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Peripheral sensitisation and loss of descending inhibition is a hallmark of chronic pruritus

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List of Abbreviations

AD: atopic dermatitis; ANOVA: analyses of variance; AUC: area under the curve; BRP: brachioradial pruritus; CDT: cold detection threshold; CMH: mechano- and heat-sensitive C-fibers; C_{MIA} fibers: mechano-insensitive C-fibers; CP: chronic pruritus; CPM: conditioned pain modulation; CPT: cold pain threshold; CS: conditioning stimulus; DMA: dynamic mechanical allodynia; HC: matched healthy controls; HPT: heat pain threshold; IENFD: intraepidermal nerve fiber density; IQR: interquartile range; MDT: mechanical detection threshold; MPS: mechanical pain sensitivity; MPT: mechanical pain threshold; NGF: nerve growth factor; NRS: numerical rating scale; PGP: protein gene product; PHS: paradoxical heat sensation; PN: chronic prurigo of nodular type; PPT: pressure pain threshold; QST: quantitative sensory testing; SD: standard deviation; TS: test stimulus; TSL: thermal sensory limen; VAS: visual analogue scale; VDT: vibration detection threshold; WDT: warmth detection threshold; WUR: wind-up ratio

ABSTRACT

Neurophysiological mechanisms leading to chronicity of pruritus are not yet fully understood and it is not known whether these mechanisms diverge between different underlying diseases of chronic pruritus. This study aimed to detect such mechanisms in chronic pruritus of various origins. One-hundred and twenty patients with chronic pruritus of inflammatory origin (atopic dermatitis), neuropathic origin (brachioradial pruritus) and chronic prurigo of nodular type, the latter as a model for chronic scratching, as well as 40 matched healthy controls participated in this study. Stimulation with cowhage induced a more intensive itch sensation compared to stimulation with other substances in all patient groups but not in healthy controls, arguing for sensitisation of cutaneous mechano- and heat-sensitive C-fibers in chronic pruritus. All patient groups showed a decreased intraepidermal nerve fibre density compared to controls. A decreased condition pain modulation effect was observed in all patient groups compared to controls, suggesting a reduced descending inhibitory system in chronic pruritus. In sum, chronic pruritus of different etiology showed a mixed peripheral and central pattern of neuronal alterations, which might contribute to the chronicity of pruritus with no differences between pruritus entities. Our findings may contribute to the development of future treatment strategies targeting these pathomechanisms.

Key words: Itch, quantitative sensory testing, conditioned pain modulation, intraepidermal nerve fibre density, pain

INTRODUCTION

Chronic pruritus (CP) is a symptom of many different etiologies with high impact on patients' quality of life (Hay et al., 2014). One reason for the current suboptimal management of CP patients (Pereira and Stander, 2017) is the ambiguity about the neurophysiological mechanisms underlying CP. There are some interesting hints about the mechanisms underlying physiologic pruritus transmission from current animal studies (Pandey et al., 2017) but studies involving CP patients investigating the mechanisms leading to chronicity in humans are still scarce. Recent studies suggested abundant interactions between the peripheral and central nervous system and immune system (Oetjen et al., 2017), but the exact structural and functional alterations are unclear. Cutaneous nerve fibres transmitting pruritus undergo peripheral sensitisation, similarly to pain (Rukwied R. et al., 2013). In the skin, several classes of C and A δ fibres are involved in pruritus transmission. Cutaneous histamine-sensitive nerve fibres are mechano-insensitive C pruriceptors (C_{MIA} fibres) (Binder et al., 2008). As these comprise only 5% of all epidermal C-fibers and histamine is not the major pruritogen in CP, sensitisation of C_{MIA} fibres alone cannot explain the development of CP. Another group of peripheral nerve fibres involved in pruritus transmission are the mechano- and heat-sensitive, histamine-insensitive C-fibers (CMH fibres); these fibres belong to the polymodal C-fiber nociceptors known to be involved in the transmission of pain (Dhand and Aminoff, 2014). CMH fibres can be activated by mucunain, a proteinase of cowhage (*Mucuna pruriens*) resulting in pruritus induction in human volunteers (Johanek et al., 2007). These two peripheral C-fiber groups transmit their activity to separate spinothalamic neuron populations indicating distinct pruritus pathways, in the periphery and spinal cord (Davidson et al., 2014). However, to date, it is unclear whether peripheral sensitisation of CMH

fibres is involved in CP patients and if this is crucial for clinically relevant pruritic diseases. Furthermore, the relative contribution of the two pruritus pathways for the continuation of pruritus is unknown. Finally, a role of A δ fibres in itch sensation has been suggested (Ringkamp et al., 2011) but their role is not defined yet for CP patients.

Pruritus also involves the brain and mapping of a pruritus matrix in healthy volunteers, and CP patients indicated similar but not identical brain networks of pruritus and pain (Carstens and Akiyama, 2016). Interestingly, simultaneous pruritic and painful stimulation activated the periaqueductal grey, which is known for its role in endogenous pain inhibition (Carstens and Akiyama, 2016). Endogenous descending inhibition, known to be involved in chronicity of pain (Lewis et al., 2012, Yarnitsky, 2015), might, therefore, play a role for inhibiting pruritus at the spinal level and could possibly be involved in CP.

In this study we used a comprehensive set of neurophysiological and morphological investigations, both in CP patients and healthy matched controls, to determine the impact of different peripheral pruritus pathways as well as the involvement of endogenous inhibition by condition pain modulation (CPM) for various entities of CP. We selected CP of inflammatory etiology (atopic dermatitis, AD), neuropathic origin (brachioradial pruritus, BRP), and chronic prurigo of nodular type (PN), the latter as a model for chronic scratching. Aim was to detect pathophysiological mechanisms of peripheral sensitisation and central inhibition involved in the development and maintenance of CP and whether these mechanisms diverge between CP of different origins.

RESULTS

Demographics

120 patients (AD: n=40, BRP: n=40, PN: n=40) and 40 healthy controls (HC) were included in the study. Demographic data and pruritus characteristics are presented in Table 1.

Experimental Pruritus Induction

All active substances induced a robust pruritus as measured by the area under the curve (AUC; median [interquartile range; IQR]: cowhage: 7.8 [2.7;18.9], histamine: 2.4 [0.4;12.1], capsaicin: 3.0 [0.9;9.1]), while the negative control (NaCl: 0.3 [0.1;1-0]) evoked minimal itch sensation (Table S1). Within each patient group and their matched HC the AUC of pruritus intensity induced by the active substances was significantly higher than the pruritus evoked by the negative control (Fig. 1a-c; $p<0.01$). Cowhage induced a higher AUC pruritus intensity compared to histamine (AD: $p=0.02$; BRP: $p=0.003$; PN: $p=0.002$) and capsaicin (AD: $p=0.002$; BRP: $p<0.001$; PN: $p=0.005$) in all patient groups, but not in controls (cowhage vs. histamine: $p>0.1$; cowhage vs. capsaicin: $p>0.05$). Within patient groups and their HCs, the AUC pruritus intensities induced by histamine and capsaicin did not differ ($p>0.1$).

In PN patients, stimulation with cowhage led to a significantly higher pruritus intensity measured by the AUC compared to matched controls ($p=0.02$), while no differences were found between the remaining patient groups and controls ($p>0.1$) or across patient groups ($p>0.1$). Regarding the other substances (histamine/capsaicin/NaCl), no differences were recorded between groups ($p>0.1$). Considering the maximum itch intensity, only PN ($p=0.009$) but not AD ($p=0.09$) or BRP ($p=0.23$) patients showed significant higher scores after stimulation with

cowhage compared to controls (Fig. 1d). No difference between patients and controls or between patient groups was observed for the other substances ($p>0.05$).

Quantitative Sensory Testing (QST)

QST data are summarised in Fig. 2 a-b (z-scores relative to their HC group) and Table 2 (raw data, mean±standard deviation (SD)). AD Patients showed a reduced mean vibration detection threshold (VDT; AD: 6.67/8±0.83, HC 7.11/8±0.59; $p=0.026$ Aß-fiber "loss of function") compared to HC, while no differences in the remaining parameters were detected. BRP patients had an increased mean warmth detection threshold compared to HC (WDT; BRP: 3.04°C±1.55, HC 2.35°C±1.05; $p=0.018$ C-fiber "loss of function"). In PN patients, all mean QST parameters were comparable to matched HC. The percentage of patients showing pathological QST scores ($z>2$ or $z<-2$) is shown in Fig. 2 c-d; there was an increased percentage of patients with pathological reduced thermal thresholds (indicating loss of function) in BRP. In contrast, more PN patients show an increased (gain of function) sensitivity to punctate mechanical stimulation (mechanical pain stimulation (MPS) and wind-up (WUR)) and painful heat.

Conditioned Pain Modulation (CPM)

Sixty-five subjects (13 AD, 16 BRP, 14 PN, 22 HC) completed the CPM assessment. Individual heat intensities able to produce a rating of around 60/100 were comparable between patient groups and controls ($p>0.05$) and pain ratings to several heat test stimuli in patients were similar to their matched HC (Table S2).

In the CPM test-line, all patient groups had similar pain intensity ratings assessed on a NRS (0-100) to the first test-stimulus $(TS)_{before}$, which were comparable to HC (Fig.

3a). In HC, pain intensity ratings decreased from 51.76±12.77 to 29.24±11.78 (Mean±SD, TS_{during}, $p \leq 0.001$, Table S3/Fig. 3a), indicating a robust endogenous inhibition (Fig. 3b). Furthermore, the mean intensity of the TS applied 5 minutes after the conditioning stimulus (CS), (TS_{after}) was still significantly reduced when compared to the TS prior to the CS ($p<0.05$, Table S3/Fig. 3a). In contrast, in each CP patient group, pain ratings to the TS applied simultaneously with the CS (TS_{during}) did not change significantly compared to first TS (TS_{before}) ($p>0.05$, Fig. 3b) indicating lack of endogenous inhibition (Fig. 3b). In accordance, there was no reduction in pain ratings to the third TS (TS_{after}) in any patient group ($p>0.05$, Fig. 3a). The immediate CPM-effect (in percentage) was significantly different between the HC (-37.68±35.02) and all patient groups (AD: -10.47±31.06, BRP: -11.68±22.75, PN -14.88±42.17; AD and BRPvsHC: $p<0.01$; PNvsHC: $p<0.05$, Fig. 3b).

Intraepidermal nerve fibre density (IENFD)

A skin biopsy to determine the IENFD was obtained from 81 patients (AD: n=27, BRP: n=33, PN: n=21) and 39 HC. Patients showed a significantly reduced IENFD compared to HC (AD: p=0.001; BRP: p=0.02; PN: p=0.003; Fig. 4). A higher IENFD in BRP compared to PN patients (p=0.03) was found, but no differences were observed between other patient groups ($p>0.1$). We observed a negative correlation in CP patients between the IENFD and WDT (r=-0.28, p=0.004), but not in HC (r=-0.11, $p>0.05$).

DISCUSSION

We performed a comprehensive phenotyping of the structural and functional alteration of the peripheral nervous system in a large cohort of CP patients with different pruritus entities. In CP patients, we observed a disturbance of the peripheral nerve fibre density, an increased sensitivity to pruritic stimuli by cowhage, suggesting a peripheral sensitisation to such stimuli. QST parameter were only slightly altered but with a similar trend as seen in patients with a painful (small-fibre) neuropathy. However, the pattern of functional and structural changes in CP of inflammatory origin, neuropathic origin and in PN was quite similar. In addition the efficacy of the descending inhibitory system was fundamentally impaired in patient groups compared to healthy controls.

In contrast to histamine-induced pruritus, pruritus induction with cowhage in pruritic skin of patients with different types of CP resulted in an increased sensory perception compared to the other active substances including histamine. This suggests a significant role of cowhage-activated epidermal CMH fibres in CP. The increased pruritus intensity following cowhage in CP patients might point to a sensitization of CMH fibres in CP. Our data are in agreement with recent observations suggesting dominance of non-histaminergic pathways in itch arising from AD (Andersen et al., 2017a) and further show the importance of these pathways in non-atopic forms of CP. Rukwied et al. described that nerve growth factor (NGF) sensitises CMH fibres for induction of pruritus by cowhage (Rukwied R. R. et al., 2013). NGF is a relevant factor in cutaneous inflammatory diseases (Mollanazar et al., 2016) and might contribute to sensitisation and also to a spontaneous activity of nerves and to the generation of pruritus (Rukwied R. et al., 2013). Spontaneous firing of cutaneous nerves was previously described in PN (Schmelz et al., 2003). It is most likely that

neuro-immune mechanisms might explain sensitisation of CMH fibres in CP, while the role of scratching indicated previously as one important etiological factor (Pereira et al., 2017) needs to be explored. Our data suggest sensitisation of cowhage-sensitive fibres to a significant level in PN when compared to age and sex-matched healthy volunteers. As for AD and BRP, in which significant higher responses to cowhage compared to other pruritogens but not between patients and controls was found, other mechanisms than in PN may contribute to the development and perpetuation of itch.

The neuronal sensitisation shown in this study was not related to a hyperinnervation as previously advocated (Kamo et al., 2011). Accordingly NGF induces sensitisation in porcine C nociceptors, which is not accompanied by increased IENFD (Hirth et al., 2013). Determination of the IENFD in all three CP entities showed a decrease of the number of cutaneous nerves crossing the basement membrane. In this study, we did not compare intraindividually the lesional IENFD to non-pruritic, normal skin of the patients. However, we performed this in several previous studies as for example in BRP (Pereira et al., 2018) and found normal IENFD values in the non-affected skin. The method used in our study was validated for detecting nerve fibre alterations such as neuropathies in the non-lesional skin (Lauria et al., 2010). In skin diseases such as AD or PN, the epidermis shows acanthosis, which impacts the quantification of the nerves. However, we extensively investigated nerve fibre anatomy in PN, and previously described a rarefication of IENFD dependent on disease duration (Schuhknecht et al., 2011) with reconstitution after healing of PN (Bobko et al., 2016). Although the nerves crossing the basement membrane were reduced, an increased intraepidermal sprouting of nerves is possible, which could explain the contradictory reports by various groups using different methods to analyse the epidermal nerve fibre structure in CP entities either describing hypoinnervation

(Martinelli-Boneschi et al., 2017, Milian-Ciesielska et al., 2017) or hyperinnervation (Kim et al., 2014, Tominaga and Takamori, 2014). Recently, using a modern three-dimensional technique, a downregulation of epidermal nerve fibers was recorded in pruritic atopic dermatitis skin confirming our results (Tan et al., 2018). The pathophysiological role of the alteration of the intraepidermal neuroanatomy is unclear; in previous studies, we speculated that the origin of the changes is due to scratching (Kim et al., 2014, Tominaga and Takamori, 2014). However, in BRP, no differences in IENFD were recorded between patients with and without scratch lesions (Pereira et al., 2018). Thus we hypothesize that different cellular and molecular mechanisms contribute to the reduced IENFD observed in CP.

QST did not detect significant functional abnormalities in any parameter assessed here in CP patients except an increase in WDT, which was only significant in BRP patients. However, the frequency of abnormal WDT is approximately 10-20% across patient groups, and all of these patients show a loss of function. Such a pattern is well related to a rarefication of IENFD, for example in patients with diabetic neuropathies and small-fibre neuropathy (Raputova et al., 2017). In addition, individual WDT assessed in patients with CP correlated well to the IENFD, pointing towards a C-fiber "loss of function" as found in small-fibre neuropathies (Scherens et al., 2009). This is in line with recently reported changes in a smaller cohort of BRP patients (Misery et al., 2014). However, a functional role of a small-fibre neuropathy for patients with CP of inflammatory origin (and PN) has not been shown before. There is no "thermal" (or "mechanical") hyperalgesia as shown in distinct subgroup of patients with painful neuropathies (Baron et al., 2017), similar to the previously termed "irritable nociceptor" profile (Demant et al., 2015, Demant et al., 2014). However, together with our stimulation experiments, it might be suggested that sensitisation of

rarefied CMH-fibers transmitting itch might play a significant role in CP and occurs regardless of the pruritus etiology.

One interesting finding is that there seem to be subgroups of patients with more pathological QST parameters than others; for example, increased responsiveness to pinprick stimuli (MPS and wind-up) in some patients with PN indicate a gain of function in A δ -fibers; this might indicate (Andersen et al., 2017a) sensitisation of mechano-sensitive A δ -fibers, presumably in some patients more than in others. Such heterogeneity of sensory signs in patients with CP is very similar to findings in patients with painful peripheral neuropathies indicating some differences in the pathophysiological mechanisms and presumably in treatment response between patients with CP (Baron et al., 2017, Maier et al., 2010).

A role of impaired central inhibition for the maintenance of chronic pain states is well documented (Martel et al., 2013, Normand et al., 2011, Wilder-Smith et al., 2010). A few studies in the last years have made an effort to identify an analogous endogenous inhibitory mechanism for CP (van Laarhoven et al., 2010). In our study healthy volunteers showed a robust inhibition of the test stimulus by using a painful conditioning stimulus indicating high endogenous inhibitory control; however, such a CPM effect was absent in all CP groups demonstrating in impaired endogenous inhibitory system in patients with CP and, of particular interest, regardless of the etiology. Although the test stimulus used here was a painful stimulus, recent data (van Laarhoven et al., 2010) suggest that endogenous inhibition on pain and itch involves a similar pathway. In healthy volunteers, experimental itch and pain evoked by electrical stimulation were inhibited by both ipsilateral and contralateral pain stimulation with the cold pressor test (Andersen et al., 2017b) suggesting that descending inhibition evoked by a painful conditioning stimulus inhibits itch and pain

in the healthy. Whether an *itch reducing itch* experimental design similar to a *pain inhibiting pain* is possible, is controversial (Andersen et al., 2017b, van Laarhoven et al., 2010). Collectively, our data indicate that an impaired central inhibitory system is possibly contributing to the maintenance of pruritus in CP states and thus to the chronicity of the condition. However, it remains unclear whether the impairment of central pain inhibition is a cause for CP or rather a consequence thereof. Possibly healthy individuals with impaired endogenous inhibition may be at higher risk to develop CP after onset of acute pruritus; alternatively, a decreased inhibitory system may develop during the course of pruritus. Analogous studies uncovering these questions have been performed in chronic pain resulting in conflicting data with some studies identifying a less effective CPM effect as a risk factor for the development of chronic pain after surgery (Ruscheweyh et al., 2017, Yarnitsky et al., 2008) and other observations failing to detect a predictive value of CPM (Grosen et al., 2013).

In conclusion, CP of different origins show a similar pattern of peripheral sensitisation of CMH fibres, rarefication of intraepidermal nerve fibres, and impaired endogenous pain inhibition. Neuropathic CP forms may additionally present with C-fiber loss of function in a subgroup of patients as well as gain of function in Ad-fibres. These peripheral and central neuronal mechanisms may contribute to the perpetuation of CP and should be considered when developing novel therapeutic strategies in these patients.

MATERIAL & METHODS

Subjects

Sample size estimates were drawn from previous studies on pruritus (Schneider et al., 2015), pain QST (Phillips et al., 2014) and CPM studies (Albu et al., 2015, Gehling et

al., 2016) and adapted for multiple testing for the three patient group design. Adult patients (aged ≥18 years) with CP conditions (AD, BRP, PN, n=120) as well as 40 sex and age-matched HC without atopic disposition were included. No disease severity assessments were performed for the included patients. Subsamples of HCs were defined before data analysis to optimise age and gender matching for each CP condition. Inclusion and exclusion criteria are presented in Table S4. We registered the study at the German registry of clinical trials (DRKS00005226, register date: 14.08.2013). Declaration of Helsinki protocols were followed. Study participants gave written and oral informed consent. The study was approved by the local ethics committee (Medical Faculty of the University of Münster, nr.: 2011-114-f-S).

Study Design

After a dermatological examination and completing routine pruritus questionnaires, all subjects underwent the same set of examinations consisting of a comprehensive clinical and physiological investigation. First, experimental pruritus was induced by local stimulation with cowhage, histamine, and capsaicin and a negative control (NaCl) at the volar forearm and assessed for 30 minutes. After a minimum waiting period of at least 2h following the last application of a pruritic stimulus, QST was performed at the volar forearms. Afterwards, following randomisation, a subset of the participants underwent the assessment of endogenous pain inhibition by CPM. Finally, a skin biopsy was obtained in order to determine the IENFD.

Experimental procedures

Experimental Pruritus Induction

Study participants were stimulated with pruritic substances (cowhage, histamine, and capsaicin) and negative control (NaCl) in a double-blind, randomised order at four pre-defined areas of the volar forearms into the pruritic non-lesional (BRP) and perilesional (AD, PN) skin. Following stimulation, pruritus intensity was assessed on a visual analogue scale (VAS; range 0-10) as long as a pruritus sensation persisted or for a maximum of 30 minutes.

Quantitative Sensory Testing (QST)

QST was performed at the volar aspect of both forearms (randomised order) near to those areas used for pruritus induction (and later skin biopsy) to enable comparison of results but with a safe time window for recovery (see above). Testing was done according to the protocol by the German Research Network for Neuropathic Pain ((Rolke et al., 2006), Supplementary Methods Section)

Conditioned Pain Modulation (CPM)

The efficiency of endogenous pain inhibition was assessed using the CPM paradigm as previously described ((Pud et al., 2009) Supplementary Methods Section). The CPM effect was defined as the percentage of the endogenous inhibitory effect and is denoted by a negative percentage value for the reduction of pain ratings of the test stimulus (TS) during conditioning stimulation (CS). In contrast, pain facilitation is expressed by a positive percentage value (Yarnitsky et al., 2015).

Determination of the intraepidermal nerve fibre density (IENFD)

After the experimental procedures, a skin biopsy was taken from the pruritic skin in patients and non-pruritic skin in HC at the volar forearm in order to determine the

IENFD, as previously described ((Schuhknecht et al., 2011) Supplementary Methods Section). The IENFD was obtained by dividing the mean number of intraepidermal nerve fibres penetrating the basal membrane by the epidermal length in mm.

Statistical analysis

Statistics were performed using SPSS software 24.0 (IBM, Armonk, NY, USA). Data was tested for normality with the Kolmogorov-Smirnov test and Q-Q residual plots. Comparisons between groups (comparisons between patient groups and controls) were performed using one-way analyses of variance (ANOVA) or unpaired t-tests as appropriate, or for non-parametric data, with the analogues Kruskal-Wallis test or the Mann-Whitney U test. For comparisons within groups repeated measures ANOVA or paired t-tests were chosen as appropriate, or for non-parametric data, the analogues Friedman test and Wilcoxon test. Correlations between variables were performed using Pearson's r for normally distributed data and Spearman's rho for non-normally distributed data. To compare QST-parameters independent of their physical dimensions, a z-transformation was performed by using the QST data and compared to the data from the age- and gender-matched controls by treating the control data mean (z=0) as the known population mean value (Rolke et al., 2006). The effect of CPM and the IENFD was calculated as mentioned above. We chose 2-tailed tests and a significance level of 0.05 for statistical comparisons. Parametric and non-parametric data are shown as mean±SD and median [IQR], respectively.

DATA AVAILABILITY STATEMENT

Data on cowhage stimulation, intraepidermal nerve fiber density and quantitative sensory testing for the corresponding diagnoses and control patients would be

necessary to interpret, and replicate the key findings of the current submission. These primary research data can be obtained by the authors upon request (pogatzki@anit.uni-muenster.de; Sonja.staender@uni-muenster.de).

CONFLICT OF INTEREST

EPZ: financial support from Mundipharma GmbH and Grunenthal for research activities, advisory and lecture fees from Grünenthal, MSD Sharp & DOHME GmbH, Mundipharma GmbH; Mundipharma International and advisory fees from Janssen-Cilag GmbH; Fresenius Kabi and AcelRx. All of these aspects are not related to the present work.

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The other authors state no conflict of interest.

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Data curation: EPZ, SST

Formal analysis: EPZ, MPP, AC, CZ, TD, DS, MR, KA, SST

Funding acquisition: EPZ, SST

Investigation: MPP, AC, CZ, TD, CR, TL, DS, KA

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Project administration: EPZ, CW, SST

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TABLES

Table 1. Demographic data of subjects.

	AD	AD matched controls	BRP	BRP matched controls	PN	PN matched controls
n	40	30	40	31	40	27
Age (years)	45 [21;74]	51 [32;62]	57 [36;82]	55 [49;65]	59 [27;79]	56 [48;65]
Sex (M:F)	21:19	16:14	18:22	15:16	19:21	16:11
Pruritus duration (months)	73 [26;258)	n.a.	57 [11;105]	n.a.	102 [38;209]	n.a.
Pruritus intensity (VAS; last 4 weeks)	7.0 [4.0;8.3]	n.a.	7.0 [3.8;8.0]	n.a.	6.0 [4.4;7.3]	n.a.

[1]Demographic data (age and sex), pruritus duration (in months) and the worst pruritus intensity of the past 4 weeks assessed on a VAS scale are shown as median [interquartile range] for patients and matched controls. There were no differences in sex between patient groups ($p>0.5$). Regarding age, AD patients were significantly younger than BRP ($p=0.001$) and PN ($p<0.001$) patients

[2]AD: atopic dermatitis; BRP: brachioradial pruritus; HC: healthy controls; n.a.: not applicable; PN: chronic prurigo of nodular type; VAS: visual analogue scale.

Table 2: QST Values of each patient group compared to matched control healthy volunteers.

	AD	AD matched controls	BRP	BRP matched controls	PN	PN matched controls
CDT [°C]	-2.66 ± 1.69	-2.72 ± 1.51	-3.84 ± 2.16	-3.04 ± 2.07	-3.12 ± 2.36	-2.94 ± 1.54
WDT [°C]	2.73 ± 1.38	2.33 ± 1.04	**3.04 ± 1.55 ***	2.35 ± 1.05	2.84 ± 1.43	2.39 ± 1.08
TSL [°C]	4.96 ± 2.41	4.70 ± 2.11	6.35 ± 4.10	4.84 ± 2.52	5.78 ± 3.00	4.78 ± 2.10
PHS [absolute]	0 [0;0]	0 [0;0]	0 [0;0]	0 [0;0]	0 [0;0]	0 [0;0]
CPT [°C]	11.79 ± 9.65	9.69 ± 8.82	6.57 ± 8.74	9.89 ± 9.28	9.92 ± 8.50	10.93 ± 9.07
HPT [°C]	45.17 ± 3.47	45.45 ± 3.90	46.53 ± 3.66	45.0 ± 4.29	44.38 ± 5.23	44.94 ± 4.20
MDT [mN]	2.79 ± 4.40	2.99 ± 3.67	3.39 ± 5.07	3.33 ± 4.14	3.39 ± 3.42	3.16 ± 3.84
MPT [mN]	96.36 ± 116.45	79.39 ± 101.84	89.89 ± 129.73	80.66 ± 105.50	78.48 ± 119.67	74.32 ± 101.98
MPS [NRS]	2.16 ± 3.86	3.24 ± 5.56	1.63 ± 2.76	3.17 ± 5.50	5.69 ± 10.14	3.61 ± 5.75
DMA [NRS]	0.23 ± 0.75	0.10 ± 0.41	0.63 ± 2.76	0.15 ± 0.49	0.31 ± 1.85	0.11 ± 0.43
WUR	2.61 ± 1.27	3.33 ± 1.93	2.96 ± 1.98	3.06 ± 2.38	2.96 ± 1.52	2.74 ± 1.59
VDT [/8]	**6.67 ± 0.83 ***	7.11 ± 0.59	6.63 ± 0.78	6.94 ± 0.71	6.81 ± 0.78	7.00 ± 0.63
PPT [kPa]	617.22 ± 208.00	664.83 ± 231.53	657.38 ± 250.10	728.08 ± 280.65	634.99 ± 311.27	710.46 ± 287.97

[1]Mean ± SD, * $p<0.05$ versus matched control.

[2]AD: Atopic dermatitis; BRP: brachioradial pruritus; CDT: cold detection threshold; CPT: cold pain threshold; DMA: dynamic mechanical allodynia; HPT: heat pain threshold; MDT: mechanical detection threshold; MPS: mechanical pain sensitivity; MPT: mechanical pain threshold; PN: chronic prurigo of nodular type; PHS: paradoxical heat sensations; PPT: pressure pain threshold; TSL: thermal sensory limen; VDT: vibration detection threshold; WDT: warmth detection threshold; WUR: wind-up ratio

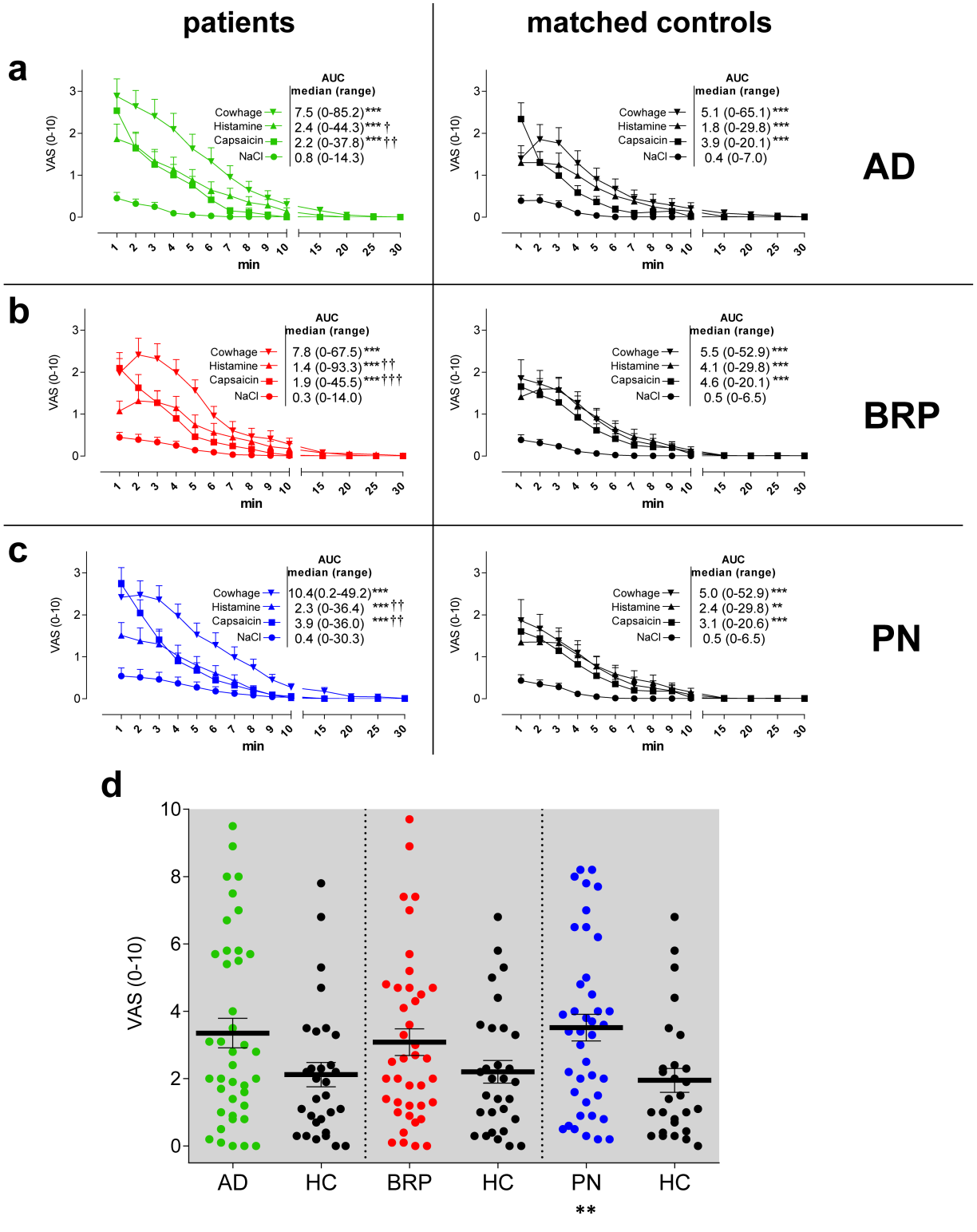
FIGURE LEGENDS

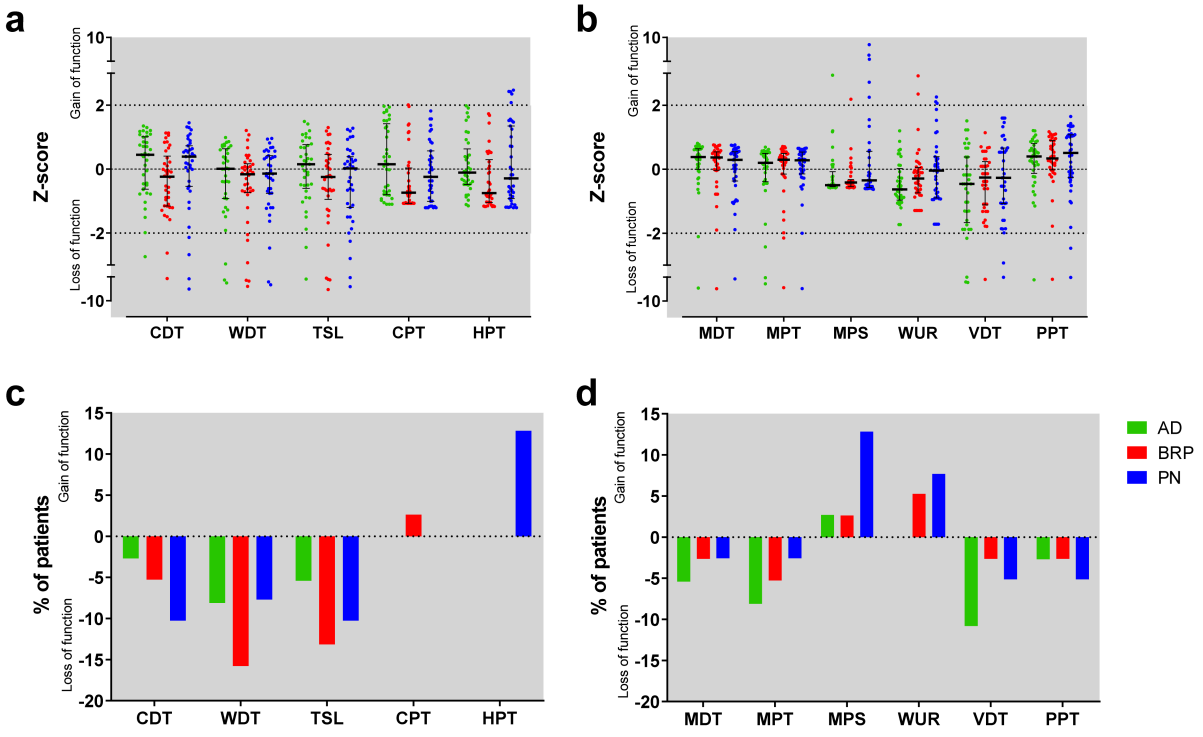
Fig. 1. Experimental pruritus induction. Cowhage, histamine or capsaicin [a-c] provoked significant higher pruritus intensity ($p \leq 0.001$) than stimulation with NaCl in all chronic pruritus (CP) patient groups (AD: atopic dermatitis; BRP: brachioradial pruritus; PN: chronic prurigo of nodular type). Cowhage induced a significant higher pruritus intensity than the other active stimulations in CP but not in HC. Area under the curve (AUC) was calculated for each participant, mean±SEM are plotted. The highest cowhage-induced itch rating [d] is displayed, mean±SEM are plotted. Only cowhage induced significant higher pruritus intensities in CP patients compared to HC (PN/HC: $p=0.009/n=40/27$). Wilcoxon Signed Rank Sum Test for related samples [a-c] (*$p<0.05$/**$p<0.01$/***$p \leq 0.001$ vs. NaCl; $^{\dagger}p<0.05$/$^{\dagger\dagger}p<0.01$/$^{\dagger\dagger\dagger}p \leq 0.001$ vs. cowhage), independent Samples Mann-Whitney U Test [d] (**$p<0.01$ vs. HC). Adjustment for multiple testing was not calculated.

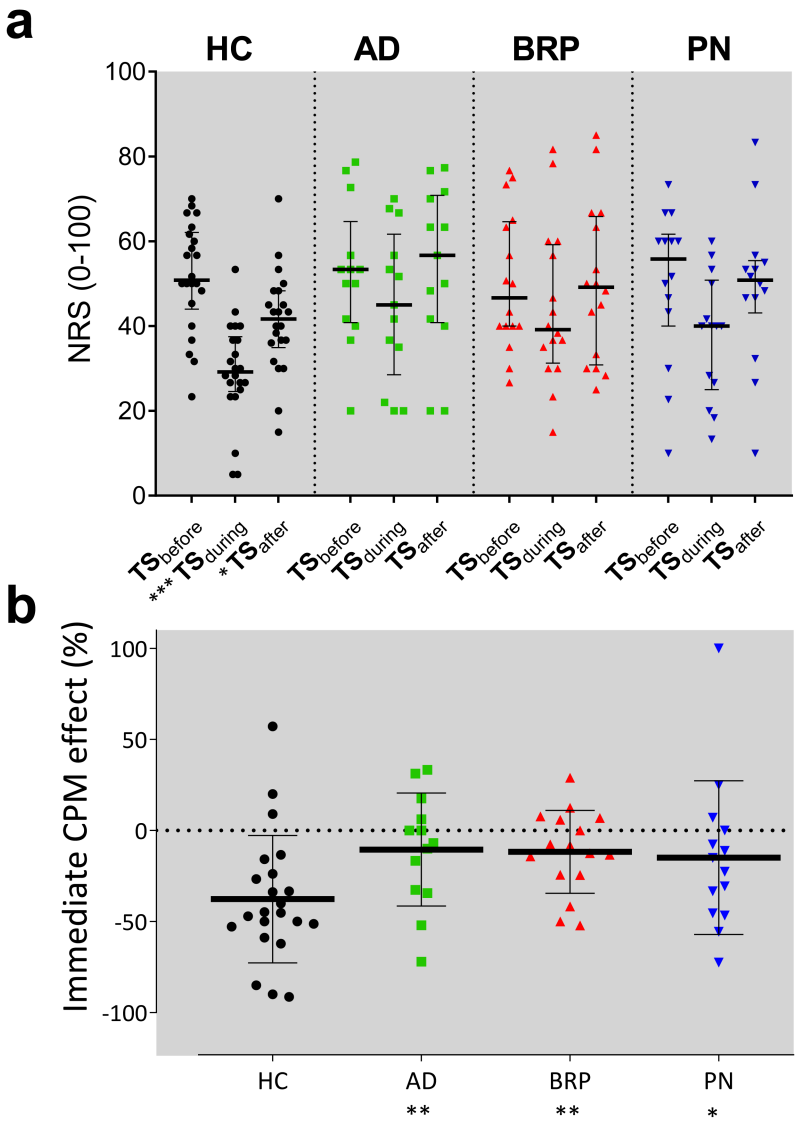
Fig. 2. Quantitative sensory testing. Thermal [a] and mechanical [b] QST parameters for each patient group (green: atopic dermatitis (AD), red: brachioradial pruritus (BRP), blue: chronic prurigo of nodular type (PN)) are shown as z-scores calculated from data of the age- and sex-matched controls enrolled in this study, single values and median (line)±interquartile range (wiskers) are plotted. Scores>0 show gain of function, scores<0 indicate loss of function. The percentage of patients with pathological QST scores ($z>2$ or $z<2$) is shown in [c] (thermal parameters) and [d] (mechanical parameters), bars represent the percentage of pathological test results (outside the 95% confidence interval) for thermal and mechanical test stimuli with gain or loss of sensory function in different patient groups.

Fig. 3. Conditioned pain modulation (CPM). Numerical rating scale (NRS)-scores [a] are shown for the test-stimulus (TS) assessed prior (TS_{before}), during (TS_{during}), and 5 minutes after (TS_{after}) the conditioning stimulus (CS) for each patient group and healthy controls; single values and median±interquartile range are plotted. There was no difference in the pain intensity of the TS_{before} and CS between groups. The immediate CPM effect is shown in [b]. Data are shown as single values and mean±standard deviation. HC, but not any patient group, showed a statistical significant CPM effect ($p \leq 0.001$). In [a] with ANOVA and Dunnett´s multiple comparison Post Hoc test (*$p<0.05$/**$p<0.01$/***$p \leq 0.001$ vs. TS_{before}), in [b] with Kruskal-Wallis test and Dunn´s multiple comparisons Post Hoc test (*$p<0.05$/**$p<0.01$ versus matched control).

Fig. 4. Intraepidermal nerve fibre density (IENFD) in chronic pruritus patients and healthy controls. Nerve fibres were stained using a PGP9.5 specific antibody, and density was calculated per mm epidermis. All chronic pruritus patients showed reduced IENFD compared to sex and age-matched healthy controls (AD/HC, n=27/30, $p<0.001$; BRP/HC, n=33/30, $p<0.05$; PN/HC, n=21/27, $p<0.01$). Independent Samples Mann-Whitney U Test. AD: atopic dermatitis; BRP: brachioradial pruritus; HC: healthy controls; IENFD: intraepidermal nerve fibre density (fibres/mm); PN: chronic prurigo of nodular type. *$p<0.05$/**$p<0.01$ vs. matched control.






a
HC
AD
BRP
PN
NRS (0-100)
100
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b
Immediate CPM effect (%)
100
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HC
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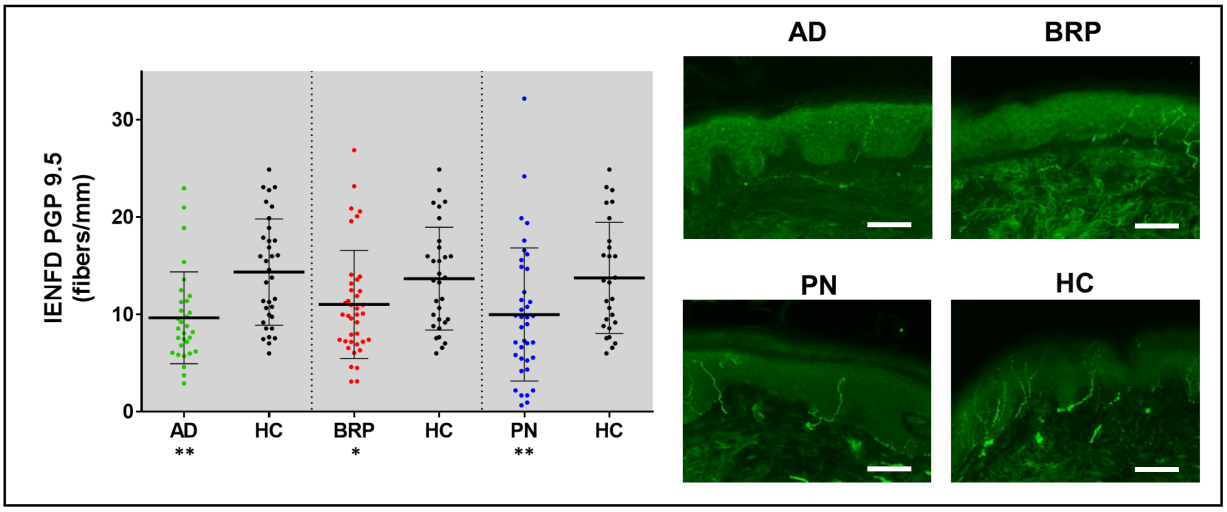

SUPPLEMENTARY METHODS SECTION

Experimental Pruritus Induction

Study participants were stimulated with pruritic substances (cowhage, histamine, and capsaicin) and negative control (NaCl) in a double-blind, randomised order at four pre-defined areas of the volar forearms into the pruritic non-lesional (BRP) and perilesional (AD, PN) skin with a 5-6 cm distance between sites. For the stimulation with cowhage, 10 to 20 active cowhage spicules were attached to a cotton bud (Johanek et al., 2007), while to perform the stimulation with histamine and capsaicin, spicules previously inactivated by autoclaving were loaded with histamine (aqueous solution:10 mg/ml) or capsaicin (aqueous buffered solution from ethanol stock solution: 200 mg/ml), respectively, and mounted on a cotton bud. Autoclaved spicules loaded with NaCl were used as negative controls. All loaded spicules were allowed to air-dry before use. Spicules were pressed into the epidermis, and pruritus intensity was assessed on a visual analogue scale (VAS; range 0-10) every minute for ten minutes and afterwards every five minutes as long as a pruritus sensation persisted or for a maximum of 30 minutes. The maximal pruritus intensity during the stimulation was calculated using the VAS assessments of the patients. There was a 10-minute interval between stimulation with the various substances.

Quantitative Sensory Testing (QST)

QST was performed at the volar aspect of both forearms (randomized order) near to those areas used for pruritus induction (and later skin biopsy) to enable comparison of results but with a save time window for recovery (see above). Testing was done according to the protocol by the German Research Network for Neuropathic Pain containing 13 different thermal and mechanical tests to assess the function of small and large nerve fibers (Rolke et al., 2006). Briefly, a 3x3 cm

contact thermode (TSA II NeuroSensory Analyzer, Medoc Ltd., Israel; baseline temperature: 32°C, ramp rate: 1.0°C/s; cut-off: 0ºC and 50ºC) was used to assess thermal thresholds. First, cold and warmth detection thresholds (CDT, WDT) were assessed by instructing participants to push a button as soon as they perceived a cold or warmth sensation to terminate the stimulation. Thereafter, alternating warm and cold stimuli were applied to assess the thermal sensory limen (TSL); patients were instructed to press a button when the thermode temperature changed from neutral to cold or warmth, and the paradoxical heat sensations (PHS) were recorded as the number of hot sensations to cold stimulation. To determine cold and heat pain thresholds (CPT, HPT) participants were asked to press a button as soon as they perceived a painful sensation. All assessments were performed in triplicate, and the mean thereof was calculated to obtain the individual thresholds.

Mechanical detection thresholds (MDT) were assessed using a series of Von Frey filaments (0.25-512mN, 0.5mm diameter; Optihair2-Set, Marstock Nervtest, Germany). Participants were stimulated with the filaments in ascending and descending order ("method of limits") and were asked to report when a stimulus was perceived. Mechanical pinprick pain was determined using a set of pins (8-512mN, 0.2mm diameter; PinPrick, MRC Systems, Heidelberg, Germany). To assess mechanical pain thresholds (MPT), the pins were applied in ascending and descending order and participants were instructed to report when a painful stimulation occurred. Using the same set of pinpricks, mechanical pain sensitivity (MPS) was determined by asking participants to rate the pain intensity evoked by the pins on a numerical rating scale (NRS; 0-100). To assess the dynamic mechanical allodynia (DMA), a brush (200-400mN), a cotton wool tip (100mN) and a cotton wisp (3mN) were applied to the skin five times each, and the resulting pain was recorded on a NRS (0-100). Each pin, as well as the three dynamic mechanical stimuli, were applied five times in a randomized, mixed order. Afterward, the wind-up ratio (WUR) was assessed as the

difference in evoked pain by a single pinprick stimulation (128mN and 256mN) and ten consecutive stimulations at 1 Hz with the same pinprick.

Using a tuning fork (64 Hz, 8/8; AESCULAP, B. Braun Company, Germany) applied to the radial styloid processus, vibration detection threshold (VDT) was determined three times at each forearm. The pressure pain threshold (PPT) was assessed by stimulating three times with a pressure algometer (1cm^2; FDN200, Wagner Instruments, USA).

Conditioned Pain Modulation (CPM)

The efficiency of endogenous pain inhibition was assessed using the CPM paradigm as previously described (Pud et al., 2009). First, thermal stimulation causing a pain intensity rating of approximately 60 (NRS, 0-100) was determined using a 9cm^2 contact thermode (TSA II NeuroSensory Analyzer, Medoc Ltd., Israel). Briefly, two series of three 7s heat stimuli (45ºC, 46ºC, and 47ºC) were applied to both forearms (first set of stimulations on the right forearm in ascending order, second set of stimulations on the left forearm in randomized order) and the temperature inducing an intensity of approximately 60 (NRS, 0-100) was chosen as the test stimulus (TS). If all of these temperatures induced pain > 65 NRS or pain < 55 (NRS 0-100), an additional series with lower (42ºC and 43ºC) or higher (48ºC and 49ºC) temperatures was performed in order to find the appropriate temperature, respectively.

Afterwards, participants received a 30s heat stimulation with the previously determined temperature at the left volar forearm (TS_{before}) and were asked to rate the pain intensity at 10s, 20 and 30s on an NRS (0-100). After a 5-minute interval, participants were instructed to immerse their contralateral hand in a 10ºC cold water-bath for 60s and rate the pain intensity to the water after 30s and 60s (conditioning stimulus, CS) on an NRS (0-100). Participants were trained to spread fingers and not to touch the bottom or the walls of the water bath. Thirty seconds after the

beginning of the CS, the test stimulus was applied again at the left volar forearm next to the previous stimulation; participants were asked to rate the pain intensity induced by the second TS at 10s, 20 and 30s (TS_{during}). Finally, after termination of TS_{during} and CS and a 5-minute break, the TS was repeated without the CS and participants were instructed to report the pain intensity induced by the TS at 10s, 20 and 30s on an NRS (TS_{after}). The immediate (prolonged) CPM effect was assessed by calculating the difference between the mean of the three pain ratings to the TS without concomitant CS minus the mean of the three pain ratings to the TS during (5 minutes after) application of the CS relative to the total pain rating to the TS without concomitant CS (CPM−effect = (Mean of three pain ratings to the TS_{before}− Mean of three pain ratings TS_{during}/TS_{before}). The CPM effect was defined as the percentage of the endogenous inhibitory effect and is denoted by a negative percentage value for the reduction of pain ratings of the TS during CS. In contrast, pain facilitation is expressed by a positive percentage value (Yarnitsky et al., 2015).

Determination of the intraepidermal nerve fibre density (IENFD)

After the experimental procedures, a skin biopsy was taken from the pruritic skin in patients and non-pruritic skin in HC at the volar forearm in order to determine the intraepidermal nerve fiber density. Under local anesthesia (Xylonest 1% ®), a 4 mm punch biopsy was taken. IENFD was assessed as previously described (Schuhknecht et al., 2011). Briefly, cryosections (30µm) obtained from each biopsy were incubated with the primary antibody against the neuron-specific hydrolase protein gene product (PGP) 9.5 (polyclonal rabbit, 1:2000; Chemicon, Temecula, CA, USA). Afterward, the secondary antibody anti-rabbit-fluorescein isothiocyanate (FITC) (1:50; pig antirabbit immunoglobulin FITC; Dako, Glostrup, Denmark) was used to stain the tissue sections. The number of intraepidermal nerve fibers penetrating the basement membrane was counted at

400x magnification in three specimens per biopsy. Using the software Olympus DP soft analySIS Image Processing (Olympus, Tokyo, Japan, v. 3.2), the length of the epidermis was determined. The IENFD was then obtained by dividing the mean number of intraepidermal nerve fibers penetrating the basal membrane by the epidermal length in mm.

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Supplementary table 1. Experimental pruritus induction. Pruritus intensity induced by cowhage, histamine, capsaicin and NaCl (negative control) was assessed for 30 minutes using the visual analogue scale. The area under the curve was calculated (VAS-score/30 min) and is shown as median [interquartile range]. AD: atopic dermatitis; AUC: area under the curve; BRP: brachioradial pruritus; Cap: capsaicin; Cow: cowhage; HC: healthy controls; His: histamine; PN: chronic prurigo of nodular type

	All subjects	**AD**	**AD matched controls**	**BRP**	**BRP matched controls**	**PN**	**PN matched controls**	**All healthy controls**
n	160	40	30	40	31	40	27	40
Cow (AUC)	7.8 [2.7;18.9]	7.5 [2.9;12.2]	5.1 [1.1;10.9]	7.8 [2.4;19.1]	5.5 [1.8;10.9]	10.4 [3.3;23.4]	5.0 [1.1;8.8]	6.2 [2.0;12.4]
His (AUC)	2.4 [0.4;12.1]	2.4 [0.3;13.9]	1.8 [0.5;12.5]	1.4 [0.4;11.2]	4.1 [1.1;15.9]	2.3 [0.5;10.9]	2.4 [0.8;16.2]	3.5. [0.8;15.1]
Cap (AUC)	3.0 [0.9;9.1]	2.2 [0.6;9.1]	3.9 [1.8;5.7]	1.9 [0.5;6.5]	4.6 [1.6;9.1]	3.9 [0.8;13.6]	3.1 [1.6;8.9]	4.6 [1.9;8.8]
NaCl (AUC)	0.3 [0.1;1-0]	0.8 [0.1;0.8]	0.4 [0.0;1.5]	0.3 [0.1;0.9]	0.5 [0.1;1.4]	0.4 [0.1;1.3]	0.5 [0.1;1.6]	0.4 [0.0;1.4]

Supplementary table 2. Temperature determination of the test stimulus (TS) for CPM paradigm. Pain ratings (NRS, 0-100) for determination of the test-stimulus (contact heat) are presented as mean ± standard deviation and the used temperature (°C) for the test stimulus (TS) in CPM paradigm (Median and 95% confidence interval of median).

AD: atopic dermatitis; BRP: brachioradial pruritus; CP: Chronic pruritus; CPM: condition pain modulation; CS: conditioning stimulus; HC: healthy controls; NRS: numerical rating scale; PN: chronic prurigo of nodular type; TS: test-stimulus

TS (°C)	HC	AD	BRP	PN	CP
45	46,69±27,60 16	27,00±23,94 10	32,14±24,47 14	35,00±19,47 13	31,76±22,27 37
46	43,93±20,77 14	23,85±11,87 7	26,82±21,33 11	36,33±18,86 12	29,93±18,72 30
47	53,77±24,32 13	35,00±14,36 9	26,40±15,35 10	46,00±23,10 12	36,483±19,80 31
48	35,83±12,01 6	39,67±17,99 9	39,70±16,22 10	33,60±15,65 5	38,42±16,26 24
TS (°C)	**47** (45 to 48)	**48** (43 to 49)	**48** (45 to 49)	**47** (45 to 48)	**48** (47 to 48)
N	**22**	**13**	**16**	**14**	**43**

Supplementary table 3. Condition Pain Modulation. Pain ratings (NRS, 0-100) for the test-stimulus (contact heat) before, during and after conditioning stimulation (immersing the contralateral hand in a 10ºC water bath) as well as for the conditioning stimulus are presented as mean ± standard deviation and mean difference, 95% confidence interval of difference. AD: atopic dermatitis; BRP: brachioradial pruritus; CPM: condition pain modulation; CS: conditioning stimulus; HC: healthy controls; NRS: numerical rating scale; PN: chronic prurigo of nodular type; TS: test-stimulus Independent Samples Mann-Whitney U Test (*p<0.05, **p<0.01, ***≤0.001 vs TS_{before})

	HC	AD	BRP	PN
TS pain prior to CS (TS_{before})	51.76 ± 12.77	52.54 ± 16.55 (-0.78. -16.17 to 14.6)	50.35 ± 16.21 (1.4. -13.05 to 15.85)	50.07 ± 18.20 (1.69. -13.35 to 16.72)
TS pain after 10s during CS	35.00 ± 13.63	40.23 ± 15.30 (-5.23. -20.61 to 10.15)	39.69 ± 17.75 (-4.69. -19.14 to 9.76)	36.43 ± 14.10 (-1.43. -16.46 to 13.61)
TS pain after 20s during CS	27.27 ± 11.82	**46.00 ± 18.81* (-18.73. -34.11 to -3.34)**	**45.31 ± 19.80** (-18.04. -32.49 to -3.59)**	38.57 ± 13.51 (-11.3. -26.33 to 3.74)
TS pain after 30s during CS	25.45 ± 13.10	**49.1 ± 22.53*** (-23.62. 39.01 to -8.24)**	**48.43 ± 21.58*** (-22.98. -37.43 to -8.53)**	38.21 ± 17.82 (-12.75. -27.79 to 2.27)
CS pain after 30s	51.36 ± 24.31	42.92 ± 24.74 (8.44. -6.94 to 23.82)	36.25 ± 27.05* (15.11. 0.67 to 26.56)	38.57 ± 25.45 (12.79. -2.24 to 27.83)
CS pain after 60s	57.38 ± 25.72	61.69 ± 23.61 (-4.31. -19.83 to 11.21)	51.25 ± 31.44 (6.13. -8.46 to 20.72)	53.46 ± 26.05 (3.92. -9.22 to 21.13)
TS post CS (TS_{after})	40.95 ± 11.94	53.77 ± 19.40 (-12.82. -28.2 to 2.57)	50.0 ± 18.65 (-9.05. -23.49 to 5.4)	49.10 ± 18.02 (-8.15. -23.28 to 6.89)
Immediate CPM effect	22.52 ± 18.22	7.44 ± 19.60 (15.08. -0.3 to 30.46)	**5.88 ± 13.07* (16.64. 2.18 to 31.09)**	12.33 ± 15.96 (10.19. -4.85 to 25.22)
Prolonged CPM effect	10.8 ± 13.14	-1.23 ± 11.11 (12.03. -3.35 to 27.42)	0.35 ± 9.26 (10.45. -4 to 24.9)	0.98 ± 12.58 (9.82. -5.21 to 24.86)

Supplementary table 4. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
• Age ≥ 18 years	• Additional skin lesions at testing site (e.g. infection, tattoos)
• Patients with chronic pruritus (duration of ≥ 6 weeks) with one of three diagnoses (see below)	• Neurological, psychosomatic or severe psychiatric disorders
• Clinically diagnosed atopic dermatitis according to the Rajka and Hanifin criteria with eczemas involving the forearms	• Skin type III-VI (Fitzpatrick)
• Brachioradial pruritus: presence of chronic pruritus on one or both forearms, MRT changes at the cervical spinal level	• Allergies to the used substances
• Chronic prurigo of nodular type: presence of pruriginous lesions on upper extremities	• Diseases that prevent study participation
• Healthy controls	• Intake of medication that influences pruritus perception 1 week prior to study begin
	• Use of urea, polidocanol, capsaicin, topical steroids, topical keratolytics, exfoliatives, tanners, topical or systemic antihistamines, naltrexone, anticonvulsants or sedatives 1 week prior to study start
	• Use of systemic steroids, UV-therapy 4 weeks prior to study begin
	• Use of topical or systemic immunmodulators, antidepressants or

drugs against migraine 4 weeks prior to study start
• Intake of non-steroidal anti-inflammatory drugs or other analgesics 7 days prior to study start
• Drug abuse
• Use of cosmetic products at the experimental days
• Participation in another study in the previous 4 weeks
• Pregnant and lactating women
• Atopic disposition or pruritus (healthy controls)