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Peripheral and central components of habituation of heat pain perception and evoked potentials in humans

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Abstract

For the neurophysiological examination of nociceptive pathways, contact-heat evoked potentials (contact-heat EPs) are elicited by repetitive brief noxious heat stimuli. Suppression of heat responses in primary nociceptive neurons during repetitive stimulation has been shown in animal models *in vivo* and *in vitro*. We now investigated whether heat pain and contact-heat EPs in humans display equivalent signs of habituation. Heat pain and EPs were elicited in 16 volunteers with a contact thermode (30 °C s^{-1}). Heat pulses at three intensities (pain threshold, moderate noxious and maximum available) were applied to the right forearm either by moving the thermode after each pulse to variable locations or when fixed to one location (inter-stimulus intervals 8–10 s). Contact-heat EPs consisted of an early negativity in temporal leads (N1), followed by a biphasic response at the vertex (N2-P2). Pain ratings and contact-heat EPs (N1 and N2-P2 components) displayed significant temperature dependence. N2-P2 correlated positively with ratings. With stimulation at variable locations, both measures slowly decreased with time constants τ of 2 min (ratings) and 12 min (EPs). With stimulation at a fixed location, habituation was much faster for both, ratings ($\tau = 10$ s) and EPs ($\tau = 33$ s). As a consequence, both measures were significantly reduced ($p < 0.005$) leading to a rightward shift of the stimulus–response function by 5 °C. In conclusion, human heat pain perception and contact-heat EPs display signs of rapid habituation when stimulation is restricted to a fixed location and thus, reflect fatigue of peripheral nociceptive neurons. Habituation within the central nervous system is slower and less pronounced.

Keywords: Nociceptor; Primary sensory neuron; Suppression; Fatigue; Contact-heat evoked potentials; Laser-evoked potentials

1. Introduction

Brief painful heat pulses, as generated by infrared lasers, have been established as a specific stimulus for nociceptive A_δ- and C-fiber afferents and are widely used to assess the function of nociceptive pathways in humans (Bromm and Treede, 1984; Plaghki and Mouraux, 2003). Laser-evoked potentials (LEPs) have been validated for the assessment of nociceptive deficits in patients with peripheral or central nervous system disorders (Bromm and Treede, 1991; Bromm and Lorenz, 1998; Treede et al., 2003; Cruccu et al., 2004).

Repeated stimulation may lead to habituation or sensitization, which alters the amplitude of averaged evoked potentials. Habituation is defined as response decrement resulting from repeated stimulation, whereas sensitization is defined as response increment resulting from novel, strong or noxious stimulation (Prescott, 1998). Although the terms habituation and sensitization are often used to describe processes within the central nervous system, peripheral mechanisms may contribute as described in the auditory and olfactory systems

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(Thornton and Coleman, 1975; Dalton, 2000). Peripheral sensitization in nociceptive nerve terminals is a well-known phenomenon. Following an injury, heat responses of nociceptive afferents are increased leading to heat hyperalgesia at the site of injury (Meyer and Campbell, 1981). However, repetitive stimulation of the receptive field of a nociceptor may also induce a reduction in discharges (''fatigue'') in both A_δ- and C-fiber nociceptive afferents (LaMotte and Campbell, 1978; Treede, 1995; Peng et al., 2003). A similar phenomenon (''tachyphylaxis'') has been found for the transduction process for noxious heat stimuli in dorsal root ganglion neurons and in the heat transduction channel TRPV1 (Tominaga et al., 1998; Schwarz et al., 2000).

Peripheral sensitization and fatigue/tachyphylaxis of nociceptive nerve endings can be avoided, if the stimulus location is shifted after each successive stimulus; this procedure is usually followed during recording of laser-evoked potentials (Bromm and Lorenz, 1998; Spiegel et al., 2000). However, even with this paradigm of variable stimulus location, LEPs and pain ratings exhibit a response decrement across stimulus repetitions, suggesting that central mechanisms contribute to habituation of human heat pain (Kazarians et al., 1995; Weiss et al., 1997; Valeriani et al., 2003).

The aim of the present study was to characterize the central and peripheral components of habituation of heat pain perception in human subjects. For this purpose, we analyzed pain ratings and evoked potentials in two paradigms: heat stimulation at a fixed location (peripheral and central mechanisms) and at variable locations (central mechanisms only). Because there is no active cooling, repetitive laser stimulation of a fixed skin site leads to local accumulation of heat, thus gradually increasing effective stimulus intensities (cf. Leandri et al., 2006). We therefore used a contact heat stimulator (Granovsky et al., 2005; Iannetti et al., 2006) that provides rapid heating with a thermofoil and active cooling by a Peltier element. Previous studies with such a device used either variable locations (Chen et al., 2001; Le Pera et al., 2002; Valeriani et al., 2002) or fixed locations (Granovsky et al., 2005; Iannetti et al., 2006) but no study has directly compared both paradigms.

2. Methods

2.1. Subjects

Experiments were performed in sixteen healthy subjects (seven female and nine male, age range 21–38 years, mean age 28.3 ± 5.0 years, mean ± standard deviation). Each subject was familiarized beforehand with the experimental procedures and gave written, informed consent. The study was approved by the Local Ethics Committee and complied with the Declaration of Helsinki. Participants were paid for attendance.

2.2. Heat stimulator

Noxious heat stimuli were delivered with a computerized thermal contact stimulator (contact-heat evoked potential stimulator, CHEPS; Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel). This device generates rapid temperature changes by use of a combination of a heating foil (27 mm in diameter; Fig. 1A) with a Peltier element for active back-cooling. Steep-ramped heat pulses were applied from a nominal baseline temperature of 32 °C using the highest heating rate available (nominally 70 °C s^{-1}) immediately followed by back-cooling (nominally −70 °C s^{-1}). Temperature data from two internal thermocouples (between heating foil and a thin plastic cover for electrical insulation; Fig. 1A) and two external miniature thermocouples (between the stimulator and the skin; IT-1E connected to BAT-12 microprobe thermometers, nominal time constant of thermocouple: 5 ms; Physitemp Instruments Inc., Clifton, NJ, USA) were recorded simultaneously with the EEG (see below, Section 2.5). The latter were used to assess the time course of the actual skin surface temperature. The higher one of the readings of the two external thermocouples was used, to account for inhomogeneities in the heating foil (see Fig. 1B–D). After each block of stimuli, the internal temperature destination signals of the device were stored, too. Thermographic control measurements of spatial distribution of thermal stimuli were done with an infrared thermocamera (Varioscan Typ 20.11; Jenoptik, Jena, Germany) at the maximum acquisition rate of about 0.5 Hz. Since the camera could not be triggered by the stimulator, only snapshots were taken to analyze spatial temperature distributions. Time courses were evaluated from the miniature thermocouples only.

2.3. Experimental protocol

Heat stimuli were applied to the volar side of the right forearm. The experimental protocol comprised four blocks of repeated heat stimulation. During each block, 30 heat pulses were delivered with an inter-stimulus interval varying between 8 and 10 s. Three different stimulus temperatures were given in randomized order within each block:

- The individual pain threshold temperature,
- The maximum peak temperature available (51 °C), and
- The mean temperature in between those (moderately noxious).

Thus, each block consisted of ten heat stimuli at the pain threshold, ten at the maximum stimulus temperature and ten moderately noxious heat stimuli. Pain threshold temperature was determined with heat ramps of 1 °C s^{-1} to avoid reaction time artefacts (Yarnitsky and Ochoa, 1990). Pain thresholds were determined as means from three consecutive stimulus repetitions.

Two different stimulus paradigms were tested in the present study:

(a) Fixed location of the thermode and
(b) Variable location.

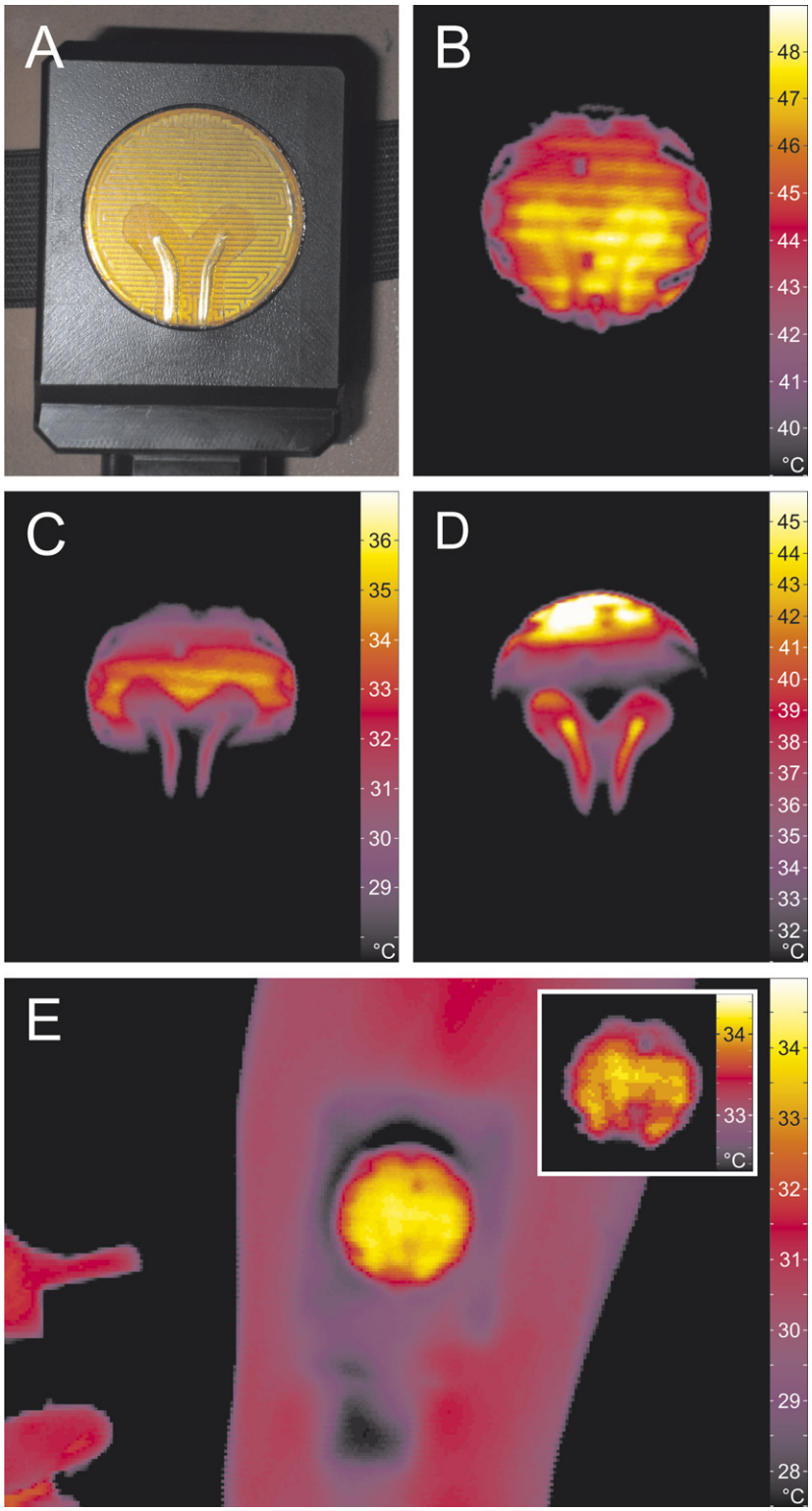

Fig. 1. Contact-heat evoked potential stimulator. (A) Picture of the contact-heat evoked potential stimulator with the round heating foil (diameter of 27 mm) embedded in a plastic cube. The heating foil comprises meander shaped conduction paths as well as two internal thermocouples fixed to the foil by a Y-shaped thin plastic cover. (B) When heating the device the conduction paths led to temperature inhomogeneities of more than 5 °C as revealed by thermography (range 40.5–50 °C, 46 ± 1.3 °C, mean ± standard deviation). Similar temperature differences were seen at the thermofoil while rapidly heating (C) and cooling (D). Here, temperature was lagging at the site of the internal thermocouples due to the insulation by the plastic cover. (E) Thermography of a volunteer's forearm immediately after removal of the thermode following stimulation with a maximum heat pulse revealed local heating of the skin by the stimulator as well as slight cooling of the surrounding area. Thermal inhomogeneities of the thermode were smoothed considerably as shown in the amplified inset (range 32.6–34.2 °C).

During the fixed location paradigm, the thermode was fixed to the volar side of the right forearm for the entire block using an elastic strip. For the variable location paradigm, the forearm was divided into five adjacent, but non-overlapping, skin districts each on the radial and ulnar side. The thermode was moved either clock-wise or counter-clock-wise (balanced across the two repetitive blocks) across these 10 sites. Each paradigm was applied twice; the sequence of paradigms was balanced across subjects (fixed-variable–variable-fixed, or variable-fixed–fixed-variable). The interval between repeated blocks was 5 min, thermode position was changed before the second fixed location paradigm. Thus, the full protocol obtained in each subject consisted of four experimental blocks, two with fixed and two with variable thermode location.

2.4. Pain ratings

Subjects rated the magnitude of pain sensation induced by each single heat pulse using a numerical rating scale (NRS) ranging from 0 (“non-painful”) to 100 (“most intense pain imaginable”). Thus, a rating of 0 indicated either a non-painful percept or that the stimulus was not detected at all.

2.5. EEG recording

Experiments were conducted in a light- and noise-reduced, electromagnetically shielded chamber kept at 24 °C. The participants were awake, sat relaxed in a reclining chair and faced a fixation point. EEG recording was triggered to the falling phase of the TTL-trigger pulse (14 ms duration) generated by the CHEPS at the onset of the heat pulse. Stimulus-triggered EEG traces (2.5-s duration including 0.5-s pre-stimulus interval) were recorded with Ag–AgCl electrodes (bandpass 0.16–500 Hz, sample rate 1000 Hz) from Fz, Cz, Pz (vs. linked earlobes) as well as T3 and T4 (vs. Fz, international 10-20-system) as described previously (Spiegel et al., 2000). A vertical electrooculogram (EOG) was recorded to monitor blink artefacts. Electrode impedance was maintained below 5 kΩ by cleaning the skin with a glass fiber eraser.

2.6. Data evaluation and statistics

For analysis of contact-heat EPs, EEG traces of each subject were averaged separately for the three stimulus temperatures and were evaluated offline. Sweeps contaminated with blink artefacts were discarded. N1 amplitude was evaluated as baseline-to-peak amplitude in the contralateral temporal lead (T3 vs. Fz). The vertex potential amplitude (N2-P2) was evaluated as peak-to-peak amplitude between the negative peak N2 and the positive peak P2 in Cz. Pain ratings were also averaged per stimulus temperature within a block. Data are presented as mean values ± standard error of the mean (SEM) if not otherwise indicated.

Temperatures simultaneously stored with EEG recording were analyzed using the averaged traces containing all sweeps of a subject at a given temperature in a given block. Voltage-signals of both BAT-12 thermometers were carefully and individually calibrated (precision of <0.2 °C) using a mercury thermometer. The thermal response time of those thermometers was determined in previous experiments (time constant of about 100 ms; Schwarz et al., 2000). Mean baseline temperature was calculated as mean temperatures during the 500 ms of the pre-stimulus interval, respectively. Mean peak temperatures and latencies were measured as well; real slopes of temperature increases under thermal load by the skin were calculated by dividing the maximal temperature change by the peak latency measured externally at the skin.

For statistical analyses of pain ratings, contact-heat EP amplitudes or latencies, two-way repeated measures analysis of variance (ANOVA; paradigm [2] × stimulus temperature [3]) and mixed-model three-way ANOVAs (gender [2] × paradigm [2] × stimulus temperature [3] or gender [2] × paradigm [2] × stimulus number [30]) were calculated. For post-hoc tests, the least significance differences (LSD) test was used. Additional group comparisons between subjects or paradigms were done using two-tailed t-tests for independent or dependent samples. Two-tailed $p < 0.05$ was considered statistically significant. Correlation analysis was performed using Pearson product moment correlation coefficients. For correlation of inter-individual data, raw values were normalized by setting ratings or contact-heat EPs obtained at the maximum stimulus intensity in the first block to 100%.

To determine the rates of habituation of subjective pain ratings we accounted for the different stimulus intensities applied in a block by normalizing all ratings individually to the mean pain rating obtained at a given temperature across both paradigms. After normalization, ratings in response to the respective stimulus in a block (i.e., stimulus 1–30) could be averaged across subjects directly. EEG sweeps were treated similarly, but since we did not do a single trial analysis, this resulted in one grand-average EP amplitude per stimulus number that was normalized to the mean EP amplitude obtained at that stimulus intensity across both paradigms. Therefore, statistical analysis of the rate of habituation was only done for the pain ratings data, since only grand-means of contact-heat EP-waveforms were available per stimulus number. Exponential decays were fitted bi- or mono-exponentially as appropriate, yielding either one or two time constants (τ):

$$F(x) = A_\infty + A_1 e^{-(x-x0)/\tau 1} + A_2 e^{-(x-x0)/\tau 2}$$

3. Results

3.1. Stimulus characteristics

The mean pain threshold for ramped contact-heat stimuli at a rate of 1 °C s^{-1} was 40.3 ± 0.7 °C (mean ± SEM, range 36–45 °C; $n = 16$), consistent with threshold temperatures for C-fiber nociceptors in man (mean ± SEM: 40.7 ± 0.4 °C; Weidner et al., 1999) and monkey (mean ± standard deviation: 41.0 ± 3.0 °C; Treede et al., 1995). Pain thresholds are essentially related to C-fiber nociceptors, whereas evoked potentials in the latency range studied here are related to A_δ-fiber nociceptors, which have higher thresholds (mean ± standard

deviation: 46.0 ± 2.9 °C; Treede et al., 1995). However, thresholds as low as 39.5 °C were reported for single A_δ-fiber nociceptors (Treede et al., 1998), and due to its size the thermode always covers the receptive fields of many nociceptors.

The maximum stimulus temperature was set to 51 °C in all experiments and the moderate noxious stimulus temperature between pain threshold and maximum was 45.8 ± 0.3 °C (43–48 °C; Table 1). The peak temperatures measured at the skin surface, however, were markedly lower than the nominal ones, and the peak latencies of the skin temperature changes were prolonged by about 50 ms as compared to the signal of the internal thermocouples. As a result, the real slopes of the temperature changes at the skin were reduced by a factor of 2–3 compared with the nominal ones (see Table 1 and Fig. 2).

In order to exclude that these differences were due to dampening of the external digital thermometer the stimulator was tested on human skin and on foam plastic covered with foil, i.e. with and without thermal load. Without thermal load, heat stimuli with nominal temperatures of 40, 46, and 51 °C induced peak temperatures of 42.0, 46.6, and 50.9 °C. With the thermal load of the human skin, peak temperatures were reduced to 39.7, 42.7, and 45.2 °C ($n = 10$, each). Thus, the differences between nominal and actual stimulus temperatures were due to thermal inertia of the skin.

3.2. Contact-heat evoked potentials and pain ratings

Fig. 2 demonstrates the contact-heat EPs in response to the maximum heat pulses in the variable location paradigm that led to pain ratings of 34 ± 7. In the majority of subjects, EEG recordings were variably contaminated with electrical artefacts that were obviously due to on- and off-pulses of the stimulator (Fig. 2, dotted lines; cf. Iannetti et al., 2006). These electrical artefacts did not affect quantitative determination of the main heat-evoked potential components N2-P2 that were observed at mean latencies of 358 ± 7.3 ms (N2) and 462 ± 8.5 ms (P2; $n = 16$, each). Since these artefacts, however, fell

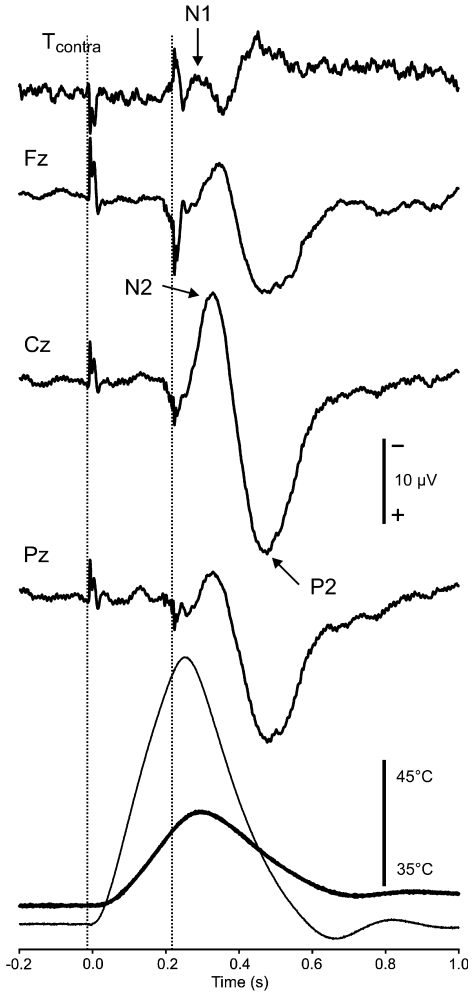

Fig. 2. Contact-heat evoked potentials. Heat-evoked potentials from the recording sites T_{contra} (referenced to Fz), Fz, Cz and Pz (referenced to linked earlobes). The contact-heat EPs comprised an early negativity (N1) that was best seen in the contralateral temporal lead (T_{contra}) as well as a biphasic component (N2-P2) with a maximum in the vertex lead Cz. Grand means across 16 subjects stimulated with noxious heat pulses of nominally 51 °C peak temperature applied to variable locations. Note the switching artefacts that occurred in 6 of the 16 subjects (dotted lines). The lowest traces represent the time course of the temperature signal from the internal thermocouples of the device (narrow line) and the resulting temperature change at the skin surface as measured with an external miniature thermocouple (bold line). The dotted lines indicate the heating interval of the thermofoil.

Table 1
Characterization of the rapid contact-heat stimuli

	Threshold temperature	Moderate temperature	Maximum temperature
Nominal settings			
Baseline temperature (°C)	32.0	32.0	32.0
Peak temperature (°C)	40.3 ± 0.7	45.8 ± 0.3	51.0 ± 0.0
Peak latencies (ms)	155 ± 5.2	199 ± 3.1	248 ± 2.6
Slope (°C s^{-1})	51.9 ± 2.63	68.9 ± 0.7	76.8 ± 0.9
External temperature measurements			
Baseline temperature (°C)	33.8 ± 0.1	33.8 ± 0.1	33.8 ± 0.1
Peak temperature (°C)	38.2 ± 0.3	40.0 ± 0.3	42.0 ± 0.3
Peak latencies (ms)	203 ± 5.3	246 ± 3.1	292 ± 2.7
Slope (°C s^{-1})	22.3 ± 1.3	26.2 ± 1.1	29.0 ± 1.1

Table 2
Mean contact-heat EPs and pain ratings: results of three-way ANOVAs

	F values	p values
N1 amplitudes (determined in 12 of 16 subjects)		
Paradigm	$F_{1,10} = 8.6$	$p < 0.05$
Temperature	$F_{2,20} = 8.6$	$p < 0.005$
Gender	$F_{1,10} = 0.03$	$p = 0.88$
Paradigm × temperature	$F_{2,20} = 2.6$	$p = 0.10$
N2-P2 amplitudes		
Paradigm	$F_{1,14} = 12.2$	$p < 0.005$
Temperature	$F_{2,28} = 78.1$	$p < 0.001$
Gender	$F_{1,14} = 1.2$	$p = 0.28$
Paradigm × temperature	$F_{2,28} = 8.4$	$p < 0.005$
Mean ratings		
Paradigm	$F_{1,14} = 18.3$	$p < 0.001$
Temperature	$F_{2,28} = 20.9$	$p < 0.001$
Gender	$F_{1,14} = 0.81$	$p = 0.38$
Paradigm × temperature	$F_{2,28} = 10.3$	$p < 0.001$
Normalized ratings		
Paradigm	$F_{1,14} = 77.4$	$p < 0.001$
Stimulus number	$F_{29,406} = 7.6$	$p < 0.001$
Gender	$F_{1,14} = 0.77$	$p = 0.40$
Paradigm × stimulus number	$F_{29,406} = 4.6$	$p < 0.001$

All other interaction terms were non-significant.

into the same time window as the early N1, this contact-heat EP component could not be determined in four subjects. Mean N1 latency was 295 ± 7.5 ms.

Amplitudes of the contact-heat EP-components N1 and N2-P2 indicated pronounced temperature dependence for both paradigms (Table 2 and Fig. 3A, B) and volunteers were able to discriminate stimulus intensities reliably (Table 2 and Fig. 3C). N2-P2 amplitude of the contact-heat EPs and pain ratings of individual subjects at the different stimulus temperatures and paradigms were positively correlated (r_{var}: 0.856 ± 0.03 [range 0.618–0.960]; r_{fix}: 0.868 ± 0.03 [range 0.510–0.976]; $n = 16$, each) indicating that the N2-P2 amplitude could predict the subjective painfulness of a contact-heat pulse. Inter-individual correlation analysis of normalized values revealed a weaker although still significant correlation of contact-heat EPs and N2-P2 amplitudes ($r = 0.688$, $p < 0.001$). These findings indicate that an increase in N2-P2 in a subject is associated with an increase in heat pain but the slope of this function differs between individuals.

3.3. Reduced heat evoked potentials and heat pain during repetitive stimulation of the same skin area

Contact-heat EP amplitudes of N1 and N2-P2 components were significantly smaller when contact-heat stimuli were applied to a fixed location than when the thermode was moved to variable locations between stimuli (Fig. 3A, B and Table 2) with the largest differences seen at the moderately noxious temperature (reduction by about 45%). Stimulation at a fixed location (Fig. 3, open circles) led to a rightward shift of the stimulus response function by nominally about 5 °C as compared to variable locations (Fig. 3, filled circles).

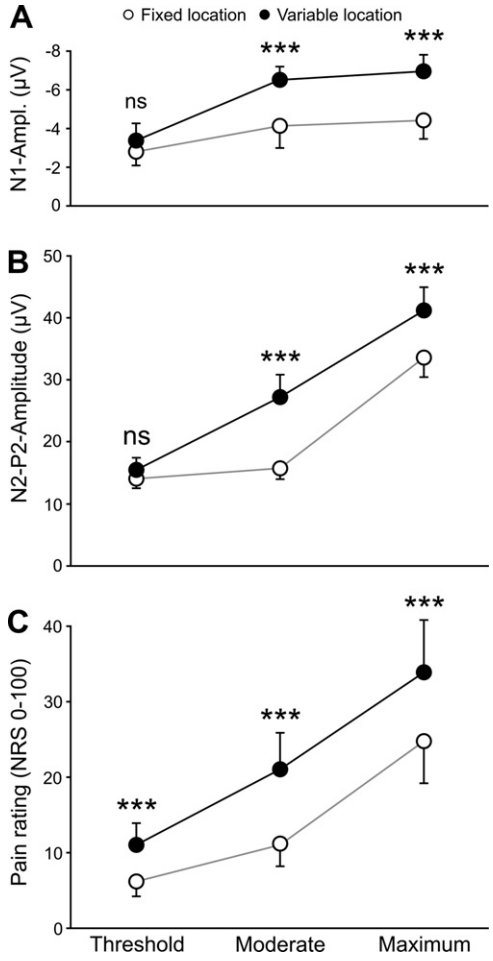

Fig. 3. Pain ratings and evoked potentials for constant and variable location. Amplitudes of the early contact-heat EP component N1 (A), the main contact-heat EP component N2-P2 (B) as well as of the subjective pain ratings (C) displayed pronounced temperature dependence in both paradigms as revealed by two-way ANOVA (see Table 2). However, contact-heat EPs (A and B) and ratings (C) were reduced in the paradigm "fixed location of the thermode" as compared to "variable location" (Table 2). NRS (0–100) 0, no pain; 100, most intense pain imaginable; mean ± SEM; $^{ns}p > 0.40$, $^{***}p < 0.001$ LSD post-hoc test.

Pain ratings largely paralleled these changes of the contact-heat EPs. Pain ratings were significantly lower at all three stimulus temperatures when the location of the thermode was not changed (Table 3 and Fig. 3C). Again, at moderate noxious stimulation pain ratings differed by a factor of approximately 2 between paradigms. Ratings at the moderate noxious temperature in the paradigm "fixed location" were similar to those in the paradigm "variable locations" stimulated at the pain threshold temperature, indicating a rightward shift of the stimulus response function by about 5 °C, too.

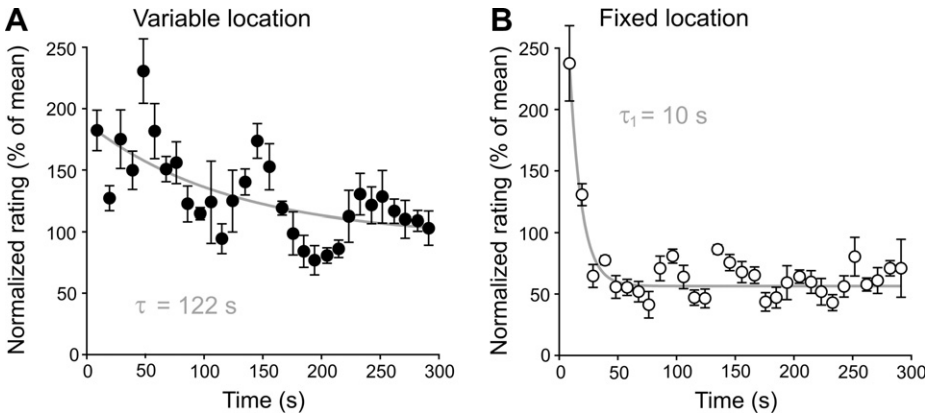

Fig. 4. Habituation of contact-heat pain is mainly due to peripheral fatigue. Normalized pain ratings (by dividing by the individual mean rating at the respective intensity) in response to the 30 contact-heat stimuli applied within the paradigms variable (A) and fixed location of the thermode (B). When the thermode was moved from stimulus to stimulus (A), ratings slowly decreased throughout the entire block with a time constant of about 120 s. (B) In contrast, when the thermode position was kept constant pain ratings decreased with a time constant of about 10 s during the first stimuli and remained almost completely constant thereafter. Mono- (A) and bi-exponential curve fittings (B) are given as solid lines, time constants τ are given as inserts.

3.4. Peripheral and central components of habituation

In order to determine time courses within runs, pain ratings and contact-heat EP-waveforms were normalized to the different noxious stimulus temperatures applied in a block. Normalized pain ratings displayed significant main effects for paradigm and for the stimulus repetition in a block as well as an interaction of those main effects (Table 2). In the paradigm "variable location" (Fig. 4A) the last three ratings were reduced by about 40% vs. the first one, whereas in the paradigm "fixed location" (Fig. 4B) the last three ratings were reduced by about 70%, which was a significantly greater response decrement ($p < 0.005$, paired t-test).

Most of that additional response decrement happened within the first three stimuli (Fig. 4B). Accordingly, a bi-exponential fit yielded a time constant for the initial decay of $\tau_1 = 10$ s, approximately the duration of one inter-stimulus interval. This small time constant τ_1 was followed by an extremely large value for τ_2 (nominal >75 days), indicating that – once established after few stimuli – no further habituation was seen. In contrast, pain ratings continuously decreased throughout the entire block when the identical sequences of heat stimuli were applied to variable locations. Bi-exponentially fitting revealed similar time constants τ_1 and τ_2, thus data were collapsed into a mono-exponential fit with a single time constant τ of 122 s (Fig. 4A). When analyzed after full-establishment of peripheral habituation, i.e. after omitting the responses to the first three heat pulses, NRS ratings were 5.9 ± 2.1, 10.1 ± 2.8 and 23.7 ± 5.5 in response to low, moderate and high heat pulses in the paradigm "fixed location" and 10.8 ± 2.9, 20.6 ± 4.8 and 33.8 ± 7.0 in the paradigm "variable location" (all $p < 0.01$ vs. fixed location, paired t-test). Thus, omitting the first three responses had only minor effects on the mean pain ratings (cf. Fig. 3C).

Across the initial three stimulus repetitions, contact-heat EPs decreased markedly when applied to a fixed location (Fig. 5B), but not in the paradigm variable location (Fig. 5A). Normalized contact-heat EP amplitudes decreased by about 50% during stimulation at a fixed location, the last three contact-heat EPs differed significantly from the first three ($p < 0.05$; Student's paired t-test). In contrast a non-significant decrease by about 10%, only, was observed when heat stimuli were applied to varying locations ($p = 0.94$). As a consequence, the last three contact-heat EP amplitudes differed significantly between paradigms ($p < 0.05$, paired t-test) but the first three did not ($p = 0.60$). Accordingly, a very slow and mono-exponential decay of the contact-heat EPs with time was observed in the paradigm "variable location" (τ of about 730 s; Fig. 5C) whereas during "fixed location", contact-heat EPs – as the ratings – bi-exponentially decreased with a small initial τ_1 (about 33 s) followed by an almost infinite τ_2 (Fig. 5D).

3.5. Habituation of heat pain does not display gender differences

Gender differences have been previously demonstrated for temporal summation of thermally (Fillingim et al., 1998) and mechanically induced pain (Sarlani and Greenspan, 2002). We therefore tested whether females and males differed with respect to habituation of heat pain. Female subjects displayed lower pain thresholds than males (39.4 ± 1.0, $n = 7$, vs. 40.9 ± 0.9, $n = 9$) but this slight difference was not significant ($p = 0.30$). Neither contact-heat EP amplitudes (N1, N2-P2) nor mean and normalized NRS ratings displayed an effect of

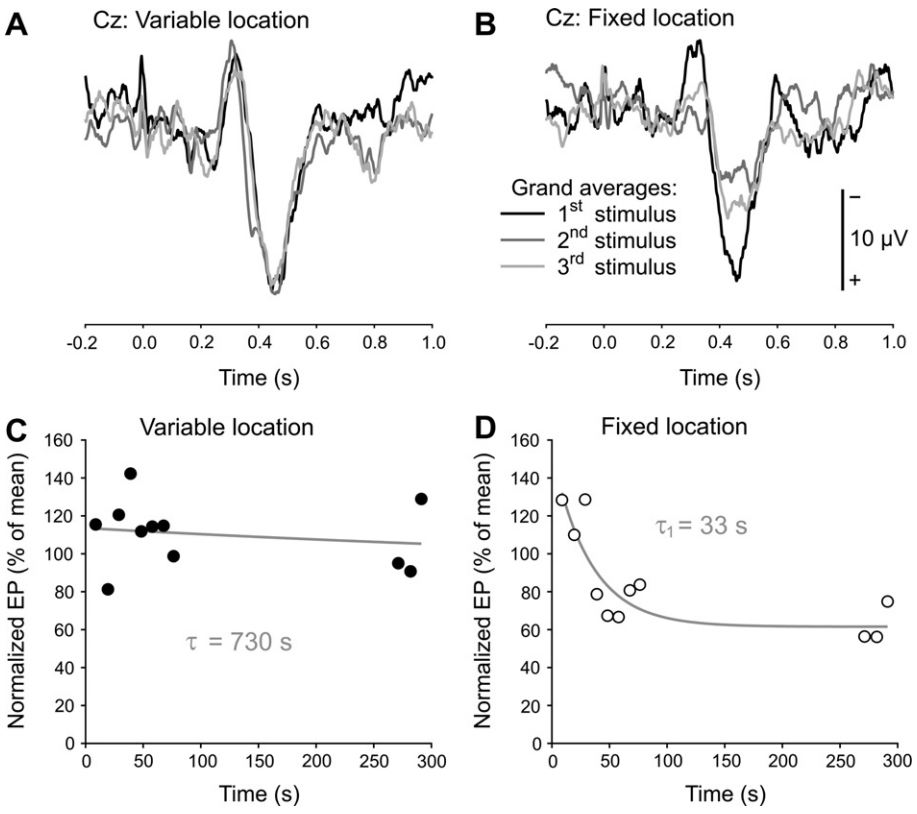

Fig. 5. Habituation of contact-heat evoked potentials is mainly due to peripheral fatigue. (A) Grand averages of the contact-heat EPs induced by the first three moderate noxious heat pulses obtained in the paradigms variable (A) and fixed location of the thermode (B). Whereas amplitudes of the N2-P2 component did not markedly change in the paradigm "variable" (A), N2-P2 gradually decreased when heat stimuli were applied to the same skin area (B). Normalized N2-P2 amplitudes (by dividing the grand averages obtained across subjects by the mean contact-heat EPs at the respective temperature) in response to the first 8 and last 3 contact-heat stimuli applied within the paradigm variable (C) and fixed location of the thermode (D). Similar to the pain ratings shown in Fig. 4, contact-heat EPs in the paradigm variable location slowly decreased throughout the entire block with a time constant τ of >10 min when the thermode was moved from stimulus to stimulus (C). When the same skin site was stimulated, contact-heat EPs decreased during the first stimuli (τ of about 33 s) independent of the stimulus temperature and remained constant thereafter (D). Mono- and bi-exponential fits as well as time constants τ are given as solid lines and inserts.

gender. None of the interaction terms including gender reached statistical significance (Table 2). Thus, no differences in habituation could be found between females and males in this study.

4. Discussion

This study has shown that contact-heat evoked vertex potentials (N2-P2) increase with stimulus intensity and are positively correlated with the concurrently evoked pain sensation. In addition, we observed an earlier negativity N1 that resembles the N1 of LEPs generated in the operculo-insular cortex (Kunde and Treede, 1993; García-Larrea et al., 2003). A mild degree of habituation was observed with time constants of 12 min for contact-heat EPs and 2 min for pain sensation, when each stimulus was applied to a separate skin site, as usually done with LEPs. However, when applied with the thermode fixed to one skin site – as usually done with the CHEPS device (Granovsky et al., 2005; Iannetti et al., 2006) – contact-heat EPs and pain sensation were markedly reduced, equivalent to a reduction in nominal stimulus temperature by 5 °C. This was due to much shorter time constants (33 s for EPs, 10 s for pain), which can be attributed to peripheral fatigue of primary nociceptive nerve terminals. Signs of sensitization were not observed, not even at the highest stimulus intensities available. In contrast to some previous studies (Fillingim et al., 1998; Sarlani and Greenspan, 2002) there were no gender differences regarding habituation of heat pain.

4.1. Habituation and sensitization

Repetitive stimulation can lead to a steady decrease in response magnitude (habituation) or to an increase (sensitization). Whereas in most sensory systems habituation dominates, sensitization is the prominent phenomenon in the nociceptive system (Perl, 1976; Woolf, 1983; Treede et al., 1992). Nevertheless, under certain conditions, habituation to repeated noxious heat stimulation has been observed (Price et al., 1977; Adriaensen

et al., 1984; Valeriani et al., 2003; Kleinböhl et al., 2006). These studies used brief stimuli of moderate intensity that do not induce tissue damage and hence avoid peripheral sensitization. In our study, the maximum temperature at the skin surface (42 °C) was well below the damage threshold, although the maximum nominal temperature (51 °C) would have suggested otherwise. We also avoided induction of central summation by using stimulus repetition rates below the critical windup frequency of 0.3 Hz (Herrero et al., 2000). Under these conditions, habituation prevails even in the nociceptive system.

4.2. Peripheral fatigue and central habituation

With fixed stimulus location, habituation of perceived pain intensity was nearly twice as pronounced (70% reduction vs. 40% reduction across 30 stimulus repetitions) and occurred ten times faster (τ of 10 s vs. 122 s) than with variable stimulus locations. Under these conditions, nociceptive input to the spinal cord progressively decreases due to peripheral fatigue of A_δ- and C-fiber nociceptors (LaMotte and Campbell, 1978; Treede et al., 1998; Peng et al., 2003). Nociceptive afferents exhibit a mixed static and dynamic response to heat stimulation; repeated heat stimuli suppress the dynamic response, but the static response remains relatively constant (Adriaensen et al., 1984; Treede, 1995). Peripheral fatigue occurs mainly across the first couple of stimuli. Tachyphylaxis of heat-evoked inward currents in nociceptive dorsal root ganglion neurons exhibits a similar time course (Schwarz et al., 2000). We now demonstrated a similarly rapid decrement of heat-evoked potentials and associated pain sensation with fixed stimulus location. These findings demonstrate that peripheral fatigue is reflected in the activity of nociceptive brain regions in humans and is relevant for perceived pain intensity. A potential limitation of this interpretation is that dishabituation may have been induced by moving the thermode. Therefore, the difference between fixed and variable stimulus location may reflect peripheral fatigue and putative differences in central habituation. It is, however, impossible to separate a central effect of spatial mismatch from that of stimulating different peripheral receptive fields. Thus, these putative differences cannot be quantified experimentally. Nevertheless, the degree of peripheral fatigue observed in primary nociceptors *in vitro* (Schwarz et al., 2000) and *in vivo* (Peng et al., 2003) is quantitatively remarkably similar to that deduced here.

When probe location was changed between successive heat pulses, habituation was of smaller magnitude but still present (Spiegel et al., 2000; Kleinböhl et al., 2006). Therefore, central synaptic transmission in the nociceptive system contributes to overall habituation. Transmission at the first relay station in the spinal cord is usually assumed to be reliable, and habituation is mostly seen in non-nociceptive neurons (Egger, 1978). However, the spinal flexor withdrawal reflex is one of the classical models of habituation (Groves et al., 1970), and the synaptic input of A_δ-fibers to lamina I spinal neurons was found to decrease by 70% with a time constant of 100 s at 1-Hz stimulation frequency (Sandkühler et al., 1997). Activity within several brain areas correlated with habituation of heat pain, but this study assessed primarily peripheral components of habituation (Becerra et al., 1999). Whether additional synapses within thalamus and/or cortex further promote central habituation of heat pain is unknown so far.

Our finding that ratings habituated more than contact-heat EPs might suggest a major contribution by brain areas upstream of the generators of EPs in primary and secondary somatosensory cortex, insula and mid-cingulate cortex (García-Larrea et al., 2003). Such pain-reducing activities have been reported for the dorsolateral prefrontal cortex (Lorenz et al., 2003; Schmahl et al., 2006). Other authors, however, have reported a similar decrement for ratings and for evoked potentials, suggesting that most processes of habituation may occur downstream of the mid-cingulate cortex (Bromm and Scharein, 1982; Weiss et al., 1997; De Tommaso et al., 2005).

4.3. Biological effects of rapid heating

Whenever skin temperature is to be changed, energy has to be transferred between thermode and tissue. Thermal conductivity of the skin is relatively poor (thermal diffusivity α 0.0005–0.0015 cm^2/s; Hensel, 1950), leading to two effects: temperature change within the skin is slower than within the thermode, and peak temperatures are lower. These differences become more pronounced, as the rate of temperature change increases and the stimulus duration decreases. Our previous studies using multi-layer Peltier devices (Wilcox and Giesler, 1984) demonstrated a small discrepancy at 1 °C/s and a major discrepancy of more than 50% at 10 °C/s nominal rate of temperature change (Tillman et al., 1995a; Magerl and Treede, 1996). In the present study, at a nominal rate of change of 70 °C/s the real slope of skin temperature change amounted to about 40% of that at the stimulator surface. Thus, modern heat foil stimulators have improved contact heating by about one order of magnitude.

For comparison with published literature, we used a slow ramp rate (1 °C/s) to determine heat pain thresholds. Pain thresholds at 70 °C/s (nominal) ramp rate may be quite different, and the above considerations suggest that they should be higher. But the opposite has been observed (Yarnitsky and Ochoa, 1990; Tillman et al., 1995b), and hence our stimuli with peak temperatures of 38–42 °C were likely above pain threshold at

that fast ramp rate. This interpretation is confirmed by our rating data, which for the lowest stimulus intensity were 5.9/100. The somewhat paradoxical rate dependence of heat pain thresholds (lower threshold at faster ramp rate) has been explained by a similar rate dependence of the peripheral transduction process (Tillman et al., 1995b; Greffrath et al., 2002).

4.4. Technical considerations of the contact-heat stimulator

Feedback-controlled contact-heat stimulators with built-in thermocouples offer the advantage that skin temperature can be controlled, a useful property for sensory physiology. Due to the thermal inertia of the skin, however, a precise control of skin temperature is only possible in the static case or for very slow changes in skin temperature (e.g. 0.1 °C/s). At the rapid heating rates needed for heat-evoked potentials, skin temperature will always lag behind stimulator temperature. For safety reasons, one cannot use the externally measured skin temperature for feedback control of the device. Therefore, skin temperatures should always be monitored during rapid heating experiments.

One might conclude that the fixed-location paradigm is preferable for brain imaging studies, because following the first couple of stimuli there is a long period of stable perceptual and cerebral responses. The fixed location paradigm, however, falls short of an ideal pain experiment, because peripheral nociceptor fatigue reduces the effective stimulus temperature by 5 °C. Clinical use of contact-heat EPs requires that they can be elicited in all healthy subjects; as with LEPs, absence or a decrease in amplitude could then be counted as pathological findings (cf. Spiegel et al., 2000). This aim was only reached at the highest temperature (nominally 51 °C, measured 42 °C) applied at variable skin locations. The need to move the thermode after each stimulus, which is done much more easily with a laser, offsets some of the advantages of a contact-heat stimulator. Further experimental use of contact-heat EPs is hampered at present, because the early component N1 is inconstant due to stimulus artefacts (Fig. 2 and Iannetti et al., 2006).

5. Conclusions

The active cooling of the contact-heat stimulator enabled the repetitive stimulation of a fixed skin location without causing burn injuries or sensitization. Under these conditions, fatigue of primary nociceptive afferents led to a rapid habituation of contact-heat EPs and perceived pain sensation. Thus, peripheral fatigue is reflected in the activity of nociceptive brain regions in humans and is relevant for perceived pain intensity, particularly for brief and non-sensitizing stimuli.

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