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Report

Perceptual Correlate of Nociceptive Long-Term Potentiation (LTP) in Humans Shares the Time Course of Early-LTP

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Klein T, Magerl W, Treede R-D. Perceptual correlate of nociceptive long-term potentiation (LTP) in humans shares the time course of early-LTP. *J Neurophysiol* 96: 3551–3555, 2006. First published October 4, 2006; doi:10.1152/jn.00755.2006. As in neocortex and hippocampus, neurons in the dorsal horn of the spinal cord develop long-term potentiation of synaptic efficacy (LTP) on high-frequency stimulation (HFS) of their afferent input, although how long LTP lasts in this nociceptive relay nucleus has not yet been addressed. Here we studied neurogenic hyperalgesia, a perceptual correlate of nociceptive LTP, in 13 healthy subjects, after HFS (5 × 1 s at 100 Hz) of superficial cutaneous afferents. HFS led to a mean upward shift of the stimulus–response function for pinprick-evoked pain (punctate mechanical hyperalgesia) in all subjects by a factor of 2.5 ($P < 0.001$) that lasted undiminished for the initial 1-h observation period. Follow-up tests until the next day revealed that this type of neurogenic hyperalgesia decayed with a $t_{1/2}$ of 3.3 h (99% CI: 3.1–3.5 h) and disappeared completely within 25.4 h (99% CI: 20.4–31.6 h). Touch-evoked pain (dynamic mechanical allodynia) developed in eight of 13 subjects, decayed with a $t_{1/2}$ of 2.9 h from the maximum and disappeared within 9.3 h. These findings suggest that a single HFS session induces nociceptive LTP in healthy subjects that corresponds to early-LTP (LTP1), implying primarily posttranslational mechanisms for this type of plasticity of human pain perception.

INTRODUCTION

Use-dependent strengthening of nociceptive synaptic transmission in the spinal cord leads to an enhanced responsiveness of dorsal horn neurons (central sensitization; Woolf and Salter 2000), which may result in an increased pain sensitivity (hyperalgesia) when it involves projection neurons of the spinothalamocortical pathway (Ikeda et al. 2003). It was previously suggested that long-term potentiation (LTP) of synaptic transmission—a common mechanism underlying learning and memory formation in many areas of the brain—is one important mechanism underlying central sensitization and its perceptual correlate neurogenic hyperalgesia (Ji et al. 2003). Thus studying use-dependent sensitization in the nociceptive system contributes to the understanding of mechanisms underlying certain acute and chronic pain states ("pain memory"), although it may also have important, more general implications for the understanding of memory formation in sensory systems (Treede et al. 2006).

Neurogenic hyperalgesia including enhanced pain perception to noxious pinprick stimuli (punctate mechanical hyperalgesia) and pain to nonnoxious light tactile stimuli (dynamic mechanical allodynia; LaMotte et al. 1991) is a cardinal sign of certain clinical conditions covering a broad time range reaching from hours (acute pain) to days (e.g., postoperative pain) to months or even years (chronic pain), suggesting that different mechanisms underlying the induction and maintenance of nociceptive LTP may be involved in these varying time courses of neurogenic hyperalgesia. However, it has not been addressed yet in either animal or human studies how long LTP in nociceptive pathways (nociceptive LTP) lasts in the spinal cord.

In other systems like the neocortex and the hippocampus it depends on numerous conditions how long LTP can and will last (reviewed by Abraham 2003). Early studies on LTP have shown that at least two stages of maintenance occur: *1*) an early-LTP, which depends primarily on posttranslational modifications and lasts up to a day (LTP1); *2*) at least two forms of late-LTP (LTP2 and LTP3, with time constants of about 3.5 and 25 days, respectively), which depends on transcriptional processes and de novo protein synthesis (Nguyen et al. 1994; Racine et al. 1983). In principle very similar posttranslational and transcriptional mechanisms were also previously described for central sensitization of the spinal cord, pointing to close parallels of hippocampal LTP and central (spinal) sensitization of the nociceptive system (Ji et al. 2003).

We recently introduced a human experimental model, which links nociceptive LTP with its perceptual consequence neurogenic hyperalgesia in the human nociceptive system (Klein et al. 2004). Here we used this model to characterize the time course of neurogenic hyperalgesia induced by high-frequency electrical stimulation (HFS) of nociceptive primary afferents, which may allow conclusions on underlying cellular and subcellular processes.

METHODS

The study was approved by the local ethics committee and was performed in healthy subjects (five female, eight male; age 24–31 yr). Each subject was familiarized with the experimental procedure before the experiments and had given written informed consent.

Conditioning stimulus

A detailed description of the conditioning electrode that consists of a ring of 10 small blunt wires (diameter: 0.25 mm each) is given elsewhere (Klein et al. 2004). The high current density arising from the specific punctate electrode configuration favors the activation of superficial nociceptive Aδ- and C-fiber afferents (Inui et al. 2002; Nilsson and Schouenborg 1999). Cathodal electrical stimuli were applied on the forearm 5 cm distal to the cubital fossa by a constant-current stimulator (model DS7H; Digitimer, Welwyn Garden City, UK), and a large surface electrode on the ipsilateral upper arm served as the anode. Stimulus intensity was adjusted at 20 × individual

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detection threshold (T) determined by applying single electrical pulses. High-frequency stimulation trains of 100 Hz for 1 s (pulse width 2 ms), repeated five times at 10-s intervals, were applied to induce nociceptive LTP.

Stimulus–response functions

Pain to punctate stimuli was tested by stimulus–response functions of pricking pain to a series of seven calibrated cylindrical punctate probes (8, 16, 32, 64, 128, 256, and 512 mN; tip diameter 0.25 mm; "pinpricks").

Allodynia was tested by three tactile stimuli that were applied with short strokes: a soft cotton wisp (about 3 mN), a cotton-tipped applicator (about 100 mN), and a soft makeup brush (about 400 mN). They activate only low-threshold mechanoreceptors (Leem et al. 1993) and are not painful in normal skin. For further details see Magerl et al. (2001).

Within one run of a stimulus–response (S/R-) function (application of seven pinprick and three tactile stimuli within 2 min) both sets of mechanical test stimuli were applied in a balanced manner so that the subject was not aware of either the sequence or the force of the mechanical stimuli. The mechanical stimuli were applied in a balanced order within a circular area at 10- to 20-mm distance from the electrode array.

Pain ratings

Subjects rated the magnitude of pain to mechanical and conditioning electrical stimuli on a numerical rating scale (NRS) ranging from 0 (nonpainful) to 100 (most intense pain imaginable). Subjects were free to use integers as well as fractions ad libitum. They were instructed to distinguish pain from the perception of touch or pressure by the presence of a sharp or slightly pricking or burning sensation.

Experimental design

Mechanical test stimuli were applied in 2-min runs (ten mechanical stimuli), alternating continuously between conditioned and the contralateral skin site during a time period of 40 min before (baseline) and 60 min (test period) after conditioning HFS followed by 20-min assessment periods at 4, 5, 8 (in three subjects), and 24 h after HFS.

Data evaluation and statistics

All pain ratings were transformed into decadic logarithmic values to achieve a normal distribution. To avoid loss of zero-values, a small constant (0.1) was added to all ratings (for theoretical background, see Magerl et al. 1998). Data are expressed as retransformed means as well as log means ± SE. Data obtained at 4 and 5 h after HFS were pooled.

Pinprick-evoked pain ratings were normalized to baseline. Punctate mechanical hyperalgesia was defined as an upward shift of the pinprick S/R-function after HFS and was quantified as the difference of log-transformed normalized pain ratings between the test and the contralateral site. This procedure is equivalent to building the ratio of original pain ratings, but avoids the skewed nonnormal distribution of ratio data. In the following, we refer to this parameter as the ratio between test and contralateral site. The individual half-lives ($t_{1/2}$) of punctate hyperalgesia and the predicted time needed to return to the baseline level ($t_{\text{full recovery}}$) were judged by using individual regression lines. The mean $t_{1/2}$ and $t_{\text{full recovery}}$ were then estimated by a sigmoid function fitted to the cumulative probability distribution. For statistical analysis two-tailed paired *t*-tests were performed for parameters of pinprick-evoked pain.

Ratings to touch-evoked pain did not allow normalization to baseline because pain was absent at baseline. Pain to light touch stimuli (dynamic mechanical allodynia) was defined as the upward shift in S/R-functions for pain evoked by light touch after conditioning HFS versus the corresponding time for the contralateral control site. Because neither original nor log-transformed data were normally distributed, allodynia was analyzed by a nonparametric Friedman ANOVA. Values of $P < 0.05$ were considered statistically significant.

RESULTS

High-frequency electrical stimulation (HFS) evoked mild to moderate pain (mean pain rating 36 of 100 corresponding to 1.561 ± 0.385 log units NRS) and resulted in a mean upward shift of the stimulus–response function to pinpricks adjacent to the conditioned skin site by a factor of 2.5 (punctate mechanical hyperalgesia; Fig. 1*A*). Punctate hyperalgesia developed significantly immediately after HFS ($P < 0.001$), increased slightly over the next 40 min, and peaked between 40 and 60 min after HFS with a mean increase of about 306% (0.486 ± 0.049 log units; Fig. 1*B*) within this time window.

After reaching its maximum, punctate mechanical hyperalgesia decreased continuously (Fig. 2*A*). It decayed with a mean half-life of 3.3 h after HFS (99% CI: 3.1–3.5 h; 0.520 ± 0.008 log units) and was predicted to return to baseline 25.4 h after HFS (99% CI: 20.4–31.6 h; 1.404 ± 0.031 log units; Fig. 2*B*).

HFS also induced an upward shift of the S/R-function to light tactile stimuli (dynamic mechanical allodynia; Fig. 3*A*).

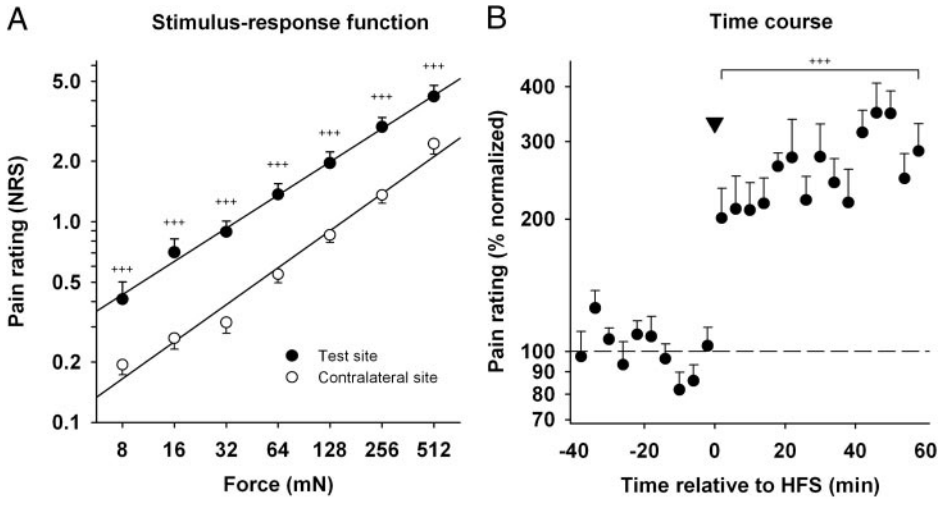

FIG. 1. Effect of conditioning high-frequency stimulation (HFS) on pinprick-evoked pain. *A*: stimulus–response (S/R-) functions for pinprick-evoked pain at the test site (filled circles) and at the contralateral site (open circles) after conditioning stimulation. After HFS, there was a parallel upward shift of this function adjacent to the conditioning skin site. Each circle represents mean pain ratings to one stimulus intensity averaged over 60 min after conditioning stimulation and all subjects. Paired *t*-test conditioned vs. contralateral site. *B*: increase of pinprick-evoked pain after HFS peaked between 40 and 60 min after HFS at about 300%. Each circle represents the ratio between conditioned and contralateral sites of normalized pain ratings to pinprick averaged over a 4-min time window, all stimulus intensities, and all subjects. Paired *t*-test vs. baseline; $^{+++}P < 0.001$, mean ± SE, $n = 13$.

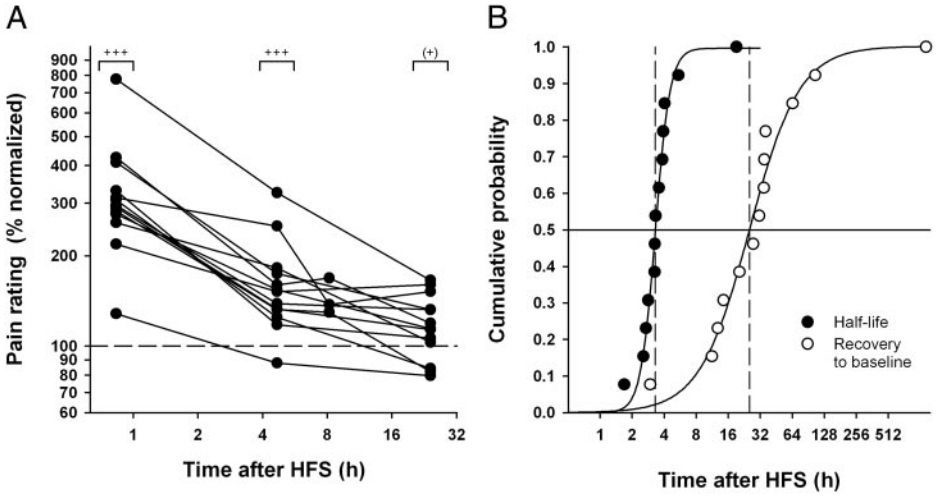

FIG. 2. Time course of punctate hyperalgesia. *A*: individual time courses of the recovery of punctate hyperalgesia. Each dot represents the ratio between conditioned and contralateral sites of normalized pain ratings to pinprick averaged over a 20-min time window. Paired *t*-test vs. baseline; $^{(+)}P < 0.10$, $^{+++}P < 0.001$. *B*: cumulative probability distribution of the individual half-lives and predicted recovery time to baseline. Mean half-life ($t_{1/2}$, 3.3 h after HFS) and predicted full recovery time ($t_{\text{full recovery}}$, 25.4 h after HFS) were estimated by a sigmoidal fit to the probability curves (at 50% probability; intersection of the horizontal black line with the sigmoidal curve). Each dot represents the individual observed half-life (filled circles) and predicted recovery time (open circles).

Allodynia developed significantly within the first 10 min after HFS ($P < 0.5$) and reached its maximum 40–60 min after HFS (Fig. 3*B*). However, because only eight of 13 subjects developed statistically significant allodynia ($P < 0.05$, data not shown), and even those subjects gave pain ratings mainly between 0.1, 0.2, or 0.3 intermingled with about 70% ratings of 0, mean pain ratings including all subjects were quite low. Because of the high interindividual variability of the time course of allodynia, we estimated the decay of allodynia on the base of the group means (Fig. 3*C*). Allodynia decayed with a half-life of 2.9 h after HFS (0.457 log units) and returned to baseline 9.3 h after HFS (0.969 log units; Fig. 3*C*).

DISCUSSION

In the present study we have shown that high-frequency electrical stimulation (HFS) of primary nociceptive afferents leads to neurogenic hyperalgesia encompassing enhanced pain perception to noxious pinprick stimuli (punctate mechanical hyperalgesia) and pain to light tactile stimuli (dynamic mechanical allodynia), reproducing our previous results (Klein et al. 2004). Next we determined the time course of the decay of neurogenic hyperalgesia (half-lives of 1.9–2.3 h), which was consistent with the time course that was described for the so-called early-LTP phenomenon (or LTP1) in several parts of the CNS (Abraham 2003). Moreover, the time courses of the decay resembled the decay of neurogenic hyperalgesia induced by intradermal capsaicin injection (LaMotte et al. 1991), suggesting that in both models of neurogenic hyperalgesia, central sensitization of nociceptive spinal dorsal horn neurons depends on the early type of LTP (LTP1) of synaptic transmission.

Mechanisms of early-LTP (LTP1) in the nociceptive system: evidence from animal studies

LTP1 of synaptic transmission in, say, the hippocampus typically occurs rapidly after the initiating event and primarily depends on posttranslational modifications such as phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (especially the GluR1 subtype) by several protein kinases (protein kinases A and C, PKA/PKC, and Ca^{2+}/calmodulin-dependent protein kinase, CaMKI I; Lynch 2004). This leads to an enhanced Ca-permeability of AMPA receptors and increased insertion into the postsynaptic

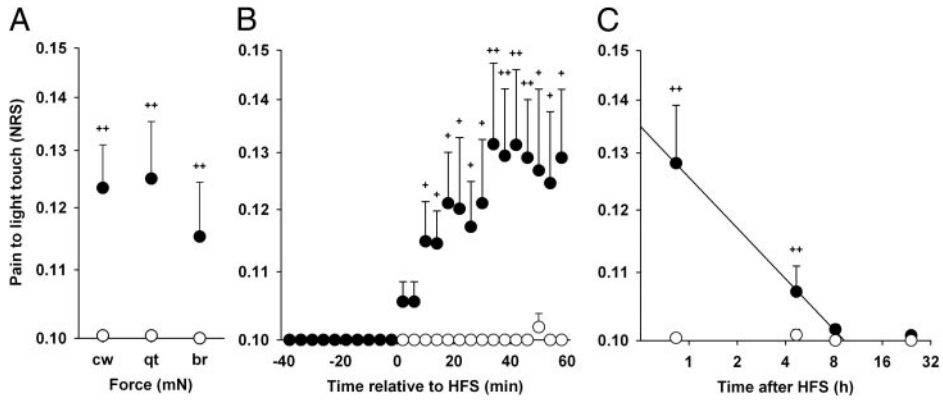

FIG. 3. Time course of dynamic mechanical allodynia *A*: S/R-function to tactile-evoked pain at the conditioned (filled circles) and the unconditioned contralateral skin sites (open circles). Each circle represents pain ratings to one stimulus intensity averaged over 60 min after conditioning stimulation and all subjects. cw, cotton wisp (about 3 mN); qt, Q-tip (about 100 mN); br, brush (about 400 mN). *B*: pain to tactile stimuli developed slowly after HFS and remained unattenuated during the first hour. Each circle represents the mean of pain ratings to tactile stimuli averaged over a 4-min time window, all stimulus intensities, and all subjects. *C*: decay of allodynia judged by the group mean. Each dot represents touch-evoked pain ratings averaged over a 20-min time window, all stimulus intensities, and all subjects. $^{+}P < 0.05$, $^{++}P < 0.01$ Friedman ANOVA conditioned vs. contralateral site. Mean ± SE, $n = 13$.

membrane (Malinow and Malenka 2002). However, transferring LTP1 into the persistent forms of LTP2 and LTP3 depends on de novo protein synthesis and requires activation of transcriptional processes (primarily LTP3; Abraham 2003).

Evidence for the involvement of mechanisms underlying LTP1 in use-dependent synaptic plasticity of the nociceptive system came from animal studies on nociceptive LTP and central sensitization in the spinal cord: blockade of PKA, PKC, and CaMKII prevented the induction of LTP1 in the spinal cord (Yang et al. 2004). Moreover, blocking protein synthesis prevented LTP2 but not the induction and development of LTP1 (Hu et al. 2003). Thus although mechanisms of LTP2 exist in the spinal cord, the time courses of punctate mechanical hyperalgesia and dynamic mechanical allodynia after a single HFS session in our study suggest that both are primarily based on posttranslational mechanism (LTP1).

Moreover, animal studies on central sensitization induced by intradermal capsaicin injection also showed the involvement of protein kinases and AMPA-receptor phosphorylation in the induction of enhanced synaptic transmission in the spinal cord, strengthening the hypothesis that nociceptive LTP (at least LTP1) may underlie some forms of central sensitization (Fang et al. 2002, 2003; Galan et al. 2004; Ji et al. 2003; Lin et al. 1996; Nagy et al. 2004).

Differences between dynamic mechanical allodynia and static punctate hyperalgesia

Both half-life and the predicted time to return to baseline for dynamic mechanical allodynia were lower than the 99% confidence interval of the respective parameters of punctate hyperalgesia but remained within the time range of LTP1. These differences in the time course were described previously in another human surrogate model of neurogenic hyperalgesia (intradermal capsaicin injection; LaMotte et al. 1991). The differential time course may depend in part on differential involvement of protein kinases in these phenomena. Selective activation of PKA in rat spinal dorsal horn neurons by forskolin, for example, led primarily to enhanced responsiveness to noxious mechanical stimuli but not to innocuous brushing stimuli (Lin et al. 2002). Our data add to the mounting evidence for different mechanisms underlying hyperalgesia and allodynia. For example, allodynia is mediated by tactile Aβ-afferents, whereas punctate hyperalgesia is primarily mediated by nociceptive Aδ-afferents (Magerl et al. 2001).

Clinical implications

Chronic pain patients often suffer from hyperalgesic pain states for months or even years, which would be compatible with late-phase LTP (i.e., LTP3). Transferring LTP1 into the persistent state of LTP3 usually requires repetition of the initiating event (Abraham et al. 1993). Repetitive nociceptive input to the CNS might be one of the underlying mechanisms for prolonged neurogenic hyperalgesia in some chronic pain patients (Baumgärtner et al. 2002; Bennett 1994; Fields et al. 1998). However, prolonged neurogenic hyperalgesia develops in only a subset of pain patients, suggesting that the susceptibility to sensitization by repetitive noxious stimulation might be enhanced in chronic pain patients, such as those arising from diversities of genetic factors (Mogil et al. 2000).

The propensity of patients to develop chronic pain resulting from use-dependent plasticity in the nociceptive system might be assessed with the surrogate model of nociceptive LTP by using the variability in magnitude and the duration of nociceptive LTP as key measures (cf. Fig. 2*A*). This might help to identify potential mechanisms and risk factors underlying chronic pain in the future. Moreover, other diseases such as Alzheimer are known to be associated with a general impairment of LTP of synaptic transmission (Rowan et al. 2005). Here the surrogate model may serve as a tool for studying deficits in synaptic plasticity in these patients.

In conclusion, the present data suggest that LTP of nociceptive synaptic transmission and its perceptual correlate neurogenic hyperalgesia after a single high-frequency stimulation protocol of nociceptive afferents fall into the time range of LTP1 and thus mainly depended on posttranslational modifications at synapses of the nociceptive system. The human surrogate model of nociceptive long-term potentiation (LTP) provides a tool to investigate use-dependent synaptic plasticity in the nociceptive system.

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