Appendix S1.

METHODS

Somatosensory stimuli for testing itch sensitivity and itch modulation by itch and pain

To measure itch sensitivity, the following quantitative sensory testing (QST) stimuli were applied to unaffected, non-scarred skin: monofilaments, electrical stimulation, and histamine iontophoresis. Participants reported the levels of itch they perceived by the stimuli on a numerical rating scale (NRS) ranging from 0 (no itch at all) to 10 (worst itch ever experienced). Histamine was also used as itching conditioning stimulus as part of a conditioned itch modulation (CIM) procedure. Itching electrical test stimuli were given before and after the histamine (14). In addition, as part of a CIM procedure investigating the inhibition of itch by pain, a CPT was applied as painful conditioning stimulus, and itching electrical test stimuli were applied before and after the CPT. The change in itch perceived by the electrical test stimuli was the main outcome measure for both CIM procedures.

The location of stimulus application was standardized as much as possible, while considering the localization of the burn injury (itch stimuli were applied to unaffected skin). Specifically, the monofilaments and electrical stimulation were applied at the side of the non-dominant hand, while histamine iontophoresis and the CPT were conducted on the side of the dominant hand. All somatosensory itch stimuli were applied to unaffected skin. In the case that both hands of a patient were affected (n=3), the patient was asked whether the CPT could be conducted on affected skin, with which all agreed. When patients were affected at the standard measurement locations, application side of the stimuli was switched contralaterally whenever possible. If this was not possible because patients were also affected on the contralateral side, electrical stimulation was conducted halfway the lateral-anterior side of the lower leg (at the m. tibialis anterior); the other stimuli were applied according to standard protocol. For 3 patients, electrical stimulation was conducted at the lower leg; this procedure was also applied for 2 healthy subjects.

Mechanical stimulation. Two Semmes-Weinstein von Frey calibrated monofilaments (Sammons Preston Rolyan, Germantown WI, USA) of 15.1 g (diameter 0.48 mm) and 75.9 g (diameter 0.71 mm) were applied twice for 2 s, using a similar procedure to that described in a previous study by our group (17).

Electrical stimulation. According to a previous study by our research group (18), electrical stimuli were applied by a constant current stimulator (Isolated Bipolar Constant Current Stimulator DS5, Digitimer, UK) attached to the inner side of the wrist through 2 surface electrodes (a disk electrode of diameter 1 cm and a reference electrode of diameter 2 cm, VCM Medical, The Netherlands). One electrode was applied 1.5 cm proximal to the triquetrum, at the centre of the inner wrist; the reference electrode was applied 2 cm below. Stimuli were applied at 50 Hz frequency with a pulse duration of 100 μs and at a continuously increasing current intensity (0.05 mA/s) up to a maximum current intensity of 5 mA. To familiarise subjects with the sensation, they were asked to indicate twice "the first moment you feel some itch" and "the first moment you feel the urge to scratch"(18). Then, the itch tolerance threshold, defined by "the first moment you cannot resist the urge to scratch", was measured twice. In-between the measurements there was an interval varying from 30 s to 4 min, dependent on the residual itch (if the level of itch was ≥2 on an NRS, the interval was extended). After each of the latter 2 threshold measurement, filler tasks (easy puzzles) were given to diminish possible influence of itch evoked by previously applied stimuli on subsequent stimuli (18). In line with our previous studies analysing itch at the electrical tolerance threshold (10, 11), the mean itch evoked during the itch tolerance threshold measurements was the main outcome for electrical stimulation.

For testing CIM efficacy by both an itching and painful stimulus, electrical test stimuli were applied at the mean intensity of IT3 before and after the heterotopically applied conditioning itch (histamine) and conditioning pain (cold pressor task) stimuli, respectively. Timing of test and conditioning stimuli was identical to in our previous study investigating CIM, with 4 min in-between test stimuli and conditioning stimuli, but this study used a more sophisticated device to induce electrical test stimuli (14, 18).

Histamine iontophoresis. Histamine was applied to investigate both itch sensitivity and CIM efficacy by an itching stimulus (histamine is the conditioning stimulus). Histamine 0.3% (as diphosphate monohydrate) was dissolved in a gel containing hydroxypropylmethylcellulose, propylene glycol and purified water (prepared by the Clinical Pharmacy and Toxicology, LUMC). The histamine content (with diphosphate) within this solution was comparable to the solution (with dihydrochloride) used previously by our research group, as calculated by the pharmacists (e.g. 14, 11). Of the solution 2.5 ml was added to a disposable iontophoresis electrode (Iogel, Chattanooga, Hixson, TN, USA), which was placed on the forearm, 2 cm distal to the lateral epicondyle of the humerus. The reference electrode was applied to the skin on the lateral side of the triceps brachial muscle. A dose controller (Chattanooga Ionto, Chattanooga Group, Hixson, TN, USA) delivered the histamine for 2.5 min at a current of 0.4 mA. Participants rated the level of itch every 30 s during the application and subsequently up to 3.5 min. Mean levels of itch during the histamine iontophoresis were calculated.

Cold pressor task (CPT). A CPT was conducted as conditioning stimulus to investigate CIM efficacy by a painful stimulus. Participants were instructed to immerse their hand up to the wrist in a Styrofoam tank of cold water at 4°C (mean 4.1, SD 0.4) until the experimenter gave the signal to discontinue the immersion, or earlier if they could no longer tolerate it. The duration of the immersion was 1 min at maximum, of which participants were unaware (S1). Participants rated the level of pain on an NRS from 0 (no pain at all) to 10 (worst pain ever experienced) every 15 s during immersion, and subsequently up to 3.5 min every 30 s. Mean levels of pain during immersion were calculated.

Computer tasks measuring automatic reactions to itch

The computer tasks measuring automatic reactions to itch were administered using E-Prime software, version 2.0 (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Selective attention to itch-related words was measured with a modified version of the Stroop task (15, S2). In this task it is assumed that the saliency of the words interferes with the colour-naming, resulting in longer response latencies, indicating selective attention (15, S2). The current Stroop task was modified by incorporating words related to itch (e.g. itchy, head lice, and scratching), as well as words from 3 non-itch-related word categories (neutral, negative, and positive) taken from the Dutch translation of the Affective Norms for English Words list (e.g. S3). A stigmatization category was also included, which is not relevant for the present research question. The 40 words (8 words, in 5 colours) of a single word category were presented simultaneously on the computer screen. The order of the word categories was randomized and the experimenter was blind for the displayed

word category. Participants were instructed to name aloud the print colour of the words displayed, as quickly and accurately as possible. The experimenter recorded the number of errors and pressed a button after the participant had finished naming the colours of the words per screen. An approach avoidance task (S4), adapted for itch, was also administered, but due to technical problems, the data had not been recorded.

Self-report questionnaires

The following self-report questionnaires, which have previously been shown to have satisfactory reliability and validity, were administered in Dutch.

The following questionnaires were administered to the patients only in order to gather information about the severity of the burn injuries and associated itch.

The 40-item *Brief Burn Specific Health Scale (BSHS-B)* was used to measure burn-specific aspects of health status in the patients (S5). Items were scored on a 5-point Likert scale (0 = extremely; 4 = not at all), which were summed to obtain a total score for the BSHS-B. Higher scores refer to better burn-specific quality of life. Cronbach's alpha for the BSHS-B total score in the present study was 0.90.

The *Burn Itch Questionnaire (BIQ)* was administered to assess severity of spontaneous itch related to the burn wounds (see also 1). Of the 22 items of the BIQ only the items of the 3 subscales were used, i.e. itch intensity (4 items), interference of itch with sleep (4 items), and interference of itch in daily life (4 items). The items were rated on a scale from 0 (no itch/totally disagree) to 9 (worst itch/totally agree). Cronbach's alpha for these subscales in the present study ranged from 0.82 to 0.96. Moreover, based on the item in which patients had marked their burn injuries in a graphic representation of the body, the localization of the burn injuries was determined (i.e. affected body areas).

The 6-item *Patient and Observer Scar Assessment Scale (POSAS)* was administered to assess scar quality (e.g. S6). Patients make use of numerical 10-point scales in which 1 represents a scar comparable with unaffected skin and 10 represents the worst scar imaginable. The total score was calculated by summing the 6 items. Cronbach's alpha of the POSAS-patient scale in the present study was 0.89.

The *Impact of Event Scale (IES)* was administered to measure post-traumatic stress (19). Patients rated the items on a 4-point scale (0 = not at all; 1 = rarely; 3 = sometimes; 5 = often). The IES consists of 15 items within 2 subscales: intrusive (Cronbach's alpha 0.86) and avoidant traumatic stress symptoms (Cronbach's alpha 0.95).

The following questionnaires were administered to both the patients with burn wounds and healthy subjects to investigate whether the groups were comparable:

The neuroticism and extraversion subscales of the *Eysenck Personality Questionnaire revised short scale (EPQ-RSS)* were administered to measure neuroticism and extraversion (S7). The total score of both subscales was obtained by calculating the sum of the 12 dichotomous (yes = 1 /no = 0) items of both subscales separately. Higher scores represent higher scores of neuroticism (Cronbach's alpha: 0.79) or extraversion (Cronbach's alpha: 0.78).

The *Body Vigilance Scale (BVS)* was administered to measure attentional focusing on bodily sensations (e.g. 16), but due to insufficient reliability (Cronbach's alpha: 0.42), the results have not been reported.

The *Hospital Anxiety and Depression Scale (HADS)* and the *Positive and Negative Affect Schedule- short version (PANAS-s)* were administered on the day of testing to measure mood state (S8–S10). The HADS consists of 7 items measuring the subscale depression (Cronbach's alpha: 0.79) and 7 items measuring the subscale anxiety (Cronbach's alpha: 0.75). Items were rated on a scale from 0 to 3, and the total score was obtained by summing the items per subscale. The PANAS-s consists of 2 subscales, positive and negative affect, with 5 items each, scored on a 5 point-Likert scale. The total scores per subscale were obtained by summing the items. Cronbach's alphas for the subscales in the present study were 0.85 and 0.93, respectively.

Statistical analyses

Prior to analyses, missing NRS scores for the induced levels of itch by histamine (in total 3 missing values of 1 patient) and pain by the CPT (in total 15 missing values of 7 subjects) were replaced using the last observation carried forward (LOCF) method. As a check, respective analyses were re-run for the variables without the LOCF method; this procedure obtained similar levels of significance and is not further reported. Before conducting the analyses, variables were checked for normal distribution and transformed when necessary. The variables itch evoked by mechanical and electrical (IT3) stimulation as well as pain evoked by the CPT were log-transformed, which resulted in normal distribution. Levels of itch induced by the somatosensory stimuli were compared between patients and healthy controls by conducting analyses of variance (ANOVAs) with condition as independent variable and the itch scores for each stimulus separately as dependent variable. Considering the low levels of itch due to mechanical stimulation, a non-parametric Mann–Whitney test had been additionally conducted. CIM efficacy by histamine and the CPT were analysed in separate repeated measures ANOVAs with the levels of itch evoked by the electrical test stimuli before and after histamine and the CPT as within-subjects factors and group as between-subjects factor. For the modified Stroop task, reaction times for the word categories were compared in a repeated measures ANOVA with condition (patient/control) as between-subjects factor and the 4 different word categories as within-subjects factor. Simple contrasts were conducted comparing the itch word category separately with the other word categories. The results of the approach avoidance task will not be reported as data are not available. Individual characteristics related to the burn incident were descriptively reported (note that the Burn Itch Questionnaire subscales were analysed for 13 patients as 2 patients had not experienced itch during the 7 days preceding completing the questionnaires), while the other individual characteristics were compared between the patients and healthy subjects in separate ANOVAs. To explore whether patients with post-traumatic stress showed a higher sensitivity to itch stimuli, less efficient CIM, and more selective attention to itch than those without post-traumatic stress, we descriptively compared the patients with post-traumatic stress with the patients without post-traumatic stress on these outcome measures.

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