimulation was determined (TSL: thermal sensory limen), as well as the number of paradoxical heat sensations (PHS). Afterwards, study participants were stimulated in ascending and descending order with a set of von Frey filaments (Optihair2-Set, Marstock Nervtest, Germany [forces between 0.25 and 512 mN; diameter: 0.5 mm]) and instructed to tell which stimulations were perceived (MDT: mechanical detection thresholds). Using a set of pinpricks (PinPrick, MRC Systems, Heidelberg, Germany [forces between 8 and 512 mN; diameter: 0.25 mm]) participants were asked to report when the stimulation was painful (MPT: mechanical pain thresholds). The stimulus-response function to pinprick stimulation (MPS: mechanical pain sensitivity) and to innocuous stimuli (cotton wisp, a cotton wool tip and a brush; ALL: dynamic mechanical allodynia) was then determined. The wind-up ratio (WUR) was assessed by measuring the difference in pain intensity induced by a single pinprick stimulation (256 mN) and by a series of 10 pinprick stimuli (256 mN, 1 Hz). Subsequently, using a tuning fork (AESCULAP, B. Braun Company, Germany; 64 Hz, 8/8 scale), the vibration detection thresholds (VDT) were assessed and, finally, pressure pain thresholds (PPT) were measured by stimulating 3 times with a pressure algometer (FDN200, Wagner Instruments, USA; 1-cm^2 probe). QST was performed on itchy skin on the forearm (n=13, 41.9 %), upper arm (n=11, 35.5%), flank or shoulder (n=7, 22.6%). Corresponding areas to the later taken biopsies were used.

Immunohistochemistry

Determination of the intraepidermal nerve fiber density (IENFD): Following the method by Schuhknecht et al. (22), the tissue was frozen in liquid nitrogen after fixation in 4% formalin and buffering in 5%, 10% and 20% sucrose. Three 35-µm cryosections were sliced by each biopsy, mounted on slides (Dako Cytomation, Glostrup, Danmark) and stained overnight with a polyclonal antibody against protein gene product 9.5 (PGP 9.5; 1:200, Zytomed Systems, Berlin, Germany). The sections were incubated with a second antibody, fluorescein isothiocyanate isomer 1 (FITC)-conjugated Swine Anti-Rabbit (1:200, Dako, Cytomation, Glostrup, Danmark). Following the guideline established by (S1), the IENFD was determined. Using a fluorescence microscope (Olympus, Modell BX43F, Tokyo, Japan), all nerves,which clearly penetrated the basement membrane, were counted. IENFD was calculated as the mean of nerve fibers per millimeter epidermal length (IENF per mm). Three specimens per biopsy were counted and the mean was determined. IENFD was compared using the Mann-Whitney U test. For correlation of IENFD and clinical parameters, we employed the Pearson's correlation coefficient.

TRP immunostaining: Stainings of 5 CP-W and 5 CP-N were analyzed exemplarily, including their matching HC. Immunocytochemistry was performed for 6 thermo-channel antibodies in different cryosections following the standards of the Department of Dermatology and Center for Chronic Pruritus, University Hospital Münster, which are similar to the ones described in Haas et al. 2010 (S2). The fixated tissue was sliced to 3–5 µm sections and thawed at room temperature. The tissue was incubated in the 2% paraformaldehyde/phosphate buffered saline (Morphisto, Frankfurt, Germany; Sigma-Aldrich, Steinheim, Germany) and saturated picric acid for 20 min. They were immunostained with rabbit anti-TRP antibody (TPRV1 1:200; Alamone Labs, Jerusalem, Israel; TRPV2 1:2000; LSBio, Seattle, USA; TRPV3 1:200; TRPV4 1:1000; TRPA1 1:400; Abcam, Cambridge, UK; TRPM8 1:200; LSBio, Eching, Germany) overnight at room temperature, followed by the incubation with secondary antibody, FITC swine anti-rabbit immunoglobulins (1:200; Dako Cytomation, Glostrup, Danmark). As a positive control, rat brains were used for each antibody.

For each antibody a standard staining of normal tissue was extensively studied before. A trained blinded observer assessed the fluorescence samples twice by using a fluorescence microscope (Olympus, Mofell CX31, Tokyo, Japan). The comparison of TRP immunostaining was performed using the Wilcoxon test.

Quantitative real time PCR: For evaluation of gene expression by means of quantitative real time PCR total RNA was isolated using the mirVana miRNA Isolation Kit (Ambion, ThermoFisher Scientific) following the manufacturers' instructions. Tissue homogenization was performed using Precellys 24 homogenizer together with the ceramic kit 1.4/2.8 mm (Bertin Corp., Rockville, US). Turbo DNAse treatment (Turbo DNA-free Kit, Ambion, ThermoFisher Scientific) was conducted for removal of contaminating DNA. Following, cDNA synthesis was performed using the iScript cDNA Synthesis Kit (BioRad, Germany). For SYBR green based qPCR following primers were used: TRPV1for: gaatgacgccgctggct, TRPV1rev cagcggctccaccaagag; TRPV3for: gcctgtaagacgaacagcaga, TRPV3rev: ctgggttccgcttctacacc; TRPM-8for: atgtgatcgctagcctggtg, TRPM8rev: cactggtgctgaaggctttg; beta actin_for: aaggagaagctgtgctacgtc, beta actin rev: aaccgctcattgccaatggtg. Relative gene expression to beta actin was calculated using the delta delta Ct approach.

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