The pattern and time course of somatosensory changes in the human UVB sunburn model reveal the presence of peripheral and central sensitization

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1. Introduction

Human pain models provide experimental information on clinical manifestations of pain mimicking some symptoms and signs of inflammatory and neuropathic pain [57] and are thus valuable tools for translational research toward a mechanism-based treatment of chronic pain [108]. By using the same induction techniques and similar readouts for pain and hyperalgesia in animal studies and clinical trials, they help to assess pharmacological target engagement at the transition from preclinical to clinical drug development (forward translation) and to imply pain mechanisms from clinical signs and symptoms (backward translation).

One of these translational pain models is ultraviolet B (UVB)-induced skin inflammation [70]. UVB irradiation causes a cutaneous stress response encompassing oxidative stress, activation of cutaneous cells like keratinocytes and fibroblasts, activation of immune cells like mast cells and neutrophils, and expression of inflammatory mediators like cytokines, chemokines, and tumor necrosis factor alpha (TNF-α) [4,16,23,27,48,93]. Although painless, UVB irradiation is noxious and elicits pronounced long-lasting inflammation-related heat and mechanical hyperalgesia at the UVB-irradiated site in men, rat, mice, and even Drosophila (primary hyperalgesia [7,40,44,88,89]). In contrast, secondary hyperalgesia adjacent to UVB-irradiated skin is controversial, ranging from no changes [12–14] to documentation of large areas of pinprick hyperalgesia suggesting central sensitization [26,38]. Functional imaging of UVB-induced thermal and mechanical hyperalgesia revealed differences in cortical processing [90].

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The time course of UVB-induced primary hyperalgesia peaks at 24–48 h, then slowly subsides [38,40]. Neither the decay of UVB-induced primary hyperalgesia beyond 72 h nor full profiles of sensory changes in the primary and secondary area have been investigated. Such detailed characterization of somatosensory changes and the comparison of primary and secondary hyperalgesia time courses may elucidate the relative contribution of peripheral and central mechanisms in this human surrogate model of pain and hyperalgesia.

Quantitative sensory testing (QST) assesses characteristic sensory patterns in pain models and in pain patients, allowing the investigation of different somatosensory modalities. A comprehensive QST battery has been proposed for standardized somatosensory assessment [85]. Reference values and reliability criteria were published [32,67,84], as were sensory profiles in large cohorts with various neuropathic pain conditions [68].

The aim of this study was to characterize magnitude and time course of sensory changes induced by UVB irradiation with a comprehensive QST battery comparing primary hyperalgesia in inflamed skin and secondary hyperalgesia in adjacent noninflamed skin with contralateral control areas. The comprehensive assessment of all somatosensory modalities will close current knowledge gaps; for example, cold hyperalgesia and blunt pressure hyperalgesia have not yet been investigated in the UVB model and may provide new findings.

2. Methods

2.1. Subjects

After approval by the ethics committee of the Medical University of Vienna and obtaining written informed consent, 22 healthy right-handed volunteers (11 women, 11 men) were enrolled. Inclusion criteria were the following: 19–40 years old, body mass index between the 15th and 85th percentile, and no medication 1 week before the study. All subjects were healthy subjects with neither acute nor chronic pain. Subjects were also excluded in case of regular medication or alcohol abuse, any disease 1 week before, acute skin disease (e.g., sunburn) in the relevant areas, pregnancy or lactation, or participation in a clinical trial 2 weeks preceding this study.

During the study, subjects were asked to abstain from nicotine, alcohol, stimulating beverages containing xanthenes, and exposure to sunlight. The study took place in the same quiet air-conditioned room (23°C) during the winter season and was always conducted by the same investigator (DLS).

Subjects were familiarized with the experimental procedures during 2 training sessions in which the QST protocol was trained in an independent skin area at the hand dorsum on 2 different days before the study session. Experiments were performed with the subject in a comfortable reclining position.

2.2. UVB model

A calibrated UVB source (Sellasol; Sellas Medizinische Geräte GmbH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm) was used to induce an inflammatory skin response to UVB light. The degree of inflammation and hyperalgesia after UVB depends on an individual’s skin type rather than skin area, and there are no significant differences between extremities [30,34,64,74]. Also, repeated UVB tends to accumulate after repeated exposure, even at suberythemal doses [62]. Thus, to avoid interaction effects, the individual minimal erythema dose (MED) was determined for all volunteers at the volar side of the forearm at 24 h after an ascending irradiation dose [38,44]. Because the response to UVB becomes less variable at doses above 2×MED [40,44], a circular spot 50 mm in diameter was irradiated with 3×MED of UVB light at the ventromedial side of either the left or right thigh according to randomization. This circular area was defined as area of primary hyperalgesia (Fig. 1). The noninflamed area surrounding the sunburn and sensitive to a pinprick test was defined as the area of secondary hyperalgesia. The contralateral leg served as an untreated control.

2.3. Experimental protocol

This study consisted of 2 experiments running in succession. In experiment 1, a comprehensive test battery of QST [84,85] was applied in all 22 volunteers at 24 h after irradiation. Four areas were evaluated: the area of sunburn (area of primary hyperalgesia) and the skin surrounding the sunburn on the irradiated thigh (area of secondary hyperalgesia), as well as 2 mirror-image control areas on the nonirradiated contralateral thigh. It was taken care that the skin sectors were not the same for mechanical and thermal testing. In the zone of secondary hyperalgesia, all tests were performed at a distance of 10–15 mm to the margin of the sunburn.

After 10 subjects, we made an interim analysis to identify the sensory parameters that exhibited significant changes during the expected peak of hyperalgesia (ie, at 24 h after UVB). The temporal

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**Fig. 1.** Local changes in capillary blood flow at 24 h after UVB irradiation at 3 times the MED. (A) A typical example of a visible local sunburn erythema at the thigh depicting a sharp demarcation and restriction of visible erythema to the irradiated circular area. (B) Respective measurement of capillary blood flow by LDI (arbitrary perfusion units, PFU) shows a similar restriction of the changes in capillary perfusion (same subject as A; false color coded). (C) Average capillary perfusion measured by LDI in the UVB sunburn (black bar) is significantly increased compared to adjacent skin (gray bar) and to control skin (mirror-image site on contralateral leg, open bar). The latter were not different in skin perfusion (n = 22).
profile of sensory changes elicited by UVB was then assessed in the next 12 subjects for only these parameters for 1–96 h after UVB (experiment 2). The time course of hyperalgesia over 96 h was studied by measurements at 1, 2, 4, 8, 24, 32, 48, 72, and 96 h after UVB irradiation. The QST parameters tested in experiment 2 that were significantly altered in the interim analysis of experiment 1 were heat pain threshold (HPT) and pressure pain threshold (PPT) in the UVB-irradiated area (primary zone), and mechanical pain threshold (MPT), stimulus–response function for mechanical pain sensitivity (MPS), and dynamic mechanical allodynia (DMA) in and adjacent to the UVB-irradiated area (primary and secondary zone, respectively; cf. [81]). Additionally, the area of hyperalgesia to pinprick and DMA was mapped. The full QST profile at 24 h was also assessed in these 12 subjects, and their data were merged with those of the first 10 subjects. Thus, for experiment 1, we obtained full QST profiles in 22 subjects altogether.

2.4. Testing procedure

The testing procedure included skin blood flow, assessment of the area of secondary hyperalgesia, and a test battery of QST in a fixed sequence starting with light mechanical stimuli and ending with thermal stimuli to minimize possible sensitization. QST was started in the area of secondary hyperalgesia and completed before testing the respective control site; then the sunburn area and its contralateral control site were followed.

The capillary blood flow was measured by laser Doppler imaging (LDI) (moorLDI; Moor Instruments Ltd., Axminster, United Kingdom). This device scans with a 2-mW helium laser across the skin surface and registers the shifted frequency from the back-scattered light. The velocity of moving erythrocytes is thereby calculated, representing a relative measure of skin perfusion (laser Doppler flux = velocity × concentration of moving erythrocytes) visually presented as a 2-dimensional color-coded picture. The laser head was positioned 35 cm above the measurement site. The scan region was 11 × 11 cm. The images were analyzed by dedicated image-processing software (Moor Instruments Ltd.). The LDI blood flow response was calculated as the mean laser Doppler flux within a square of 2 × 2 cm that was centered in the respective sites. This variable (expressed in arbitrary units, aU) served as readout for inflammation intensity.

2.4.1. Mapping the area of secondary hyperalgesia and DMA

The area of secondary hyperalgesia adjacent to the UVB-irradiated site was mapped with a 256 mN pinprick stimulus along 8 lines providing the shape of an octagon [38]. This stimulus activates cutaneous nociceptors [35] and was always suprathreshold for pricking pain. Mapping of the hyperalgesia area started along the proximal–distal axis of the thigh at the most proximal site of the sunburn (R1 in Fig. 2) and more than 10 cm distant from the border of the erythema and advanced toward the erythema at 5 mm steps in >5 s intervals to preclude windup-like summation effects. The border of hyperalgesic areas was identified from an abrupt increase of pain to the pinprick and confirmed by testing at the next step inside the hyperalgesia area, and also by an abrupt decrease when moving outside of the hyperalgesia area. The transition from normal to hyperalgesic skin exhibited a sharp demarcation of the hyperalgesic area with a substantial step in painfulness at the border. Moreover, pain levels are relatively constant within the secondary hyperalgesia area, as demonstrated previously [46]. This way, the testing proceeded clockwise for all 8 spots. A typical example of a hyperalgesia mapping is shown in Fig. 2A. The 8 spots representing the border of the hyperalgesia area were marked with a pen, and the distance to the center of the sunburn was measured. The area of pinprick hyperalgesia was determined from these 8

Fig. 2. Hyperalgesia to pinprick stimuli (secondary hyperalgesia) and to light touch (DMA) at 24 h after UVB irradiation. (A) Typical example of mapping secondary hyperalgesia to pinprick stimuli. In the center of the picture, inflammatory erythema is visible, which was induced by UVB irradiation. Outside the inflamed area, hyperalgesia is mapped in 0.5 cm steps starting proximal (R1) and completed clockwise (R2–R8). The borders were identified by a steplike increase of pain sensitivity at the 8 spokes, forming an octagonal area of secondary hyperalgesia. (B) Typical example of hyperalgesia to pinprick stimuli in a subject at 24 h after UVB depicted as pain ratings in the areas of primary hyperalgesia (1 H) and secondary hyperalgesia (2 H) compared to normal skin (outside). The subject displayed prominent hyperalgesia to pinprick in the UVB area, but hyperalgesia was also present in adjacent skin compared to skin outside the zone of secondary hyperalgesia. The degree of hyperalgesia exhibits a sudden drop immediately outside of the UVB area, demonstrating the difference between primary and secondary hyperalgesia. (C) Radius of the areas of DMA (stippled bar) and hyperalgesia to pinprick (gray bar), which spread significantly beyond the border of the UVB-irradiated area (black bar, n = 22). *P < .01, **P < .0001 (paired t test).
distances by calculating the area as \( \pi \times \text{geometric mean of the 8 radii} \). To avoid bias between sessions (during experiment 2), the marks were removed immediately after completion of QST. Afterward, the area of DMA around the erythema was assessed by stroking the skin with a soft brush in the same fashion. The criterion for the occurrence of allodynia was the perception of a component of burning or pricking adding to the sensation of touch. Otherwise, the procedure and calculation of the area were the same as for the pinprick test.

2.4.2. Quantitative sensory testing

The QST battery complied to the protocol of the German Research Network on Neuropathic Pain (DFNS) [67,84,85]. In brief, this QST battery consisted of 7 tests measuring 13 parameters. The investigator performing QST was trained according to DFNS specifications [31]. The sequence of testing was fixed because it has been demonstrated that changing the order of testing may result in interaction effects [36,41]. Thus, random application of the sensory testing would have caused an (unwanted) increase of variability of data. In order to prevent these interaction effects, we used the fixed standard order of testing specified in the original instructions to the DFNS QST protocol.

2.4.2.1. Thermal detection thresholds, thermal pain thresholds, and paradoxical heat sensation to contact heat stimuli. Thermal thresholds for cold detection and warm detection as well as cold pain (CPT) and heat pain (HPT) were assessed by the method of limits with a TSA-II NeuroSensory Analyzer (Medoc, Israel) connected to a Peltier thermode (16 mm). It was placed on the thigh and secured with an elastic strap, just tight enough to ensure complete contact between thermode and skin. Baseline temperature and secured with an elastic strap, just tight enough to ensure complete contact between thermode and skin. Baseline temperature was 32°C, ascending rate was 1°C/s, return rate was 10°C/s, and the interval between the 2 measurements was 10 s. Detection was signaled by a button press, and thresholds were evaluated 3 times and averaged. Because thermoreceptors are primarily detectors of temperature change [24,51–53,59,89,97], thermal detection thresholds were calculated as difference in baseline temperature.

Additionally, the thermal sensory limen (the difference limen for alternating cold and warm stimuli) was tested by alternating warm and cold stimuli (3 each in an up–down manner), starting at a baseline temperature of 32°C with a rate of 1°C/s and immediately changing the direction of the temperature change when the subject pressed a button to signal detection (thermal sensory limen for alternating cold and warm stimuli). At any button press, the subject was instructed to report the quality of the detected thermal stimulus.

When cold and warm stimuli are rapidly alternated, mild, cold stimuli may be misperceived as hot or burning pain—that is, as paradoxical heat sensations (PHS). PHS, or the erroneous perception of cold stimuli as hot or burning pain, was determined during the thermal sensory limen procedure by counting the number of incorrect responses to the 3 cold stimuli. Nociceptors are primarily detectors of noxious temperatures [89,97,103]. Thus, thermal pain thresholds are given as absolute values and not as difference from baseline temperature.

2.4.2.2. Mechanical detection threshold to von Frey hairs. Mechanical detection threshold was assessed by using a standardized set of modified von Frey hairs (Optihair; Marstock Nervtest, Marburg, Germany) exerting forces between 0.25 and 512 mN (rounded tip, 0.5 mm in diameter). Thresholds were determined by the method of limits with the geometric mean of a series of 5 ascending and descending stimulus intensities.

2.4.2.3. MPT to pinprick stimuli. MPT was tested with a set of 7 custom-made weighted pinprick stimuli (The PinPrick; MRC Systems, Heidelberg, Germany) with fixed stimulus intensities (8, 16, 32, 64, 128, 256, and 512 mN) and cylindrical tips 0.25 mm in diameter. Painful stimuli were to be identified by the appearance of a sharp or pricking component. Thresholds were determined by the method of limits with the geometric mean of a series of 5 ascending and descending stimulus intensities.

2.4.2.4. MPS for pinprick stimuli and DMA to light tactile stimuli. A stimulus–response function for MPS using the same 7 weighted pinprick stimuli as for MPT was determined by means of a numerical rating scale (NRS, ranging 0–100). The same was done to determine DMA with light touch of a cotton wisp (3 mN), a cotton wool tip (100 mN), and a brush (SENSELab-Brush 05; Somedic, Hörby, Sweden; 200–400 mN). Five series of all 10 stimuli were applied in a pseudorandomized order. MPS was calculated as the geometric mean of all pain ratings for pinprick (ie, static) stimuli and DMA as the geometric mean of all light tactile (ie, dynamic) stimuli by using log-transformed data. Additionally, an average stimulus–response function of the respective stimuli was calculated.

2.4.2.5. Windup ratio for repetitive pinprick stimuli. The magnitude of perceived pain to 5 series of 10 pinprick stimuli (256 mN, repeated at a 1/s rate) was compared to single pinprick stimuli. Volunteers were asked to rate pain after the single stimulus and at the end of the train with a NRS (0–100). The mean pain rating of trains divided by the mean pain rating to the single stimulus was calculated as windup ratio.

2.4.2.6. Vibration detection threshold. The vibration detection threshold (VDT) was assessed with a Rydel-Seiffer tuning fork (64 Hz, 8/8 scale) in 3 series of descending stimulus intensities. The tuning fork was excited to give a clearly suprathreshold stimulus at the resonance frequency. The amplitude of the vibration then subsides slowly, and eventually the amplitudes become too low to be identified (that is, the vibration perception “disappears”). At this point, the level of excision is read from the calibrated scale of the tuning fork and provides the detection threshold for vibration when the stimulus drops below threshold (thus, VDT is called a disappearance threshold).

2.4.2.7. Pressure pain threshold. The PPT was determined with a manual pressure algometer (Wagner Instruments, Greenwich, CT) with a contact area of 1 cm². The algometer was put on the skin surface, and pressure was slowly increased at a ramp rate of 50 kPa/s until pain threshold was reached. PPT assessment was repeated 3 times and calculated as the geometric mean of the 3 assessments.

2.5. Statistical analysis

As in previous studies, most values, with the exception of CPT, HPT, VDT, and PHS, were transformed into decadic logarithm for theoretical and empirical reasons; according to Stevens power law, all psychophysical data should be log transformed, and we have empirically found that this transformation achieves a secondary normal distribution for most QST parameters [84,85]. Although the theoretical rules of psychophysics also apply to thermal pain (CPT, HPT) and vibration (VDT), the metric of determining these thresholds in our QST protocol lacks a rational choice of zero. Thus, log transformation may apply theoretically for CPT, HPT, and VDT but cannot be applied for practical reasons. To avoid the loss of zero values in the pain ratings, a small constant (0.1) was added to all pain ratings before log transformation [65].
All data are presented as mean and standard error of the mean (mean ± SEM). For this purpose, data of log transformed QST parameters were retransformed to values representing the original unit of each test. The respective parameters were compared by paired t tests, except for pain to light touch (DMA), for which no normal distribution was achieved by any transformation, and thus was tested by nonparametric paired testing (Friedman ANOVA). Finally, parameters of the test sites were normalized to the control site by calculating the z transform: \( z = (\text{value}_{\text{test site}} - \text{mean}_{\text{control sites}})/\text{SD}_{\text{control sites}} \). The z scores indicate how far and in what direction the QST parameters deviated from control skin. Z values above 0 indicate a gain of function (more sensitive). P values of <.05 were considered statistically significant.

3. Results

All 22 volunteers of experiments 1 and 2 completed the study. None of the subjects experienced any kind of spontaneous pain or pain-related sensation during or at any other time after UVB irradiation. At 24 h after irradiation, an erythema of the skin (first-degree burn) was visible in all subjects. In none of the subjects did we observe a second-degree burn. This erythema was strictly confined to the irradiated area, and no signs of an axon reflex flare were observed. A typical example of a sunburn erythema and the respective blood flow measured by LDI is shown in Fig. 1A and B. Quantitative estimates of capillary blood flow by LDI confirmed that blood flow was significantly increased in the sunburn area to 412 ± 44 aU compared to the contralateral control skin (69 ± 7 aU, \( \text{mean} \pm \text{SEM} \)). For this purpose, data of log transformed QST parameter estimates of capillary blood flow by LDI confirmed that blood flow was significantly increased in the sunburn area to 412 ± 44 aU compared to the contralateral control skin (69 ± 7 aU, \( \text{mean} \pm \text{SEM} \)). In contrast, no significant change of blood flow was observed in the area surrounding the sunburn (71 ± 6 aU, \( \text{mean} \pm \text{SEM} \)).

### 3.1. Pattern of sensory changes (experiment 1)

Large areas of pinprick hyperalgesia surrounding the sunburn (71 ± 6 aU, \( \text{mean} \pm \text{SEM} \)) were present in all subjects (mean area, 218 ± 32 cm\(^2\)). A typical example is shown in Fig. 2A. Average radius was 77.4 ± 6.7 mm—that is, hyperalgesia spread significantly beyond the outer diameter of the UVB irradiation by 52.4 ± 6.7 mm (\( P < .001; \text{Fig. 2C} \)). Areas of DMA were smaller. They occurred in the majority of subjects, but not in all (15 of 22; mean area: 37 ± 6 cm\(^2\)). Lateral spread beyond the outer margin of the UVB irradiation was small (7.5 ± 2.4 mm; \( \text{Fig. 2C} \)) but nevertheless significant (\( P < .01; \text{Fig. 2G} \)). The lateral spread of secondary hyperalgesia and DMA were significantly correlated (\( r = 0.64, P < .001 \)).

Table 1 lists the responses to different somatosensory stimuli in and around the UVB sunburn compared to control skin at 24 h after UVB challenge.

### 3.1.1. Pattern of sensory changes in the primary area (UVB irradiated)

In the primary area of the sunburn, there were no significant changes in nonnociceptive modalities except for a mild hyperesthesia to cold stimulation (cold detection threshold, \( \Delta T = 3.4^\circ \text{C} \) vs 4.8°C in the control area; \( P < .01 \)). In contrast, significant hyperalgesia was present in the sunburn area to all tested pain modalities (UVB compared to mirror-image control side; Table 1): CPT increased by 4.6°C (CPT, 19.9 ± 1.7°C vs 15.3 ± 2.0°C, \( P < .05 \)), HPT dropped by 6.0°C (HPT, 38.6 ± 0.4°C vs 44.6 ± 0.7°C, \( P < .001 \)), MPT dropped by 72% compared to control value (MPT, 19 mN vs 66 mN; \( P < .001 \)), and PPT dropped by 31% compared to control value (PPT, 258 kPa vs 374 kPa; \( P < .001 \)). Pain rating to pinprick stimuli increased to 298% of control value (MPS, 2.02 NRS vs 0.68 NRS; \( P < .001 \)). In the sunburn area, pinprick hyperalgesia was present in all subjects and highly significant at any stimulus force; for example, pain ratings at the highest force of 512 mN were increased from 5.5 to 15 NRS (\( \text{Fig. 3A, right} \)). Moreover, DMA was present in 15 of 22 subjects. Although DMA was relatively mild with low pain ratings (\( \text{Fig. 3A, left} \); \( \text{Fig. 4B} \)), it was nevertheless highly significant (\( P < .001 \) across all tactile stimuli, and at least \( P < .005 \) for every tactile stimulus type, when tested separately).

### Table 1

<table>
<thead>
<tr>
<th>QST parameter</th>
<th>Description</th>
<th>Stats on...</th>
<th>Primary hyperalgesia (UVB area)</th>
<th>Secondary hyperalgesia</th>
<th>Contralateral control</th>
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<td></td>
<td></td>
<td></td>
<td>Raw data</td>
<td>Log data</td>
<td>Raw data</td>
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<td>Cold detection threshold</td>
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<td>0.527 ± 0.048**</td>
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<td>Thermal sensory limen</td>
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<td>Cold pain threshold</td>
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<td>Heat pain threshold</td>
<td>Raw</td>
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<td>MPT</td>
<td>Mechanical pain threshold</td>
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<td>MPS</td>
<td>Mechanical pain sensitivity</td>
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<td>MDT</td>
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<td>VDT</td>
<td>Vibrations detection threshold</td>
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<td>PHS</td>
<td>Paradoxical heat sensation</td>
<td>Log</td>
<td>0.50 ± 0.21</td>
<td>0.50 ± 0.23</td>
<td>0.66 ± 0.19</td>
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</table>

QST, quantitative sensory testing; UVB, ultraviolet B; NRS, numerical rating scale (0–100).

* Statistics were done on either raw data (raw) or log-transformed data (log); data are presented as mean ± standard errors of the mean, \( n = 22 \).
* Parameters for primary and secondary hyperalgesias followed for time course according to interitem analysis after \( n = 10 \).
* Nonparametric test statistic (Friedman analysis of variance).

\( \cdot \) \( P < .05 \) (significant increases, paired t test vs normal skin on contralateral leg).
\( ** \) \( P < .01 \) (significant increases, paired t test vs normal skin on contralateral leg).
\( *** \) \( P < .001 \) (significant increases, paired t test vs normal skin on contralateral leg).
\( \cdot \) \( P < .05 \) (significant decreases, paired t test vs normal skin on contralateral leg).
\( ** \) \( P < .01 \) (significant decreases, paired t test vs normal skin on contralateral leg).
\( *** \) \( P < .001 \) (significant decreases, paired t test vs normal skin on contralateral leg).
in the zone of secondary hyperalgesia (at least significantly more pronounced when tested against the respective values at higher stimulus forces. CW, cotton wisp; QT, Q-tip; BR, brush.

Fig. 3. Hyperalgesia to light touch (DMA, left) and to pinprick stimuli (secondary hyperalgesia, right panels) at 24 h after UVB irradiation in UVB-irradiated skin (A) and in skin adjacent to the UVB area (B) compared to control skin (open circles): (A) After UVB, there is highly significant DMA (pain to all 3 types of light tactile stimuli used for testing) and hyperalgesia to pinprick (leftward/upward shift of stimulus response function to calibrated pinprick stimulation in the skin area that was irradiated by UVB. (B) DMA was also present in skin adjacent to the UVB area (secondary hyperalgesia; marginal to brush, \( P < .001 \)). Pinprick testing (right panel) also exhibits a leftward/upward shift of the SR-function, statistically significant at higher stimulus forces. CW, cotton wisp; QT, Q-tip; BR, brush. *\( P < .05 \), **\( P < .01 \), ***\( P < .001 \) (paired \( t \) test).

All parameters indicating primary hyperalgesia were significantly more pronounced when tested against the respective values in the zone of secondary hyperalgesia (at least \( P < .001 \) for HPT, PPT, and MPT, and for DMA, and \( P < .02 \) for cold pain). Notably, windup of pain sensation to repeated pinprick stimuli was not enhanced, and there was even a subtle but significant decrease in the UVB-irradiated area (\( P < .05 \); Table 1).

3.1.2. Pattern of sensory changes in the secondary area (adjacent to UVB irradiation)

Although sensory changes were much less pronounced in the area adjacent to the UVB sunburn, there were still gains in MPS that suggested secondary hyperalgesia (Table 1). There was a significant drop of DMA (pinprick stimuli) to 64% of control value (MPT, 43 mN vs 66 mN; \( P < .02 \)) and a corresponding increase in pain ratings to 130% of control value (MPS, 0.88 NRS vs 0.68 NRS; \( P < .02 \)), significant especially at higher stimulus forces. For example, pain ratings at the highest force of 512 mN were increased from 5.5 to 8.5 NRS (Fig. 3B, right). Moreover, there was significant DMA to tactile stimuli (DMA) adjacent to the UVB burn in 6 of 22 subjects (DMA, 0.32 NRS vs 0.10 NRS; \( P < .01 \) across all tactile stimuli; see Fig. 3B, left, and Fig. 4B). Changes in MPT and DMA were significantly correlated between primary and secondary hyperalgesia areas (\( r = 0.58, P < .01 \) and \( r = 0.73, P < .001 \), respectively). Thermal pain thresholds and PPT, as well as all nonnociceptive sensory thresholds, were not significantly altered in the secondary test area.

The sensory changes in the primary and secondary hyperalgesia areas at 24 h after UVB irradiation are summarized in standardized form in SD units of the control site (z profiles), thus allowing direct comparison of changes in all somatosensory modalities despite their different physical units of measurement (Fig. 4A). Hyperalgesia to pinprick stimuli was present in the primary and secondary areas. Sensitivity in MPT was approximately 3 and 1 SD above normal skin, respectively. Likewise, sensitivity in mechanical pain ratings (MPS) was approximately 1.5 and 0.4 standard deviations above normal skin, respectively. In contrast, thermal pain thresholds (cold and heat pain), as well as PPT, were only altered in the primary area (shifts approximately 0.5, 2.0, and 1.2 standard deviations above normal skin for CPT, HPT, and PPT, respectively). Moreover, there was significant allodynia in the primary and secondary areas, as already described, but there were no differences in the occurrence of PHS between hyperalgesic and normal skin areas (Fig. 4B).

3.2. Time course of UVB induced hyperalgesia (experiment 2)

The development and decay of sensory changes was followed in a subgroup of subjects (\( n = 12 \)) for 1 to 96 h after UVB irradiation in those QST pain parameters (HPT, PPT, MPT, MPS, DMA) that were significantly changed in an interim analysis (\( n = 10 \)) at the 24 h time point in experiment 1. The time courses of changes in cold detection and cold pain sensitivity were not followed because they were less prominent, and statistical significance of changes only became apparent in the full sample of 22 subjects.

3.2.1. Time courses of sensory changes in the primary area (UVB irradiated)

HPT and PPT were significantly changed only in the UVB area (Fig. 5). A trend toward heat hyperalgesia was already observed at the earliest time point of testing at 1 h after UVB, and it became significant at 2 h (HPT at 2 h, 40.7 ± 0.9°C vs 43.7 ± 1.0°C, \( P < .001 \) ). HPT dropped gradually to reach a maximal extent at 24 and 32 h after UVB (HPT at 32 h, 38.0 ± 0.4°C vs 44.8 ± 1.1°C, \( P < .001 \) ), and it returned slowly toward baseline thereafter. However, there was still substantial heat hyperalgesia at 96 h after UVB (HPT at 96 h, 41.7 ± 1.1°C vs 45.3 ± 1.0°C, \( P < .001 \); Fig. 5A). Hyperalgesia to blunt pressure was subtle during the first 8 h after UVB and reached significance only at 24 h with a maximal expression between 24 and 48 h (PPT at 32 h, 253 kPa vs 410 kPa; \( \log_{10} \) values, 2.613 ± 0.042 vs 2.402 ± 0.040 \( P < .001 \); Fig. 5B). Although still observed in some subjects, it was no longer significant at 96 h.

Testing MPT by calibrated pinpricks in the UVB-irradiated area revealed a prominent hyperalgesia already at the earliest time point of testing (approximately 30% drop in the pain threshold), which increased steadily to reach a maximal expression at 32 h after UVB. Thereafter, the mechanical hyperalgesia declined rapidly. However, it was still significant at the latest time point of testing at 96 h (Fig. 6B).

Subjects also rated the pain elicited by calibrated pinpricks (MPS) and light tactile stimulators (DMA). Like MPT, pain ratings also revealed a clear hyperalgesia with a pain increase of approximately 50% already at 1 h after UVB, which steadily increased to an approximate 5-fold painfulness compared to normal skin at 32 h. Thereafter the mechanical hyperalgesia declined but remained significant until 96 h (Fig. 7A).
Pain to light stroking of the skin with cotton wisps, Q-tips, or soft brushes (DMA), which was never observed in normal skin, was seen in some subjects at any time of testing in the UVB area. DMA was significantly present in the UVB-irradiated zone of primary hyperalgesia in the time window between 8 and 96 h, peaked at 32 h, and declined thereafter (Fig. 7B).

3.2.2. Time courses of sensory changes in the secondary area (adjacent to UVB irradiation)

MPT and MPS were significantly changed, and DMA was present in skin adjacent to the UVB burn (Figs. 6 and 7). Hyperalgesia to pinprick stimuli started very early and spread gradually beyond the outer margin of the UVB area. Lateral spreading was significant already at 4 h after UVB and reached peak values at 32 h (radius, 70.5 ± 9.7 mm, P < .001; Fig. 6A). Thereafter, the areas of hyperalgesia gradually shrank. However, secondary hyperalgesia was still significantly spread beyond the UVB area at 72 h and vanished at 96 h.

MPT tested at 15 mm outside of the UVB-irradiated area was also lowered in the skin adjacent to the UVB area. This secondary hyperalgesia waxed and waned during the first few hours and reached a peak at 32 h after UVB, and it stayed significant until

Fig. 4. Changes in perception of natural somatosensory stimuli in the UVB-irradiated area and in adjacent skin (QST profile). The magnitude of change is expressed in units of standard deviations in normal skin (z scores). A gain in sensitivity is depicted as positive values. (A) The QST profile in the sunburn area (black circles) exhibited cold hyperesthesia, and hyperalgesia to all nociceptive stimuli namely lowered thresholds for cold pain (CPT), heat pain (HPT), mechanical pain (MPT), pressure pain (PPT), and an increase in mechanical pain sensation (MPS). Pain summation tested as windup to mechanical pain stimuli (WUR) was not increased but significantly decreased. Skin adjacent to the sunburn (gray circles) exhibited only hyperalgesia to mechanical stimuli, namely a drop in threshold (MPT) and an increase in pain sensation (MPS). (B) DMA was present in the sunburn area, and also to a lesser degree in adjacent skin. Reported PHS was similar in and adjacent to the sunburn but did not differ from normal skin. Mean ± SEM (n = 22). *P < .05, **P < .01, ***P < .001 (paired t test), ††P < .01, †††P < .001 (Friedman ANOVA).

Fig. 5. Time courses of hyperalgesia to heat (A) and blunt pressure (B) in the UVB-irradiated skin area (primary hyperalgesia). (A) Repeated testing in normal skin exhibited a gradual increase in HPT, while in the UVB area it dropped significantly. Heat hyperalgesia peaked 24–32 h and slowly returned toward normal, but heat hyperalgesia was still highly significant at 96 h after UVB. (B) Repeated testing in normal skin exhibited also a subtle gradual increase in PPT, while they dropped significantly in the UVB area. The hyperalgesia to blunt pressure peaked 24–48 h and slowly returned toward normal thereafter. **P < .01, ***P < .001 (paired t test, n = 12).
96 h of observation (Fig. 6B). Pain ratings to pinprick stimuli revealed a similar time course and also peaked at 32 h. However, as estimated by pain ratings (MPS), secondary hyperalgesia to pinprick was lost at 72 and 96 h (Fig. 7A).

DMA was also present adjacent to the UVB area. Like secondary hyperalgesia to pinpricks, it peaked at 32 h and declined thereafter. While in the UVB-irradiated zone of primary hyperalgesia DMA was significantly present in the time window between 8 and 96 h, it reached significance in the adjacent secondary area only at its peak at 32 h (Fig. 7B).

4. Discussion

By using the comprehensive QST protocol of the DFNS [85], we confirmed pronounced heat and pinprick hyperalgesia in the sunburn area. Mild DMA, which was not present in all subjects, correlated strongly with pinprick hyperalgesia. Our new finding of hyperalgesia to blunt pressure and cold completed a pattern of generalized hyperalgesia to all stimulus modalities and suggest that sensory findings in complex regional pain syndrome or chemotherapy-induced neuropathic pain may be modeled...
experimentally by UVB irradiation [11,33]. Hyperesthesia to innocuous cooling suggested sensitization of cold-sensitive pathways.

UVB elicited large areas of secondary hyperalgesia to pinprick and a small rim of DMA extending beyond the primary zone. However, as in other cutaneous injury models, including capsaicin injections and high-frequency electrical stimulation, heat hyperalgesia did not extend beyond the primary zone [2,28,58,61,71,78,81,103]. Primary and secondary hyperalgesia developed rapidly in parallel within hours, peaked after 24–32 h, and lasted for 4 days and more.

4.1. Cutaneous inflammation and peripheral sensitization

UVB damages epidermal keratinocytes and fibroblast and activates dose-dependently various inflammatory processes and the cutaneous stress system in animals and humans [10,12,29,40,44,63,91,93,96]. Consequently, in the primary hyperalgesia area (ie, the area irradiated by UVB), inflammation is the prime candidate for eliciting hyperalgesia via peripheral sensitization of nociceptors [69,71,82,87,88].

Histopathologic changes documented in UVB-irradiated skin include dermal edema, endothelial swelling, mast cell degranulation, Langerhans cell depletion, and cellular infiltration with neutrophils and monocytes. Within hours, biochemical changes encompass release of histamine, kinins, and reactive oxygen species, sequential release of cyclooxygenase, mainly COX2 and lipoxygenase-derived arachidonic acid products inducing TNF-α release of cyclooxygenase, mainly COX2 and lipoxygenase-derived arachidonic acid products inducing TNF-α, and degradation of inhibitory IκB and down-regulation of multiple genes interpreted as enhanced energy production and translation, and suppressed transcription, differentiation, and transport [1,27,102]. At 24 h, anti-inflammatory cytokines (interleukin 4, interleukin 10) are up-regulated, while sensitivity of the NGF receptor trkA is enhanced by irreversible inactivation of tyrosin phosphatases, and there is a close correspondence of this pattern in animal and human skin [4,9,15,20,27,34,72,76,79,80,83,87,98]. UVB activates various immediate early genes (c-fos, c-jun) and kinases, primarily the JNK/SAPK MAP kinases [5,50,55]. Gene expression profiles in human keratinocytes to broadband UVB revealed up- and down-regulation of multiple genes interpreted as enhanced energy production and translation, and suppressed transcription, differentiation, and transport [1,27,102].

4.2. Primary hyperalgesia and peripheral sensitization

In UVB-irradiated skin, pain sensitivity to all modalities was significantly increased vs contralateral normal and adjacent nonirradiated skin, and in the same area, capillary blood flow was 6-fold increased and sharply demarcated from the immediate vicinity. Whereas heat hyperalgesia, mechanical hyperalgesia, and DMA are well known in both rats and humans [25,40], cold hyperalgesia was previously only described in rat [25], but had never been tested or published in humans. We believe that the significant cold hyperalgesia is a relevant novel finding because cold hyperalgesia also occurs in several neuropathic pain conditions—in particular, in some chemotherapy-induced neuropathic pain [11,68] and in complex regional pain syndrome—in particular, in some mixed chemotherapy-induced neuropathic pain [11,68] and in complex regional pain syndrome, which is interestingly initiated by an inflammatory event [33]. However, cold hyperalgesia has generally been elusive in human experimental models [57], where it was only significant in the topical menthol model [106], adding UVB irradiation to the list of cold hyperalgesia models. Hyperalgesia to blunt pressure, which is the most pronounced positive sensory sign in complex regional pain syndrome and nerve-injury-related neuropathic pain in humans [33], was also pronounced and long-lasting in the UVB model. Thus, our data indicate that the UVB model is relevant to 2 additional and common clinical pain syndromes.

UVB irradiation sensitizes rat polymodal nociceptors to heat, with a time course similar to that of heat hyperalgesia. Sensitization to heat seems highly conserved and occurs even in drosophila via TNF-α and downstream sensitization of TRP (transient response potential) channels [8]. Consistent with sensory changes being primarily mediated by inflammatory peripheral sensitization [13,27], nonsteroidal anti-inflammatory drugs reduced UVB-induced thermal hyperalgesia in rats and humans [12,54,95,99,100,105,107], and a selective TRPV1 antagonist abrogated UVB-induced heat hyperalgesia [18]. In contrast, opioids had marginal effects even at high concentration [75].

Data on UVB sensitization of cold-sensitive primary afferents are missing in the literature. Their potential mechanisms can thus only be inferred from other pain models. On the basis of recent electrophysiological and behavioral data, we propose that cold hyperalgesia after UVB may be mediated by sensitization of the irritant receptor TRPA1 coexpressed with TRPV1 on polymodal C-nociceptors [22,43,73]. The additional novel finding of UVB-induced cold hyperesthesia, which was never tested and thus never found before, is likely not mediated by the same mechanism because the TRPA1 receptor is involved in nonnoxious cold perception. We propose that sensitization of the menthol-sensitive cold receptor TRPM8 may be the relevant membrane process behind UVB-induced cold hyperesthesia [89,106], suggesting the interesting hypothesis that UVB may modify members of several TRP channel families.

Primary hyperalgesia developed rapidly after UVB irradiation (to pinprick within 1 h, to heat within 2 h). NGF injection induces a similar hyperalgesia as UVB irradiation, but with a much slower time course [86]. Likewise, the chemokine CXCL5 sensitizes to mechanical stimuli at >24 h [27], matching hyperalgesia to blunt pressure. However, it did not match pinprick hyperalgesia, which is more likely mediated by the rapamycin-sensitive mTOR pathway [45,49]. Interestingly, there is little evidence for sensitization of mechanically sensitive nociceptors by UVB. A comprehensive study [14] found that mechanical sensitivity of nociceptors remained either unchanged (C-nociceptors) or even reduced (Aδ nociceptors). Only one nociceptor species, namely heat-insensitive C-nociceptors, enhanced their mechanical responses [14]. TNF-α may be central because it mimicked UVB-induced peripheral and central sensitization of rapid onset (<1 h), activation of local COX and p38 MAP kinase, spinal Akt phosphorylation, and TRPV1 and GluR1 trafficking [19,21,50,77,94,109].

4.3. Secondary hyperalgesia and central sensitization

There is consensus that UVB irradiation elicits pronounced primary hyperalgesia. However, the occurrence of secondary hyperalgesia, as found in other painful injury models [13,56,60,66,92,103], is controversial. Similar to previous human studies [38,39,100] and a recent rat study [26], we found secondary pinprick hyperalgesia (lower thresholds, increased pain ratings) and DMA, whereas others failed in the same model, probably as a result of different stimulation techniques [12,13,27]. Their differences in mean pinprick thresholds between normal skin and skin adjacent to UVB irradiation were similar to our study but did not reach statistical significance, probably as a result of a lack of power. In contrast, their von Frey hair used for mapping (10 g) was too soft to become painful even in hyperalgesic skin [65]. By using suprathreshold stimuli such as 150 g von Frey hair [38,39] or 128 mN pinprick (this study) and abrupt pain increase as criteria, we found that secondary
hyperalgesia to pinprick extended more than 50 mm beyond the UVB-irradiated area.

4.4. Central sensitization without pain?

Broadband ultraviolet elicited spontaneous activity in polymodal C-nociceptors after 30 min [3,101] and enhanced background discharge in dorsal horn neurons [104]. UVB, however, induced neither nociceptor excitation nor spontaneous pain in humans or animals [14,27,38,44].

The absence of evidence for UVB-induced ongoing pain creates the apparent paradox of hyperalgesia of central origin without nociceptive input. The absence of pain, however, is not conclusive evidence of absence of nociceptive-input, low-level, noxious heat-induced secondary hyperalgesia and DMA before reaching HPT [17]. Also, inflammatory low-frequency input can sensitize spinal nociceptive neurons [47]. We suggest that low-frequency nociceptive input—below the threshold for conscious pain perception—sensitizes central nociceptive neurons in the UVB model.

4.5. Technical considerations

Secondary hyperalgesia ran in parallel with primary hyperalgesia. In the absence of UVB-induced nociceptor activation, sensitization in the primary area facilitates activation by physically very weak thermal stimuli or touching the area [49,82]. Thus, sensory testing may have been a source of nociceptor excitation, indirectly causing central sensitization and secondary hyperalgesia. We can dismiss this possibility for several reasons. First, we arranged the sequence of testing such that skin adjacent to UVB (ie, potential secondary hyperalgesia) was always tested first. Second, secondary hyperalgesia was already significant at 1 h after UVB when no prior testing had occurred. It was maximal at 24–32 h, when there was the longest delay to previous testing. Finally, it was equally pronounced in both subject cohorts, regardless of previous testing.

4.6. Conclusion

UVB irradiation induced primary hyperalgesia generalized across all pain modalities (heat, cold, blunt pressure, pinprick) mainly by sensitizing peripheral nociceptors. Its time course suggests a key role for TNF-α. UVB irradiation also elicited a moderate degree of secondary hyperalgesia to pinprick and DMA to tactile stimuli in adjacent skin areas, collectively suggesting the presence of central sensitization. In aggregate, the UVB sunburn model is a translational human model for mechanisms of inflammatory pain facilitating preclinical stages of development of peripherally acting drugs potentially suppressing nociceptor excitation and/or sensitization. In contrast, the UVB model may be of limited value for development of centrally acting drugs suppressing synaptic transmission and/or facilitation.

Conflict of interest statement

The authors report no conflict of interest.

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