Psychophysical Evidence for Long-Term Potentiation of C-Fiber and Aδ-Fiber Pathways in Humans by Analysis of Pain Descriptors

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Hansen N, Klein T, Magerl W, and Treed R-D. Psychophysical evidence for long-term potentiation of C-fiber and Aδ-fiber pathways in humans by analysis of pain descriptors. J Neurophysiol 97: 2559–2563, 2007. First published January 10, 2007; doi:10.1152/jn.01125.2006. Long-term potentiation of human pain perception (nociceptive LTP) to single electrical test stimuli was induced by high-frequency stimulation (HFS) of cutaneous nociceptive afferents. Numerical pain ratings and a list of sensory pain descriptors disclosed the same magnitude of nociceptive LTP (23% increase for >60 min, P < 0.001), whereas affective pain descriptors were not significantly enhanced. Factor analysis of the sensory pain descriptors showed that facilitation was restricted to two factors characterized by hot and burning (+41%) and piercing and stinging (+21%, both P < 0.01), whereas a factor represented by throbbing and beating was not significantly increased (+9%, P = 0.47). The increased perception of the burning pain quality for >1 h after HFS is interpreted as a LTP-like facilitation of the conditioned cutaneous C-fiber pathway. Additionally, the increase of the stinging pain quality supplied evidence for facilitation of a sharpness-sensitive Aδ-fiber pathway.

INTRODUCTION

Long-term potentiation (LTP) of synaptic efficacy, an ubiquitous mechanism of learning and memory formation, is believed to play also a major role in central sensitization of the nociceptive system underlying neurogenic hyperalgesia (Cooke and Bliss 2006; Sandkühler 2000; Treede and Magerl 1995; Woolf and Salter 2000). It has been shown that brief electrical high-frequency stimulation of C-fiber afferents (100 Hz for 1 s) is capable of inducing LTP of synaptic transmission in nociceptive neurons of the superficial spinal dorsal horn (Ikeda et al. 2003; Liu and Sandkühler 1997; Randic et al. 1993). Recently, we have shown that a long-lasting facilitation of perceived pain intensity at the conditioned site can be induced in human subjects by the same electrical high-frequency stimulation protocol (Klein et al. 2004). We suggested that in humans this increased pain sensitivity at the conditioned site is a perceptual correlate of LTP in the conditioned pathway.

However, on the basis of the previous study, it remained unclear which nociceptive fiber types (C- and/or Aδ-fibers) are involved in mediating the facilitated pain intensity. Fortunately, human psychophysical experiments offer the unique advantage to obtain detailed description of the subjective experience and thus a more complete picture of the multidimensional pain experience beyond mere estimation of pain magnitudes (Gracely 2006). Thus the relative involvement of nociceptive fiber types in mediating nociceptive LTP may be deduced from analyzing another aspect of the sensory dimension, namely pain qualities (Sinclair 1967). The aim of this study was to determine if the induction of nociceptive LTP in humans is accompanied by respective changes of sensory pain qualities and whether possible sensory changes can be narrowed down to specific pain qualities such as “burning” that is predominantly related to C-fiber mediated input (Magerl et al. 1999; Ochoa and Torebjörk 1989) or “stinging” that is predominantly related to Aδ-fiber mediated input (Mackenzie et al. 1975; Magerl et al. 1999).

METHODS

Subjects

Twelve healthy subjects (7 male and 5 female, 21–57 yr, mean age: 27 yr) participated. The study was approved by the local ethics committee, and each subject gave written, informed consent after being familiarized with the experimental procedure. Subjects were blinded regarding the rationale of the study.

Conditioning stimuli and experimental setting

Cathodal electrical stimuli were applied via a circular array (diameter: 6 mm) of 10 punctate electrodes (diameter: 0.2 mm, each) on the forearm 5 cm distal to the cubital fossa via a constant current stimulator (DS7H; Digitimer, Welwyn Garden City, UK), a large surface electrode on the ipsilateral upper arm served as the anode (for electrodes) (cf. Klein et al. 2004). The small tip diameters favor activation of superficial nociceptive afferents (Bromm and Meier 1984; Inui et al. 2002; Kaube et al. 2000; Nilsson and Schouenborg 1999). High-frequency stimulation (HFS) by 100 Hz (pulse width: 2 ms) was used to induce LTP (Klein et al. 2004). These conditioning stimuli were given as five 1-s trains every 10 s (i.e., with 9-s breaks).

Each subject participated in four experimental sessions with four different stimulus intensities: They were adjusted at either 10 or 20 × individual detection threshold (T cf. Klein et al. 2004) or at fixed stimulus intensities of 1.5 or 3 mA. The order of the four experimental sessions was balanced across subjects. The contralateral forearm served as an unconditioned control site. The rationale for the use of different stimulus intensities was to test if the reliability of conditioning would be improved by a particular combination of HFS features (fixed/variable, high/low stimulus intensities).

Test stimuli

Every 3 min, pain sensitivity was tested by three single rectangular electrical test stimuli (pulse width: 2 ms) at 10 × T (T = 0.14 ± 0.07
sensory dimension of the pain experience (Rainville et al. 1992).

Pain psychophysics

Pain sensation was assessed in two different ways: first, subjects rated the sensory dimension of the electrically evoked pain intensity on a numerical rating scale (NRS) ranging from 0 (not painful) to 100 (most intense pain imaginable). Subjects were free to use integers as well as fractions ad libitum. Second, the quality of perceived pain was examined by a list of verbal descriptors (“Schmerzempfindungsskala,” SES) (Geissner 1996). The SES is the standard instrument of the German chapter of the International Association for the Study of Pain encompassing 10 descriptors on a sensory and 14 descriptors on an affective subscale (for German words and English translations according to Chen and Treede 1985; Stein and Mendl 1988 see supplemental Table 2). Subject rated every descriptor on a four level categorical scale (0 = not appropriate; 1 = somewhat appropriate, 2 = largely appropriate, 3 = fully appropriate) once after the first run of baseline testing (prior to HFS) and three times after HFS (15, 35, and 55 min). The sensory score of SES is the mean of all sensory descriptors; the affective score of SES is the mean of all affective descriptors.

Data evaluation and statistics

ANALYSIS OF PAIN INTENSITY BY NRS PAIN RATING. NRS pain ratings were transformed into decadic logarithmic values to achieve a (secondary) normal distribution. A small constant (0.1) was added to all ratings to avoid loss of zero-values (cf. Magerl et al. 1998). Perceptual LTP was quantified as the difference of log-transformed pain ratings between the conditioned test site and the contralateral control site equivalent to a percent difference (cf. Klein et al. 2004). Normalized NRS data were then analyzed by a two-way repeated-measures ANOVA (factors: conditioning HFS stimuli and before vs. after HFS) and least significant difference (LSD) post hoc tests.

ANALYSIS OF PAIN QUALITY BY SES RATING. For the analysis of the time course of the SES scores (before vs. after HFS), individual SES scores were normalized to control site by calculating the difference between test and control site. Normalized sensory and affective SES scores were included in a three-way repeated-measures ANOVA (factors: conditioning HFS stimuli, affective vs. sensory SES score, and before vs. after HFS) and LSD post hoc tests to determine differences between affective and sensory descriptors and to delineate the locus of HFS-induced changes.

For every descriptor at baseline, we tested if it was used to a significant extent (paired t-test vs. 0). For comparison across descriptors at baseline, we have performed one-way repeated-measures ANOVA and have combined it with multiple level tests of significance that correct for the multiplicity of post hoc tests (Duncan multiple stage test).

Factor analysis of the intercorrelation matrix of the sensory pain descriptors after single electrical test pulses at baseline (10 × 10 × array of single item correlations) was performed to break down the pattern of ratings into meaningful sensory qualities. Factors with Eigenvalues of ≥1 were retained and were rotated using the VARIMAX method. VARIMAX yields orthogonal orientation of the factors. As its name implies the method maximizes the variance across factors. In other words, it aims at maximal separation of identified factors such that factor loadings (i.e., the degree that a single item represents this factor) are maximized for items within a factor and at the same time minimized for items not part of this factor. In plain words, it yields a factorial solution where the independence of resulting factors is optimized. We have chosen this method on theoretical grounds because it is most likely to yield a solution of separated sensory channels.

The effect of HFS on pain qualities in the conditioned skin site were contrasted to the unconditioned control site for mean affective and sensory SES scores, and for the pain factors specified by factor analysis of baseline ratings prior to HFS.

Data are expressed as means ± SE. P values <0.05 were considered statistically significant.

RESULTS

At baseline, single electrical test stimuli at 10 × T evoked mild pain of similar magnitude both at the test site (mean = 9.8/100 NRS; 0.993 ± 0.047 log NRS) and at the control site (mean = 9.3/100 NRS; 0.968 ± 0.055 log NRS). HFS of nociceptive primary afferents resulted in a highly significant increase of perceived pain intensity at the conditioned site reaching a plateau at ~20 min after HFS and remaining stable for the rest of the observation period with an average increase above the control site, i.e., nociceptive LTP of ~23% [log

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1 The online version of this article contains supplemental data.

FIG. 1. Long-term potentiation of pain sensation after conditioning stimulation [high-frequency stimulation (HFS)]. A: pain ratings obtained by NRS rating (●, normalized to percentage of control site) become significantly increased compared with the control site at 15 min after HFS and reach a stable plateau lasting beyond the end of the observation period. B: pain ratings obtained by sensory descriptors (●) of the Schmerzempfindungsskala (SES) at the test site become significantly increased after HFS throughout the observation period, whereas affective descriptors were unchanged (▲). ***P < 0.001; post hoc LSD test, n = 48
NRS: +0.089 ± 0.018 vs. control site, \( F(4,176) = 17.17, P < 0.001, \text{Fig. 1A} \). No significant difference of pain LTP was observed among the four different stimulus protocols \( [F(3,44) = 0.09, P = 0.96] \).

Perceived pain was investigated in more detail using the SES list of pain descriptors, divided into an affective and sensory subscale. Sensory descriptors (mean sensory score: 0.47 ± 0.04) were rated significantly higher than affective descriptors (mean affective score: 0.06 ± 0.01, \( P < 0.001 \), paired \( t \)-test). There was no difference between test and control site before conditioning stimulation \( (P = 0.30, \text{each}) \). However, at any time after HFS, there was a significant long-term increase of the sensory SES score \( (P < 0.001, \text{LSD test}; \text{Fig. 1B}) \) by 23% on average \( (0.10 \pm 0.03 \text{ vs. control site}) \). In contrast, affective SES scores after HFS did not differ between test and control site \( (P > 0.40 \text{ each, LSD test}) \). The four different stimulus protocols had no significant influence on these effects \( [F(3,44) = 0.50, P = 0.69] \).

The analysis of individual SES pain descriptors revealed that beyond an overall difference of rating magnitude between affective and sensory descriptors there were also marked differences within the profile of SES sensory descriptors \( [F(9,855) = 28.77, P < 0.001] \). The rating of the descriptor “stinging” \( (1.26 \pm 0.07) \) was rated consistently higher than any other descriptor \( (P < 0.001, \text{Duncan multiple stage test}; \text{Fig. 2A}) \). Also, some of the sensory descriptors tended to show some increase after HFS, while others did not (\text{Fig. 2B, see also supplement 1}).

To elucidate the pattern of changes, a factor analysis of the sensory SES descriptors at baseline was performed resulting in two distinct factors with Eigenvalues of 4.57 and 2.67 (\text{Table 1}). Based on the factor loadings of descriptors after VARIMAX rotation, the minor one of these factors represented by the descriptors “throbbing” and “beating” and explaining \( 30\% \) of variance was interpreted as representing a perception of “deep pain/blunt pressure.” The major factor explaining \( 42\% \) of

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\text{TABLE 1. Factor analysis of sensory pain descriptors at baseline, i.e., before HFS}
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<tr>
<td>Cutting</td>
<td>0.78*</td>
<td>Cutting</td>
<td>0.62</td>
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<tr>
<td>Beating</td>
<td>0.04</td>
<td>Burning</td>
<td>0.82*</td>
</tr>
<tr>
<td>Burning</td>
<td>0.83*</td>
<td>Tearing</td>
<td>0.34</td>
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<tr>
<td>Tearing</td>
<td>0.74*</td>
<td>Scalding</td>
<td>0.87*</td>
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<tr>
<td>Throbbing</td>
<td>0.08</td>
<td>Stinging</td>
<td>0.18</td>
</tr>
<tr>
<td>Scalding</td>
<td>0.87*</td>
<td>Pounding</td>
<td>0.10</td>
</tr>
<tr>
<td>Stinging</td>
<td>0.61*</td>
<td>Hot</td>
<td>0.89*</td>
</tr>
<tr>
<td>Pounding</td>
<td>0.09</td>
<td>Piercing</td>
<td>0.23</td>
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<tr>
<td>Piercing</td>
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<td>Percent variance explained</td>
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HFS, high-frequency stimulation. *Factor loadings >0.70, #factor loadings <0.70, but difference to alternative factor >0.50.

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The variance was interpreted as representing “superficial pain.” A second factor analysis on the seven descriptors loading on the “superficial pain” factor identified that this factor can be further subdivided into two subfactors of comparable weight: one, represented by the descriptors hot and burning, was interpreted as representing “heat pain” and the second, represented by the descriptors piercing and stinging, was interpreted as representing “sharp mechanical pain.” Both subfactors explained ~28 and 25% of variance, respectively, after VARIMAX rotation.

Analyzing the effect of HFS on the different components of the pain experience we found not only that LTP was limited to the sensory dimension (Fig. 3A) but also that within the sensory dimension only descriptors representing superficial pain were significantly potentiated by 28% (+0.12 ± 0.04, \( P < 0.01 \), LSD test) but not those descriptors representing deep pain/blunt pressure (+9%, +0.03 ± 0.03, \( P = 0.47 \), LSD test; Fig. 3B). Moreover, both major submodalities of superficial pain, namely heat pain and sharp mechanical pain, were significantly potentiated by 41 and 21%, respectively (+0.14 ± 0.06, \( P < 0.01 \), and +0.12 ± 0.04, \( P < 0.05 \), respectively, LSD test; Fig. 3C).

![Fig. 3](Image)

**Fig. 3.** SES pain ratings after HFS at the test and control site. A: pain ratings on the sensory, but not affective subscale, are potentiated by HFS. B: one factor of the sensory subscale (interpreted as “superficial pain”) was potentiated, whereas the other factor (interpreted as “deep pain/blunt pressure”) remained unchanged. C: both submodalities that define the superficial pain factor by the typical descriptor burning (C-fiber related quality, “heat pain”) and stinging (A\( \delta \)-fiber related quality, “sharp mechanical pain”) were significantly potentiated after HFS. \(*P < 0.05\), \(**P < 0.01\); post hoc LSD test, \( n = 48 \)

**DISCUSSION**

This study reproduced previous results on LTP of pain intensity (Klein et al. 2004) using a numerical rating scale (NRS), of which the upper end was labeled “most intense pain imaginable” instead of the commonly used “most unbearable pain imaginable” (cf. Gracely 2006). Likewise, using a list of pain descriptors (SES) (Geissner 1996), we found exactly the same degree of facilitation on the SES sensory subscale. In contrast, ratings on the affective subscale were significantly lower (close to 0). This is consistent with previous data on the relationship of pain and unpleasantness demonstrating that the shorter a pain stimulus the less important is its affective rating (Chen and Treede 1985; Rainville et al. 1992). Moreover, the already low ratings on the affective subscale were also not facilitated, suggesting that the affective dimension of the pain experience was of a lesser importance. Alternatively, we cannot exclude that it may due to a lack of sensitivity of the SES, and much more painful stimuli might be necessary to evoke a more substantial affective pain experiences.

Analysis of the quality of perception offers a psychophysical gateway to the afferent fiber type involved in the perception (Sinclair 1967). It has been shown that stimuli exciting nociceptive C-fibers and eliciting a sensation of “second pain” were predominantly described as hot or burning, whereas stimulation of A\( \delta \)-nociceptors predominantly elicits a pricking pain sensation (Bragard et al. 1996; Mackenzie et al. 1975; Magerl et al. 1999; Sinclair and Stokes 1964). Further support of this relationship is derived from direct observation combining microneurography and microstimulation of identified nociceptors with psychophysical assessment of magnitude, time course, and quality of perceived sensations (Ochoa and Torebjörk 1989; Schmelz et al. 2000).

Analyzing the descriptors used to describe the pain sensation in our study revealed that stinging was the prevailing descriptor at baseline (more than twice the magnitude of the next highest descriptor, burning) suggesting that pain perception at baseline is predominantly mediated by A\( \delta \)-fibers. In contrast, perceived pain after HFS exhibited predominantly a potentiation of the C-fiber mediated percepts hot, burning, and scalding (subsumed under the factor “heat pain”, >40% of potentiation), whereas the dominant A\( \delta \)-fiber mediated pain percepts piercing, stinging, and tearing were potentiated to a much smaller extent (subsumed under the factor “sharp mechanical pain”, by <20%). Moreover, the submodality “deep pain/blunt pressure” represented by the descriptors throbbing and beating was almost unchanged after HFS; this is in accordance with the finding that blunt pressure stimuli are only facilitated when a component of peripheral sensitization is involved (Kilo et al. 1994; Treede et al. 2002). This pattern is consistent with the assumption that a substantial potentiation occurred in that C-fiber pathway, which is also held responsible for the induction of nociceptive LTP. This has been described previously in animal models as homosynaptic facilitation in spinal pain pathways (Randic et al. 1993; Sandkühler 2000). Thus the data in the present study suggest that the increase of the burning pain quality is a psychophysical correlate of homosynaptic nociceptive LTP mediated by the conditioned C-fiber pathway in humans.

Stinging pain sensation can be induced by either heat or mechanical stimuli and is always due to activation of an
Aδ-nociceptor pathway (Adriaensen et al. 1983; Greenspan and McGillis 1991; Mackenzie et al. 1975; Magearl et al. 1999; Ziegler et al. 1999). The homosynaptic facilitation of an Aδ-fiber pathway is unlikely to account for the HFS-induced increase of this Aδ-fiber-mediated sensation because the activation of Aδ-fibers by HFS was not sufficient to induce LTP in the nociceptive system of animals but rather induced long-term depression (Cheng and Randic 2003; Randic et al. 1993; Sandkuhler et al. 1997). Thus although present at the site of conditioning stimulation the increase of ratings for sensory descriptors associated with “sharp mechanical pain” probably depends on heterosynaptic facilitation and thus may represent a psychophysical correlate of heterosynaptic nociceptive LTP.

The analysis revealed that the sensory descriptors hold valuable information on potential mechanisms of LTP in pain pathways. We suggest that the hyperalgesia at the site of conditioning stimulation after HFS as judged by a numerical rating scale comprised two different components: facilitation of burning pain based on homosynaptic LTP of a C-fiber pathway and a less pronounced facilitation of stinging pain based on heterosynaptic LTP of an Aδ-fiber pathway.

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REFERENCES


