

Original article

Temperature modulated histamine-itch in lesional and nonlesional skin in atopic eczema – a combined psychophysical and neuroimaging study

Background: Itch is the major symptom of many allergic diseases; yet it is still difficult to measure objectively. The aim of this study was to use an evaluated itch stimulus model in lesional (LS) and nonlesional (NLS) atopic eczema (AE) skin and to characterize cerebral responses using functional magnetic resonance imaging (fMRI).

Methods: Thermal modulation was performed on a histamine stimulus in randomized order on LS or NLS in rapid alternating order from 32°C (warm) to 25°C (cold). Subjective itch ratings were recorded. Additionally, fMRI measurements were used to analyze the cerebral processing ($n = 13$). Healthy skin (HS) of age-matched volunteers served as control ($n = 9$).

Results: Mean VAS itch intensity was significantly ($P < 0.0001$) higher during the relative cold [$55.2 \pm 8.3\%$ (LS); $48.6 \pm 8.2\%$ (NLS)] compared to the relative warm blocks [$36.0 \pm 7.3\%$ (LS); $33.7 \pm 7.6\%$ (NLS)]. Compared to HS, the itch response was delayed in LS and NLS. Itch intensity was perceived highest in LS, followed by NLS and HS.

For NLS, fMRI revealed at the beginning of the itch provocation a cerebral deactivation pattern in itch processing structures (thalamus, prefrontal, cingulate, insular, somatosensory and motor cortex). During the course of stimulation, the cerebral deactivation was reduced with time and instead an activation of the basal ganglia occurred. In contrast LS showed an activation instead of deactivation pattern already at the beginning of the stimulation in the above mentioned structures.

Conclusions: Moderate short-term temperature modulation led to a reproducible, significant enhancement of histamine-induced itch with the strongest effect in LS.

The differences in itch perception and itch kinetics between healthy volunteers and NLS in patients point towards an ongoing central inhibitory activity in patients with AE, especially at the beginning of the itch provocation.

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Key words: atopic dermatitis; atopic eczema; cerebral itch processing; functional magnetic resonance imaging; histamine; Itch; lesional skin.

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The sensation of itch – defined as ‘unpleasant sensation that provokes the desire to scratch’ (1) – is the most prevalent subjective symptom of inflammatory skin diseases (2, 3). It plays a key role in atopic eczema (4–7) and leads to a serious impairment of the quality of life of affected patients (8–10). The pathophysiology of this widespread disease (11), however, still remains incompletely understood in spite of numerous studies (12). So

far, no specific sensory receptors are described. Electrophysiological recordings have shown that spinothalamic lamina I neurons – a subpopulation of unmyelinated chemoreceptors – are selectively sensitive to histamine demonstrating a specialized central pathway (13). Peripherally unmyelinated C-neurons have been described as itch fibers (14) coinciding with histamine itch under pathophysiologic conditions in chronic pruritus (15).

It is well known that histamine and acetylcholine (ACh) provoke itch by direct binding to ‘c-fiber related sensory receptors’ and several mediators such as neuropeptides, proteases or cytokines indirectly via histamine release (2, 12, 16–18). Although other mediators than histamine have been reported to induce itch in atopic

Abbreviations: Ach, acetylcholine; EIQ, Eppendorf Itch Questionnaire; fMRI, functional magnetic resonance imaging; HS, healthy skin; LS, lesional skin; NLS, nonlesional skin; SCORAD, scoring atopic dermatitis; SD, standard deviation; VAS, visual analogue scale.

eczema (19), histamine is the best-known pruritogen in humans (16). It is released in an early phase of inflammation from mast cells, which are also involved in the inflammatory process of atopic eczema (20).

There is only limited data as to whether itch in lesional or nonlesional skin of patients with atopic eczema is processed differently – peripherally (21, 22) or centrally. The exact mechanisms and roles for central sensitization of itch in specific clinical conditions still need to be explored, whereas the importance of central sensitization in patients with chronic pain is generally accepted (16, 23). Clinically, a parallel between patients with chronic pain and with chronic itch has been shown: both respond with burning pain instead of pure itch to a histamine stimulus (24–26). Furthermore, ACh which induces pain in the controls, evokes pure pruritus in patients with acute eczema, and a ‘mixture’ of pain and itch in patients just free from eczema (27). An enhanced itch intensity in lesional atopic skin compared to nonlesional atopic skin has been shown (28) and a general qualitative description of itch in patients with atopic eczema has been reported (10). However, a qualitative comparison between lesional and nonlesional itch in atopic eczema with additional comparison to healthy skin – exploring a possible modulating role of the CNS – is still lacking.

Moreover, human studies on the physiology and pathophysiology of the itch sensation in atopic eczema patients [e.g. functional magnetic resonance imaging (fMRI) studies] have been hampered by the lack of an efficient and manageable ‘on-off’ stimulus for a long time. Short-term alternating temperature modulation of histamine-induced itch has recently been shown to provide such ‘on-off’ characteristics in healthy volunteers (29, 30).

The aim of our study was to investigate this ‘on-off’ stimulation model in patients with atopic eczema and to evaluate the effect of short-term alternating temperature modulation on histamine-induced itch in lesional, nonlesional and healthy skin using quantitative as well as qualitative scales. Additionally, we investigated the cerebral processing of these stimuli by fMRI.

Methods

Subjects

Ten right-handed male patients with atopic eczema (SCORAD >20; mean: 40; SD ± 18) and a mean age of 27.6 years (SD ± 4.3 years) were included for the psychophysical part of this study. These patients and three additional right-handed male patients ($n = 13$; age range: 18–36 years) were also investigated with fMRI.

Experiments in lesional and nonlesional skin were carried out in a randomized cross-over design, each patients being their own control.

Patients taking drugs or using topical immunosuppressive treatment at the upper right extremity were asked to stop medication for

10 days before the experiment to avoid possible side-effects or treatment related changes in itch perception.

Nine right-handed male volunteers with a mean age of 29.3 years (SD ± 2.6 years) served as control group. Right-handedness of all subjects was confirmed by the Edinburgh Handedness Inventory (31). Healthy volunteers with a history of allergy, atopic eczema, inflammatory skin disease or regular intake of drugs were excluded from the study.

The study was approved by the Ethics Committee of the Technische Universität München and conducted according to the Declaration of Helsinki. Participants gave written informed consent.

Study design

Before experiments, the patients were randomized to start either with the evaluation of itch in LS or NLS, which was carried out on separate days with a time lag of at least one week. LS was defined as erythema, papules and at least slight scaling; NLS was defined as noneczematous skin on inspection.

A histamine stimulus to induce itch was applied to the volar aspect of the dominant right forearm. 1% histaminedihydrochloride was applied as a single drop in aqueous solution on the skin, followed by a superficial puncture of this skin region with a special lancet (8). This results in a deposit of the histamine solution at the dermal–epidermal junction, where the terminals of itch-related C-fibers are located (32, 33). This model has been validated for studies of experimental itch (32, 34). After a latency of 35 s (median), an itch sensation develops with a peak itch intensity 120 s after application. Depending on the dose, the duration of a supra-threshold itch sensation ranges between 4 and 12 min. This histamine stimulus has been shown to guarantee an intense itch sensation, neither with induction of pain nor any other sensation than itch (32). Although histamine is not the central mediator involved in itch transmission in atopic eczema it is the only current reliable model for experimental itch in humans.

Sixty seconds after application of the histamine stimulus, the skin temperature was modulated by a TSA II NeuroSensory Analyzer® thermode (Medoc Ltd, Advanced Medical Systems, Ramat Yishai, Israel), which is capable of heating or cooling the skin as needed via a skin thermode placed exactly above the stimulus area. Fourteen identical cycles were applied: Each cycle started with a warm block producing a constant skin temperature of 32°C for 20 s then changing within 1.5 s (ramp 5°C/s) to a cold block of 25°C also lasting for 20 s. The applied temperature range was the result of extensive pilot trials (29).

After an interval of at least 15 min, during which the subject reported no further itching, a second session of histamine stimulation with alternating temperature modulation was performed on a different skin area.

Itch assessment

During temperature modulation, itch intensity was rated on a computerized visual analogue scale (VAS) ranging from 0 to 100 at 4-s intervals, dividing warm blocks into the intervals 1–5, cold blocks into the intervals 6–10. Time intervals necessary for the NeuroSensory Analyzer® thermode to reach its designated temperature were 1.5 s between interval 5 and 6, and 1.5 s between interval 10 and 1; these time intervals were excluded from the statistical analyses.

At one-third of the VAS (33/100) the intervention point ‘scratch threshold’ was defined. Above this threshold, each individual felt the clear-cut desire to scratch, which, however, was not permitted nor

done. Itch intensity was quantitatively expressed in percent of the VAS (29, 32, 35).

At the end of the session, the Eppendorf Itch Questionnaire (EIQ) (10), a validated instrument for qualitative and quantitative assessment of pruritus containing 80 items, was completed by the subjects. Each item is rated with regard to the itch sensation on a five point scale from 0 (not applicable) to 4 (very applicable); this was done for the cold block as well as for the warm block. Descriptive and emotional items were calculated as mean rating loads (10). The descriptive EIQ items 'more when cold', 'less when cold', 'more when warm' and 'less when warm' were excluded, as they could not be rated with regard to warm and cold blocks. As each of the volunteers was asked, whether a 'sensation of pain' was present during the experiment, the item 'painful' was excluded as well.

Functional magnetic resonance imaging

The fMRI investigation was performed 1–2 weeks after the psychophysical measurements. Every patient was investigated on two different fMRI scanning occasions/days 1 min after saline or histamine application, respectively in NLS or LS in pseudorandom order which were separated at least 1 week apart from each other. On each scanning day, all patients underwent four fMRI runs (two with saline and two with histamine stimulation) to increase the statistical power. During the fMRI runs, the sensory perception of saline/histamine was modulated by short-term alternating temperature stimulation as described above. The application and stimulation procedure for the 0.9% saline stimulus was identical to the above described procedure with the 1.0% histamine dihydrochloride stimulus.

Between the fMRI runs, there was a resting period of 30 min to guarantee identical starting conditions without a persisting itch sensation carried over from the fMRI run before. During one fMRI run, 10 cycles with alternating blocks of warm and cold stimulation were carried out.

fMRI was performed on a Siemens Symphony 1.5 Tesla MRI scanner with echo planar imaging (EPI) sequences (155 images, first five images discarded because of T1 equilibration effects, matrix: 64×64 ; TE: 50 ms; TR: 3000 ms; alpha: 90°; FOV: 192 mm, 28 axial slices; resulting voxel size: $3 \times 3 \times 5$ mm). Preprocessing and statistical analyses were conducted with SPM2, available from the Wellcome Department of Imaging Neuroscience, London, UK (36). The fMRI data were realigned to correct for motion artifacts, normalized to standard reference space according to the EPI template of SPM (mean brain of 305 healthy subjects determined at the Montreal Neurological Institute) (37) and resampled with a $3 \times 3 \times 3$ mm voxel size. Finally, the data were smoothed with an isotropic Gaussian kernel of 8 mm full-width at half maximum (FWHM) to account for anatomical individual variances and to improve the signal-to-noise ratio.

Statistical analysis

If not mentioned otherwise mean values $\pm$ SD are stated. Temperature blocks were compared with respect to VAS and EIQ using a two sample *t*-test for dependent (lesional vs. nonlesional atopic eczema skin) or independent (atopic eczema skin vs. healthy volunteer skin) variables. The intervals 1–10 were analyzed by a one-way ANOVA. In case of significance, bivariate *post hoc t*-tests were performed using Bonferroni correction for multiple testing. *P*-values of <0.05 were considered as statistically significant. All tests were performed two-tailed. Statistical analysis was performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA).

fMRI statistical analyses were first carried out on a single subject level using a fixed-effects general linear model to calculate activation maps for the histamine and saline conditions. Activation maps were then applied for group analysis to draw a statistical conclusion for the population (random-effects analysis). These maps were thresholded at $P < 0.005$ uncorrected for multiple comparisons with a minimum cluster size of five voxels (135 mm^3) uncorrected for multiple comparisons. According to previous results of H_2^{15}O -PET and fMRI studies, we expected activation of the thalamus, primary somatosensory (S1), secondary somatosensory (S2), cingulate cortex, insular, parietal and prefrontal cortex (35, 38–43).

We calculated itch-specific activation maps for the 25°C stimulation period separately for nonlesional and lesional skin. Thereby, the histamine scans were subtracted from the according time segments of the 25°C stimulation periods of the saline scans. Cerebral regions with lower activation during histamine compared to saline condition were defined as deactivated. The resulting itch-specific activation maps were intended to reveal brain regions which are more, or less active in comparison to saline. In addition to the activation map of the whole 20-s stimulation period, we also calculated four additional truncated activation maps (first 4, first 8, first 12, first 16 s of the 25°C stimulation period). These additional truncated activation maps allow the visualization of the dynamic activation changes in the brain.

Results

Quantitative assessment of itch intensity (VAS)

Histamine application. All subjects reported itch without pain within 40 s after histamine application.

Temperature modulation: cold vs. warm blocks. In each cycle, itch intensity was generally perceived as higher during the 25°C-blocks than during the 32°C-blocks and higher in lesional skin (LS) as compared to nonlesional skin (NLS) and to healthy skin (HS) of the control subjects (Fig. 1).

Atopic skin. Mean itch intensity was $55.2 \pm 8.3\%$ (LS) and $48.6 \pm 8.2\%$ (NLS) during the 25°C-block (intervals 6–10); $36.0 \pm 7.3\%$ (LS) and $33.7 \pm 7.6\%$ (NLS) during the 32°C-block (intervals 1–5) with a highly significant difference ($P < 0.0001$) between the two temperature blocks (paired *t*-test).

Mean itch intensity was highest during the 25°C-interval nine [13–16 s after beginning of cold induction; 61.5 ± 17.0 (LS); 54.7 ± 19.6 (NLS)] and lowest in LS during the 32°C-interval four (13–16 s after beginning of warmth induction; $30.2 \pm 12.7\%$) and in NLS during the 32°C-interval five (17–20 s after beginning of warmth induction; $27.6 \pm 11.1\%$) with a highly significant ($P < 0.0001$) difference between intervals (one-way ANOVA, Fig. 2).

Mean itch intensity was significantly ($P < 0.01$) higher in each of the 25°C-intervals 7–9 as compared to each of the 32°C-intervals 2–5 in LS as well as NLS. Differences between the end of the 32°-block (interval 5) and the beginning of the 25°C-block (interval 6) as well as

between the end of the 25°C-block (interval 10) and the beginning of the 32°C-block (interval 1) were significant ($P < 0.01$) in LS as well as NLS (Fig. 2).

Lesional vs. nonlesional atopic skin. Total mean itch intensity (intervals 1–10) was perceived higher in LS (45.6 ± 18.0) compared to NLS (41.0 ± 18.1 ; paired t -test, $P < 0.001$). Mean itch intensity during the 25°C-block (intervals 6–10) was significantly (paired t -test, $P = 0.003$) higher in LS ($55.2 \pm 8.3\%$) compared to NLS ($48.6 \pm 8.2\%$). No significant differences in total mean itch intensity between LS and NLS were found for the 32°C-block.

Atopic skin (lesional and nonlesional) vs. healthy skin. LS and NLS showed a delayed increase of the itch intensity with a delayed peak (interval nine; 13–16 s after beginning of cold induction) compared to HS (interval 8; 9–12 s after beginning of cold induction).

Compared to HS (42.3 ± 9.3) total mean itch intensity was generally perceived more intense in LS (45.6 ± 12.5) and slightly less intense in NLS (41.0 ± 10.7); The biggest difference in mean itch intensity was perceived between LS and HS in the late phase of the 25°C-blocks and when changing to the 32°C-blocks (intervals 8, 9, 10 and 1) (LS 57.3 ± 6.4 ; HS 48.8 ± 7.1 ; $P < 0.001$). Comparing NLS with HS intervals five and six showed a significant difference (NLS 31.5 ± 5.5 ; HS 38.8 ± 6.8 ; $P = 0.003$).

Qualitative assessment of itch intensity (EIQ)

In the EIQ mean descriptive rating loads were significantly ($P < 0.0001$) higher for the 25°C-blocks (NLS: 1.63 ± 0.82 ; LS: 1.78 ± 0.83) compared to the 32°C-blocks (NLS: 0.91 ± 0.62 ; LS: 0.94 ± 0.57). The mean emotional ratings were also significantly ($P < 0.0001$) higher for the 25°C-blocks (NLS: 1.34 ± 0.72 ; LS: 1.68 ± 0.61) compared to the 32°C-blocks (NLS: 0.53 ± 0.41 ; LS: 0.61 ± 0.41).

On a single item level, many descriptive items (Fig. 3A) were rated significantly ($P < 0.01$) higher for the 25°C-blocks compared to the 32°C-blocks in LS: 'pricking', 'biting', 'stinging', 'burning', 'cold', 'soft', 'sharp', 'pointed', 'itching', 'pinching', 'stroking' and 'mosquito bite-like'; and in NLS: 'pricking', 'cold', 'pointed', 'itching'.

The control item 'warm' was rated significantly higher for the warm block in LS ($P = 0.001$) and NLS ($P = 0.006$). The descriptive items 'cold' and 'itching' reached the highest significance level ($P < 0.001$) in LS and in NLS.

The following emotional items (Fig. 3B) were rated significantly ($P < 0.01$) higher for the 25°C-blocks compared to the 32°C-blocks in LS: 'uncontrollable', 'bothersome', 'wearing', 'unpleasant', 'I only feel the itch', 'my only desire: no itch', 'severe', 'stubborn' and 'unbearable', the last two items reached the highest significance level ($P < 0.001$). In NLS, only 'awful' was rated significantly ($P < 0.01$) higher for the 25°C-blocks.

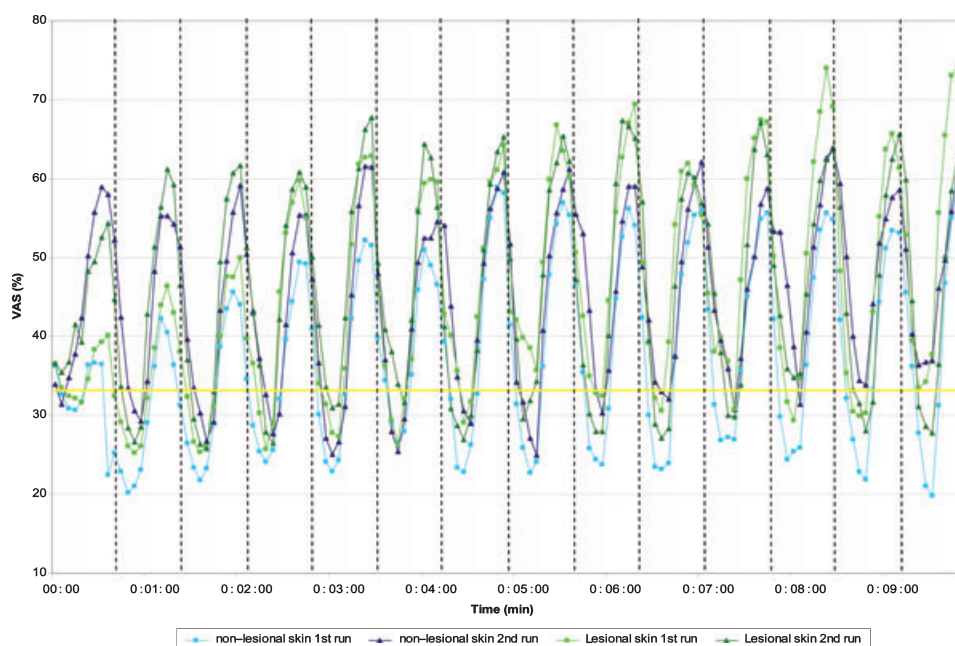


Figure 1. Showing the reproducibility of itch modulation in patients with atopic eczema: Mean itch intensity (VAS) of all atopic eczema patients ($n = 10$) in lesional skin (green) and nonlesional skin (blue) of the two sessions (square and lighter coloring: first session; triangle and darker coloring: second session). The 14 cycles (each lasting 43 s, beginning with a 21.5 s warm block [32°C; red background] and ending with a 21.5 s cold block [25°C; blue background]) are separated by the black gridlines. The yellow line represents the scratch threshold (33% itch-intensity).

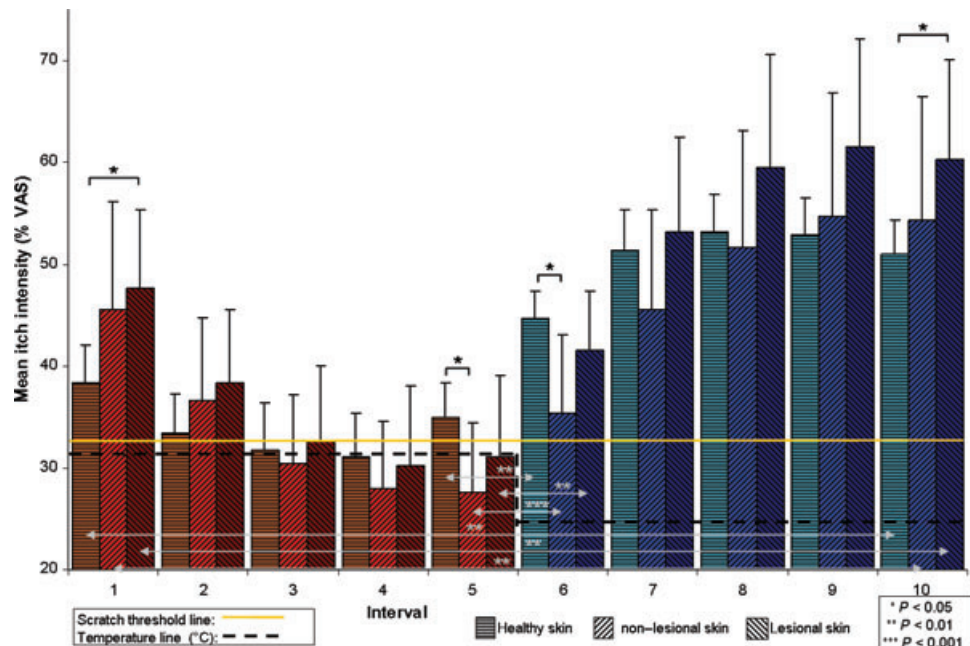


Figure 2. Demonstrating increased itch intensity on lower temperature and in lesional compared to nonlesional skin as well as delayed onset of itch modulation in patients with atopic eczema. Mean itch intensity of both sessions at the various temperature intervals ($n = 10$). As indicated by the dotted line the red columns numbered 1–5 indicate warm temperature intervals (32°C), whereas the blue columns numbered 6–10 mark relatively cold temperature intervals (25°C), each lasting 4 s. Columns with horizontal line pattern represent healthy skin, columns with ascending line pattern represent nonlesional AE skin, columns with downward line pattern represent lesional AE skin. The yellow line represents the scratch threshold (33% itch-intensity). Asterisks indicate selected significant differences between intervals. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Lesional vs. nonlesional atopic skin. Concerning the 25°C-blocks, the mean emotional ratings were significantly ($P < 0.001$) higher for LS (1.68 ± 0.61) compared to NLS (1.34 ± 0.72).

Atopic skin vs. healthy skin. There were also differences in EIQ-rating between patients with atopic eczema and healthy volunteers:

Concerning the 25°C-blocks, the mean emotional ratings were significantly ($P < 0.001$) higher for LS (1.68 ± 0.61) as well as NLS (1.34 ± 0.72) compared to HS (0.88 ± 0.77). Concerning the 32°C-blocks, the mean emotional ratings were significantly ($P < 0.001$) higher only for LS (0.61 ± 0.41) compared to HS (0.38 ± 0.35).

Descriptive items showed no significant differences between atopic eczema skin (LS or NLS) and HS.

Four emotional items were rated significantly ($P < 0.01$) higher during the 25°C-blocks for LS compared to HS on a single item level: 'unmanageable', 'my only desire: no itch', 'uncontrollable' and 'I only feel the itch'; the latter reached the highest significance level ($P < 0.001$).

Itch intensity during the fMRI measurements

For the histamine condition, a significant difference in the subjective itch ratings was noted between the 25°C and

the 32°C stimulation period for LS as well as for NLS ($P < 0.001$). The itch intensity during the 25°C stimulation was 67.9 ± 17.9 for LS and 62.1 ± 17.9 for NLS. During the 32°C stimulation it was 33.5 ± 26.3 in LS and 27.9 ± 22.1 in NLS.

For the saline control condition, there was no significant difference between the 25°C stimulation (15.8 ± 21.8 in NLS; 29.7 ± 23.5 in LS) and the 32°C stimulation (9.5 ± 15.9 in NLS; 19.4 ± 16.7 in LS).

Itch-specific brain activation

Histamine-induced itch in NLS at 25°C stimulation was reflected by a *deactivation* of brain structures, such as the primary and secondary somatosensory, insular, cingulate and prefrontal cortex, supplementary motor area, premotor areas and basal ganglia (Fig. 4A). These deactivations were less pronounced in the later course of 25°C stimulation with a parallel activation increase in the basal ganglia.

In contrast, histamine-induced itch in LS at 25°C stimulation was associated with an *activation* of the basal ganglia, insular cortex, prefrontal areas and parietal cortex. Deactivations were seen in the parietal, temporal, primary and secondary somatosensory, cingulate cortex, premotor and prefrontal areas (Fig. 4B), but were of a lesser degree as for NLS.

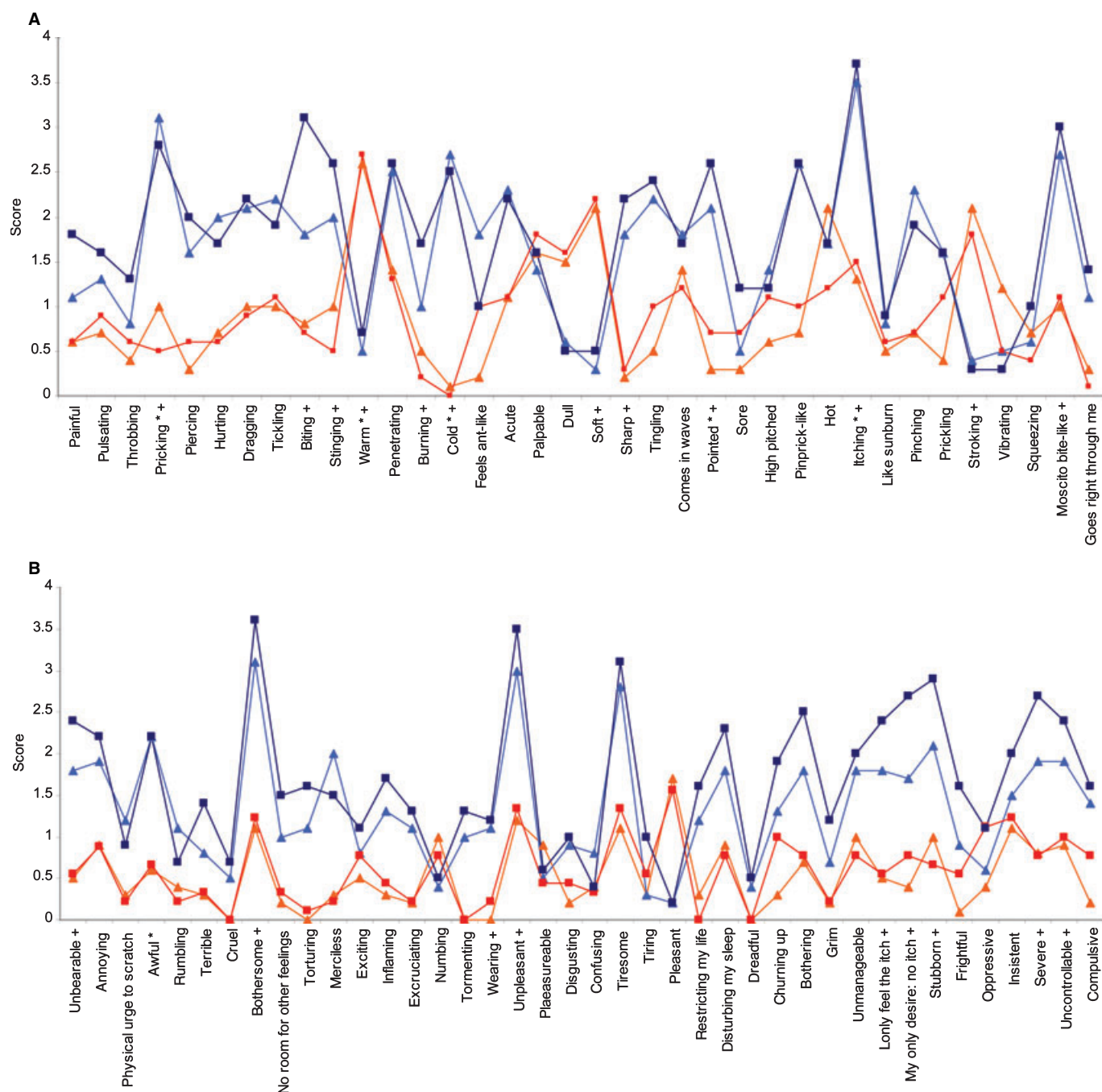


Figure 3. Item rating of the Eppendorf Itch Questionnaire in patients with atopic eczema: The red lines represent warm temperature blocks (32°C), whereas the blue lines represent relative short-term cold temperature blocks (25°C). The red and blue squares represent lesional skin, whereas the red and blue triangles represent nonlesional skin. The crosses indicate significant differences ($^+P < 0.01$) between 32 vs 25°C stimulation in lesional skin; the asterisks indicate significant differences ($*P < 0.01$) between 32 vs 25°C stimulation in nonlesional skin. A, Descriptive items; B, Emotional items.

Discussion

So far, no controlled study investigated the effect of short-term alternating temperature modulation on

experimentally induced itch in patients with atopic eczema. It is also the first study to investigate the differences in the cerebral processing of histamine-induced itch in lesional and nonlesional skin. With the

new stimulus paradigm, characterizations of different skin conditions and their effects on the cerebral processing were possible. This means that results of a brain scan under controlled stimulus conditions can differentiate lesional from nonlesional skin areas in atopic eczema.

Psychophysics

In spite of the common knowledge that intensive cold inhibits itch (34, 44, 45), but in accordance with two previous studies in healthy volunteers (29, 30), the results of our study show a reproducible, significant enhancement of histamine-induced itch by a short-term moderate temperature decrease in lesional as well as in nonlesional skin of patients with atopic eczema. When taking into account the definition of itch as a sensation provoking the desire to scratch, the stimulus paradigm shows an 'on/off' phenomenon: Mean itch intensity was above scratch threshold during the whole cold blocks, while more than half of the warm blocks remained below the scratch threshold level. Patients were also able to correctly discriminate the warm from the cold stimulation according to the ratings of the qualitative EIQ-items.

Although histamine is not the main itch mediator in atopic dermatitis it is so far the only reliable and evaluated experimental stimulus model for experimental itch (32); so far studies on the cerebral processing of itch in healthy volunteers have all been carried out with histamine (30, 35, 38–43, 46–48).

However, in another study in healthy volunteers, thermal stimuli failed to modulate experimental itch (49). This discrepancy might be explained by methodical differences, such as the use of histamine iontophoresis (instead of the skin prick test), which has been shown to induce only a moderate initial itch intensity compared to our skin puncture stimulus model (32). Different (noxious) temperature ranges, thermode sizes and thermode localization might be other reasons for differences between studies.

Mean itch intensity was perceived as more intense in lesional skin compared to nonlesional as well as healthy control skin. These findings confirm previous results of other groups (21, 28, 50).

One new finding is the fact that patients with atopic eczema (NLS as well as LS) have a delay in their itch response reaching their highest peak of itch intensity (slightly) later than healthy volunteers.

Concerning the EIQ, patients with atopic eczema in comparison to healthy controls showed the highest significance level for items which have previously been shown to be disease specific ('unbearable', 'severe', 'uncontrollable', 'I only feel the itch', 'my only desire: no itch', 'bothersome', 'wearing', 'unpleasant', 'stubborn'). These results underline the validity and specificity of the EIQ for atopic eczema (10).

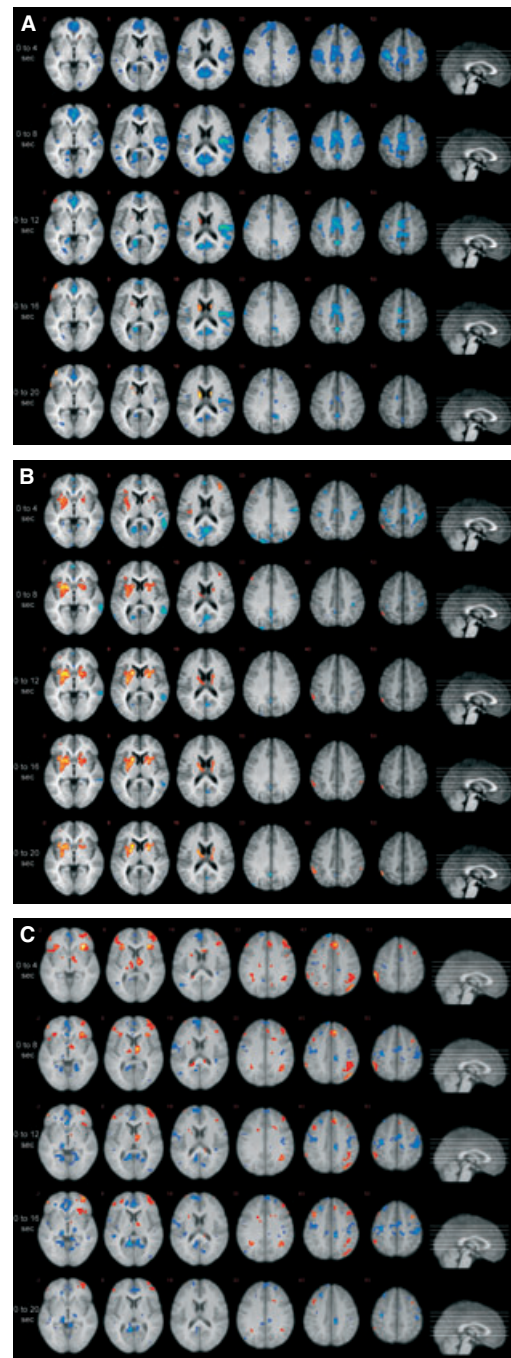


Figure 4. Cerebral activation (red color) and deactivation (blue color) patterns induced by itch provocation. (A) Nonlesional skin of patients with atopic eczema (B) lesional skin of patients with atopic eczema (C) healthy control skin. Activation maps of histamine-induced itch compared to saline were calculated for the first 4, 8, 12, 16 and 20 s of the 25°C stimulation period and are superimposed on the averaged normalized T1 weighted brain of all subjects. A dynamic process with increase and decrease of regional brain activation can be observed in the time course of the 25°C stimulation period. The statistical parametric maps were thresholded at $P < 0.005$. The right side of the image corresponds to right side of the brain (neurological convention).

Interestingly, the emotional quality ratings of itch perception of the EIQ differed strongly between all three groups, while descriptive itch quality ratings showed no significant differences between groups. Itchy lesional skin areas resulted in the highest ratings; and although mean itch intensity was perceived as slightly higher in healthy control skin compared to nonlesional atopic skin, emotional itch questionnaire ratings were significantly higher for nonlesional skin pointing out the emotional extra-component of itch in atopic eczema even in nonaffected areas.

Possible underlying mechanisms. A possibly underlying cutaneous mechanism for the biphasic itch induction by short-term alternating temperature modulation of histamine could be that high tissue concentrations of histamine lead to increased activation of itch fibers by influencing the threshold of itch specific receptors (18). Cooling is sensed by peripheral thermoreceptors, the main transduction mechanism of which is probably a cold- and menthol-activated ion channel, transient receptor potential (melastatin)-8 (TRPM8) (51, 52). TRPM8 is activated by chemical cooling agents (such as menthol or eucalyptol) or when ambient temperature drops below 26°C and depolarises sensory neurons (51, 53, 54); it can adapt to long-term variations in baseline temperature to sensitively detect small temperature changes and is selectively expressed on a certain subpopulation of A and C-type sensory afferents (55). The process between ion channel closure and reopening after depolarization occurs within seconds (56). TRPM8 is not the only thermosensitive element in cold receptors and interacts with other ionic currents to shape cold receptor activity, but seems to play an essential and predominant role in thermosensation over a wide range of cold temperatures (51). Temperature change from 25 to 32°C also enhances the sensitivity and efficacy of voltage activation for TRPV1 (57), which undergoes changes in ion selectivity upon channel activation with a maximum potential shift 21 seconds after activation (58). Pruritogens such as histamine could act on these receptors (34, 59, 60), their pruritic effect might, however, intermittently be overlapped or even enhanced when the receptors are at threshold temperature of activation, e.g. with the described short-term alternating temperature modulation.

A conceivable explanation on a spinal level for the increase of itch sensation is that the stimulation of A-delta fibers by cooling on a fast but low intensity level (temperature decrease of 5°C per second from 32 to 25°C) might lead to a temporary central disinhibition of pruritoceptive neurons, thereby enhancing pruritoceptive responses.

Central mechanisms of disinhibition have been discussed for the paradoxical heat sensation, where the perception of heat is reported when the skin temperature is innocuously cooled (61). Here the insular cortex, an

important area for thermosensory perception, is thought to play a major role (62). We have recently described the insular cortex to be involved in itch processing (30).

Neuroimaging. As mentioned above, the psychophysical data revealed that in nonlesional skin, patients have a delayed itch sensation in response to the temperature decrease as compared to lesional skin or to the skin of healthy volunteers. Peripheral mechanisms with a heightened threshold of pruriceptors (sub-population of C-fiber neurons) might contribute to this observation, but central mechanisms are in our view more likely to explain this phenomenon. The neuroimaging results support this hypothesis.

In a previous investigation of our group, histamine-induced itch in healthy control skin produced an activation of brain regions, such as the thalamus, pre-SMA, anterior insular, inferior parietal and dorsolateral prefrontal cortex and a decreased activation of the orbitofrontal, medial frontal, mid-cingulate and primary motor cortex as compared to the saline condition (30) (Fig. 4C). These regions are known to be involved in the encoding of sensory, attentive, emotional, evaluative and motivational aspects of itch (30, 35, 39, 40, 42, 43, 47, 63).

In the current study, a very similar activation pattern was observed in lesional skin (Fig. 4B). In contrast to healthy control and lesional skin, a profound deactivation pattern occurred when provoking itch in nonlesional skin (Fig. 4A). These different itch-specific brain activation/deactivation patterns are striking. In our view, the deactivation pattern during the provocation of itch in nonlesional skin reflects a neurobiological attempt to suppress the perception of itch (somatosensory areas) as well as the desire to scratch (premotor and supplementary motor areas). This hypothesis would explain why the initial itch sensation is delayed in nonlesional skin (Interval six and seven, Fig. 2).

In the course of stimulation the itch intensity ratings are increasing (Interval 8–10, Fig. 2), which is in accordance with our neuroimaging results (Fig. 4A), where the initial deactivation pattern changes to an increasing activation in the basal ganglia and lateral prefrontal areas. A feasible explanation might be related to temporal summation processes: when the amount of pruriceptive input reaches a critical threshold, the firing of itch specific neurons cannot further be suppressed by central mechanisms (24). Moreover, after exceeding the inhibition threshold of pruriceptive input, the firing seems to be prolonged in the patient group, which is reflected by the delayed decline of itch responses during the warm blocks (Interval 1–2, Fig. 2).

Although deactivations (negative fMRI BOLD signal) have been observed in many fMRI studies, its interpretation remains controversial. Recent studies suggest that the negative fMRI BOLD signal response reflects both an

active inhibitory role as well as neuronal deactivation depending on the interplay between hemodynamics and metabolism (64–66). This is not the first study reporting cerebral deactivations in itch processing: Deactivation of limbic structures in itch processing of healthy controls has been observed previously, which has been attributed to the stressful character of the itch stimulation and the urge to scratch (43) as well as the process of scratching itself (48).

A possible explanation for the differences in brain activation between nonlesional skin and lesional skin could be related to the magnitude of inflammation induced by histamine in lesional skin vs nonlesional skin. As a matter of intensity central processing may be 'prioritized'.

As this is the first study comparing lesional with nonlesional atopic eczema skin as well as healthy control skin, comparisons to other studies are difficult. Leknes et al. (42) were the first to investigate the cerebral processing of allergen-induced itch postulating a dysfunction of striato-thalamo-orbitofrontal circuits, which are believed to underlie the failure to regulate the motivational drive in disorders associated with strong urges, e.g., addiction and obsessive compulsive disorder. Schneider et al. (67) demonstrated significant differences in central imaging of histamine-induced itch between patients with atopic dermatitis in nonlesional skin and healthy subjects in a study correlating itch

intensity with cerebral activation using positron emission tomography.

Our study supports a peripheral as well as a central component in the pathophysiology of chronic itch in patients suffering from atopic eczema. On the one hand, pathological skin conditions, such as a skin barrier abnormality and local inflammation processes in atopic eczema patients (68, 69) point to a peripherally increased sensitivity to itch stimuli. On the other hand, central filter mechanisms might counteract this pathological skin condition to a certain point when the threshold of the pruriceptive input is exceeded.

Conclusion

Short-term moderate temperature modulation allows to rapidly enhance histamine-induced itch in patients with atopic eczema and to perform reliable fMRI studies on itch. The observed differences in kinetics and intensity of itch perception in lesional vs nonlesional or healthy skin are reflected by different cerebral activation patterns. The deactivation in various itch processing brain structures during itch provocation in nonlesional skin might be regarded as an attempt of the brain to suppress the itch perception. In the future, therapeutic strategies of atopic itch should therefore also target central nervous system mechanisms.

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