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Analysis of hyperalgesia time courses in humans after painful electrical high-frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity

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ABSTRACT

Electrical high-frequency stimulation (HFS) of skin afferents elicits long-term potentiation (LTP)-like hyperalgesia in humans. Time courses were evaluated in the facilitating (homotopic) or facilitated (heterotopic) pathways to delineate the relative contributions of early or late LTP-like pain plasticity. HFS in healthy subjects (n = 55) elicited highly significant pain increases to electrical stimuli via the conditioning electrode (to 145% of control, homotopic pain LTP) and to pinprick stimuli in adjacent skin (to 190% of control, secondary hyperalgesia). Individual time courses in subjects expressing a sufficient magnitude of hyperalgesia (>20% pain increase, n = 28) revealed similar half-lives of homotopic pain LTP and secondary hyperalgesia of 6.9 h and 4.9 h ($\log_{10}$ mean 0.839 ± 0.395 and 0.687 ± 0.306) and times to full recovery of 48 h and 24 h ($\log_{10}$ mean 1.679 ± 0.790 and 1.373 ± 0.611). Time course and peak magnitudes were not correlated between (r = −0.19 to +0.21, NS), nor within both readout (r = 0.29 and 0.31, NS). In most subjects, time courses were consistent with early LTP1. Notably, in some subjects (10 of 28), estimated times to full recovery were much longer (>10 days), possibly indicating development of late LTP2-like pain plasticity. Dynamic mechanical allodynia (only present in 16 of 55 subjects) lasted for a shorter time than secondary hyperalgesia. Three different readouts of nociceptive central sensitization suggest that brief intense nociceptive input elicits early LTP1 of pain sensation (based on posttranslational modifications), but susceptible subjects may already develop longer-lasting late LTP2 (based on transcriptional modifications). These findings support the hypothesis that LTP may contribute to the development of persistent pain disorders.

1. Introduction

Long-term potentiation (LTP) of synaptic transmission is considered a neurobiological key mechanism underlying learning and memory formation. LTP-like synaptic plasticity plays a role in various central nervous pathologies [10]. Subtypes of LTP have been extensively studied in neocortex and hippocampus [2] and exhibit different time courses: early LTP (or LTP1), lasting a few hours to up to 1 day, which depends primarily on posttranslational modifications (eg, phosphorylation); and late LTP (encompassing LTP2 and LTP3), which depends on transcriptional processes and de novo protein synthesis and shows time constants of about 3.5 days to up to 25 days [31,36]. More recently, it has been demonstrated in rat hippocampus that an undisturbed LTP trace can last longer than 1 year, possibly for a lifetime [3].

Within the nociceptive system, LTP of C-fibre-mediated nociceptive transmission on neurons located in the superficial and deep dorsal horn [12,38] is considered to be a mechanism responsible for an increased responsiveness of dorsal horn neurons. All studies have focussed on LTP within the stimulated pathway (homosynaptic component of central sensitization [14,40]) in principle reflecting synaptic strengthening in synapses subjected to repetitive activation (input specificity). A multitude of studies, however, suggest that behaviourally relevant central changes, which have been described in several inflammatory and neurogenic pain models [37,43,45] and which underlie secondary mechanical hyperalgesia in an area spatially remote from the conditioned skin site, involve heterosynaptic facilitation processes

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("classical" activity-dependent central sensitization [14]). Here a facilitating pathway consisting of a specific subset of capsaicin-sensitive nociceptive C fibres [28,41,42] accounting for the induction of central sensitization can be distinguished from a facilitated pathway consisting of capsaicin-insensitive Aδ fibres accounting for the expression of secondary hyperalgesia to mechanical pinprick stimuli [28,49]. Overall, the combination of both the homosynaptic potentiation of conditioning nociceptor inputs and the heterosynaptic facilitation of nonnociceptive and nociceptive afferents is considered to constitute central sensitization [24]. In a human surrogate model of nociceptive LTP induced by high-frequency electrical stimulation, we have previously shown perceptual correlates for both components of central sensitization, namely hyperalgesia to electrical stimuli at the site of conditioning stimulation (homotopic pain LTP), which we considered a perceptual correlate of "homosynaptic" LTP within the facilitating pathway, and mechanical hyperalgesia in adjacent skin (secondary hyperalgesia) as a perceptual correlate of "classical" heterosynaptically mediated central sensitization [19]. The time course of secondary hyperalgesia resembled early LTP (LTP1) in different human pain models [16,23], suggesting that classical central sensitization induced by brief, vigorous afferent barrages is linked to posttranslational modifications of synaptic processing.

In the present study, we have analyzed the relationship of individual magnitudes and time courses of perceptual correlates of the facilitating (homotopic pain LTP) and facilitated (secondary hyperalgesia) pathways to reveal to which extent both phenomena share the same mechanisms. Moreover, we have analyzed whether the time courses of pain plasticity provide information on a possible transition to long-lasting (chronic) components of hyperalgesia. This may have important implications in understanding the relative importance of homosynaptic and heterosynaptic mechanisms for behaviourally relevant central sensitization processes in humans.

2. Materials and methods

The study was conducted in 55 healthy volunteers (26 men, 29 women; mean age 23.9 years) at 2 locations (Mannheim and Muenster) and was approved by the local ethic committees. All subjects were introduced to the experimental procedures beforehand and provided written informed consent. Subjects were not informed about the scientific background and hypotheses of the study. Exclusion criteria were any history of chronic pain or any chronic or acute disease or known psychological disorders as well as drug abuse in anamnesis. Subjects did not receive any medication 3 days before the study. During the testing session, subjects were seated in a reclining chair with their arms placed on armrests.

2.1. Test stimuli

Baseline pain sensitivity and changes of pain sensitivity during an experimental day were tested with 2 different approaches: single electrical pulses applied via the conditioning electrode (homotopic), and mechanical test stimuli consisting of a set of pinprick and tactile mechanical stimulators (see below) for testing adjacent to the conditioned area (ie, at 15 mm distance to the border of the conditioning electrode; heterotopic).

To preferentially activate epidermal nerve fibres, all electrical stimuli were applied via a circular electrode array (diameter 6 mm) consisting of 10 punctate electrodes (diameter 250 μm each), which were mounted in a small plastic frame (diameter 22 mm [19]). These electrode arrays served as the cathode and were placed bilaterally on the subjects' forearms 5 cm distal of the cubital fossa, one serving as test, one as control electrode. A large surface electrode served as the anode and was placed on the upper arm of each site. Electrical stimuli were applied via a constant current stimulator (DS7H; Digitimer, Welwyn Garden City, UK). Detection thresholds were determined as the geometrical mean of 5 just subthreshold and just suprathreshold electrical stimuli using an up/down method of constant stimuli with a 10% step width [6]. Stimulus intensity for electrical test stimuli was adjusted at 10 times individual detection thresholds (10 × T).

The area surrounding the conditioning electrode was tested with standardized punctate probes (pinprick) with 0.25 mm in tip diameter as described previously [19,27], exerting forces of 8, 16, 32, 64, 128, 256, and 512 mN, which were applied in a pseudo-randomized order. These stimuli were shown previously to detect secondary hyperalgesia in areas surrounding an injury site [6,23,28,29,49]. Dynamic mechanical allodynia (DMA) was tested using standardized light touch stimuli: (1) a cotton wisp applying a force of ~3 mN, (2) a Q-tip, fixed in a flexible plastic mount, exerting a force of ~100 mN when slightly bent, and (3) a standardized brush applying forces of 200–400 mN (Senselab Brush-05, Somedic, Sweden). Electrical and mechanical stimuli were applied alternately, starting with electrical stimuli during each run.

2.2. Pain ratings

To evaluate the pain intensity of subjects, a numerical rating scale from 0 (no pain) to 100 (most intense pain imaginable) was used for all test stimuli and the conditioning stimuli. 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.

2.3. Conditioning stimuli

At the test sites, repetitive trains of high frequency stimulation (HFS; 100 Hz) were applied 5 times for 1 s each (10-s interstimulus interval; 2-ms single pulse duration) at 10× detection threshold through the electrode described above. Subjects were asked to rate the pain intensity after each train of conditioning HFS.

2.4. Experimental protocol

Ten minutes after HFS, electrical and mechanical testing was continued in the same manner for further 60 min from 10–70 min after HFS. The last 20 min (50–70 min after HFS) were averaged for the 1-h post-HFS value. Testing was repeated for 20 min each (for a total of 5 blocks of tests) at 2, 4, 8, and 24 h after HFS. The electrodes remained attached to the skin throughout baseline testing to 8 h after HFS. In 39 subjects, the electrode was removed after 8 h and reattached for the final test session at 24 h after HFS.

2.5. Data evaluation and statistics

2.5.1. Magnitudes of homotopic pain LTP, secondary hyperalgesia and DMA

Ratings of electrically induced pain (EPS) and mechanically induced pain (MPS and DMA) were transformed into decadic logarithmic values to obtain a secondary normal distribution. A small constant of 0.1 was added to pain ratings for all mechanical stimuli (light stroking tactile and pinprick) and electrical stimuli to avoid a loss of zero values (for theoretical background, see [27]). All ratings for pinprick and electrical test stimuli were normalized to average baseline ratings (percentage of baseline) by calculating the difference between log-transformed pain ratings before and after HFS. This procedure is equivalent to building a ratio of the original pain

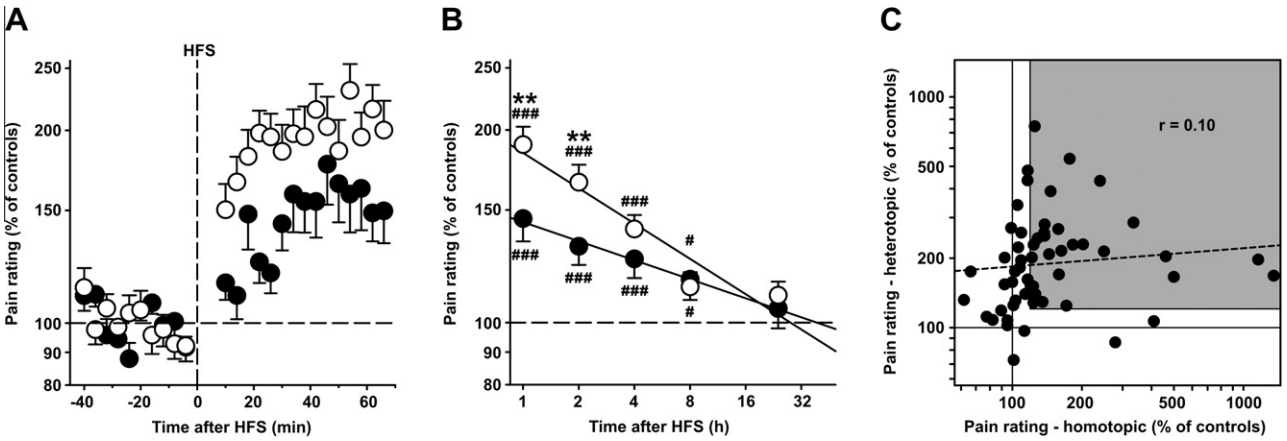

Fig. 1. Effect of HFS on homotopic electrical (solid symbols) and heterotopic mechanical (open symbols) pain stimuli. (A) After HFS pain, ratings in the homotopic test area (solid circles) and in the heterotopic test area (open circles) increased slowly and reached a plateau level at approximately 30 min, which was maintained for the next 40 min. The magnitude of homotopic pain LTP was significantly smaller than that of secondary hyperalgesia (145% vs 190%; $P < .01$). (B) Homotopic pain LTP and secondary hyperalgesia decreased monotonically over time after HFS, with similar time courses reaching baseline at approximately 30–40 h after HFS. Regression lines depict the calculated time course of homotopic pain LTP and secondary hyperalgesia based on the assumption of monoexponential decay. Circles represent the $\log_{10}$ mean (±SEM) of pain ratings normalized to baseline and the contralateral control area across all subjects ($n = 55$; 24 h: $n = 39$). (C) Scatter plot of individual magnitudes of homotopic pain LTP and secondary hyperalgesia assessed at 50–70 min after HFS. There was no correlation between both components of hyperalgesia. Subjects in whom the magnitude of homotopic pain LTP and secondary hyperalgesia exceeded a 20% increase either at 1 or 2 h after HFS relative to the contralateral control area in the homotopic and heterotopic test site (grey area) were selected for further analysis. $^{\#}P < .05$; $^{\#\#\#}P < .001$; paired t test vs control $^{**}P < .01$; paired t test, homotopic pain LTP vs secondary hyperalgesia.

ratings. In the same way, HFS-induced local changes of sensitivity were calculated as the difference of baseline-normalized log-transformed pain rating between the test and the unconditioned control site. Because DMA is lacking in normal skin of healthy subjects, data for DMA were not normalized and are shown as raw values.

Paired t tests were performed for comparison of the magnitudes of electrical and mechanical pain sensitivity before and at 1, 2, 4, 8, and 24 h after HFS. Data are presented as retransformed means as well as $\log_{10}$ mean values ± standard error of the mean (SEM).

2.5.2. Time courses of homotopic pain LTP and secondary hyperalgesia

The time courses of the decay of homotopic pain LTP and secondary hyperalgesia were estimated as follows. Mean half-lives ($t_{1/2}$) and the predicted times to a full recovery of pain sensitivity to baseline level ($t_{full\ recovery}$) were judged by log (pain increase) vs log (time) regression analysis for all subjects ($n = 55$) at 1, 2, 4, 8, and 24 h after HFS.

Individual half-lives ($t_{½}$) and the individual predicted times to a full recovery of pain sensitivity to baseline level ($t_{full\ recovery}$) were calculated in a subgroup (magnitude of both, homotopic pain LTP and secondary hyperalgesia >20% pain increase within 2 h after HFS; $n = 28$, 13 men and 15 women) by individual regression. The mean half-life ($t_{½}$) and time to recovery to baseline ($t_{full\ recovery}$) were then estimated by a Gaussian fit of the cumulative normal distribution and compared by nonparametric Friedman analysis of variance (ANOVA).

2.5.3. Correlation analyses

Because the magnitudes of homotopic pain LTP and secondary hyperalgesia were normally distributed, the correlation between homotopic pain LTP and secondary hyperalgesia as well as the correlation at different time points within homotopic pain LTP and secondary hyperalgesia, respectively, were performed by Pearson's correlation. To calculate mean correlation coefficients across different measuring times, the single correlation coefficients were transformed into the arcus tangens hyperbolicus (Fisher transformation [4]). Resulting arithmetic means of the arcus tangens hyperbolicus were retransformed into mean correlation coefficients by the tangens hyperbolicus function.

Because individual time courses in the subgroup sufficiently expressing hyperalgesia ($n = 28$) were not normally distributed, the correlation between the magnitude of homotopic pain LTP and secondary hyperalgesia and the related time course was analysed by Spearman's rank correlation.

3. Results

3.1. Induction of homotopic pain LTP, secondary hyperalgesia, and DMA

Conditioning HFS evoked mild to moderate pain, slowly increasing from the first to fifth train of HFS (35 to 46; mean pain rating 42 of 100, $\log_{10}$ mean 1.625 ± 0.044; $n = 55$).

Both pain evoked by electrical test stimuli at the site of conditioning HFS (homotopic pain LTP) and pain evoked by mechanical stimuli in an area adjacent to the conditioned skin site (secondary hyperalgesia) increased after HFS until reaching a plateau at approximately 30 min after HFS (Fig. 1A). Both components of hyperalgesia remained stable over 1 h after HFS, resulting in a peak homotopic pain LTP of 145% ($\log_{10}$ mean ± SEM 0.162 ± 0.036) and a peak secondary hyperalgesia of 190% ($\log_{10}$ mean ± SEM 0.278 ± 0.028, $P < .01$ vs secondary hyperalgesia; Table 1) averaged over 50–70 min after HFS compared to baseline. Homotopic pain LTP and secondary hyperalgesia remained significantly above the unconditioned control site over 8 h after HFS (Fig. 1B).

Table 1
Magnitude of homotopic pain LTP vs secondary hyperalgesia after HFS (n = 55).

Characteristic	Magnitude	
	LTP at 1 h (peak)	LTP at 1–8 h
Homotopic pain LTP,% increase ($\log_{10}$ mean ± SEM)	45% (0.162 ± 0.036)	30% (0.115 ± 0.026)
Secondary hyperalgesia,% increase ($\log_{10}$ mean ± SEM)	90% (0.278 ± 0.028)	51% (0.180 ± 0.021)
Statistics homotopic pain-LTP vs secondary hyperalgesia[a]	$P < .01$	$P < .05$
Correlation homotopic pain-LTP vs secondary hyperalgesia[b]	$r = 0.10$ (P = .46)	$r = 0.21$ (P = .13)

[a] By t test for comparison of the magnitudes after HFS.
[b] Pearson's correlation.

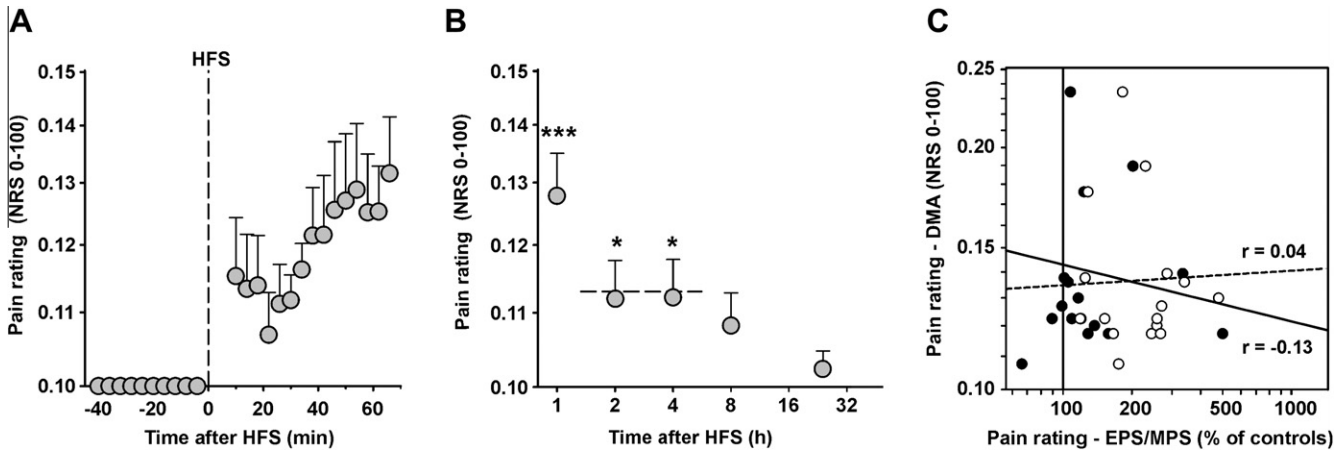

Fig. 2. Effect of HFS on sensitivity to light tactile stroking stimuli (cotton wool tip, Q-tip, brush) indicating development of DMA in a subgroup of subjects (n = 16 of 55). (A) After HFS, DMA developed gradually during the first hour, reaching a plateau level at approximately 60 min after HFS (n = 16). (B) DMA decayed to below half-maximal level (horizontal dashed line) already at 2 h after HFS (ie, at approximately 1 h after peak level). Circles represent the $\log_{10}$ mean (±SEM) of pain ratings to stroking tactile stimuli. (C) Scatter plot of individual magnitudes of homotopic pain LTP (open symbols; EPS) and secondary hyperalgesia (solid symbols; MPS) assessed at 50–70 min after HFS vs magnitude of dynamical mechanical allodynia (DMA). $^{***}P < .001$, $^{*}P < .05$; paired t test vs control site.

Approximately one third of participants also displayed HFS-induced DMA (16 of 55, 29%). Pain ratings to tactile stimulation also built slowly during the first hour after HFS (Fig. 2A), and peak allodynia pain ratings usually occurred at 50–70 min after HFS (except for 2 subjects with a shorter-lived DMA) and were 0.136 ($\log_{10}$ mean ± SEM −0.867 ± 0.022, $P < .001$).

3.2. Early (LTP1)-like and late (LTP2)-like time courses of homotopic pain LTP, secondary hyperalgesia, and DMA

Homotopic pain LTP and secondary hyperalgesia decayed with similar time courses when estimated by linear regression analysis across the means of the whole study population at the different time points (population half-life, 6.2 vs 5.3 h; extrapolated time to full recovery, 37.9 vs 28.5 h; Table 1). In contrast, across the subpopulation expressing allodynia (16 of 55), the decay of DMA was much faster with an approximate half-life short of 2 h. Although 14 of 16 expressed DMA at 1 h, only 5 of 16 subjects still exhibited DMA at 2 h. Likewise, at 2 h after HFS, DMA had also decayed to less than half maximal magnitude (Fig. 2B).

To further analyze the relationship between individual time courses of homotopic pain LTP and secondary hyperalgesia as well as between time courses and the individual magnitude of homotopic pain LTP and secondary hyperalgesia (see below), we selected a subgroup of subjects who had developed sufficient hyperalgesia (a pain rating increase in both test sites by at least 20% at either 1 or 2 h after HFS, n = 28; Fig 1C). Of the 27 subjects not meeting these criteria (no LTP), 18 subjects had insufficient magnitude of homotopic pain LTP, 2 insufficient magnitude of secondary hyperalgesia, and 7 subjects both. Despite a somewhat higher magnitude of pain increases, the population data in this subpopulation exhibited similar time courses compared to the whole group of subjects or the LTP subgroup (Table 2).

Individual time courses of homotopic pain LTP and secondary hyperalgesia for those 28 subjects are displayed in Fig. 3A and C. Parameters of time course (half-life, time of full recovery) varied widely across subjects. The estimated half-lives or extrapolated times to full recovery (as estimated by regression analysis) revealed that these functions followed the rules of psychometric functions (cumulative normal distribution; Gaussian) over log(time), ie, that the distribution of log half-lives or times of full recovery were normally distributed for both homotopic pain LTP and secondary hyperalgesia (Fig. 3B and D; Table 2). Mean ($\log_{10}$ mean ± standard deviation [SD]) half-lives as judged from these psychometric function were 6.9 h ($\log_{10}$ mean ± SD 0.839 ± 0.395) for homotopic pain LTP (n = 21) and 4.9 h ($\log_{10}$ mean ± SD 0.687 ± 0.306) for secondary hyperalgesia (n = 24), whereas time to full recovery was 47.7 ($\log_{10}$ mean ± SD

Table 2
Time courses of homotopic pain LTP and secondary hyperalgesia.[a]

Characteristic	HFS pain, mean ($\log_{10}$ mean ± SEM)	Half-life (h) (see indices)	Extrapolated time to full recovery (h) (see indices)
Homotopic pain LTP Population mean (n = 55)[b]	42.2 (1.625 ± 0.044)	6.2	37.9
No LTP subpopulation (n = 27)[c]	49.0 (1.690 ± 0.063)	2.5	6.1
LTP1 subpopulation (n = 28)[d]	36.5 (1.562 ± 0.061)	6.8	46.2
LTP1 subpopulation: individual mean (n = 28)[e]		6.9 (0.839 ± 0.395)	47.7 (1.679 ± 0.790)
Secondary hyperalgesia Population mean (n = 55)[b]		5.3	28.5
No LTP subpopulation (n = 27)[c]		5.3	28.2
LTP1 subpopulation (n = 28)[d]		5.3	28.5
LTP1 subpopulation: individual mean (n = 28)[e]		4.9 (0.687 ± 0.306)	23.6 (1.373 ± 0.611)

[a] Data show half-lives and extrapolated time to full recovery of homotopic pain LTP and secondary hyperalgesia after regression analysis.
[b] Mean values for all subjects investigated (n = 55; population mean).
[c] Mean values for a subpopulation with less than 20% of homotopic pain-LTP and secondary hyperalgesia in the first 2 h after HFS (n = 27; no LTP subpopulation mean).
[d] Mean values for a subpopulation with more than 20% of homotopic pain-LTP and secondary hyperalgesia in the first 2 hours after HFS (n = 28; LTP1 subpopulation mean).
[e] Mean, $\log_{10}$ mean, and distribution parameters (SD) derived from Gaussian fit of individual time courses for the LTP1 subpopulation (n = 28).

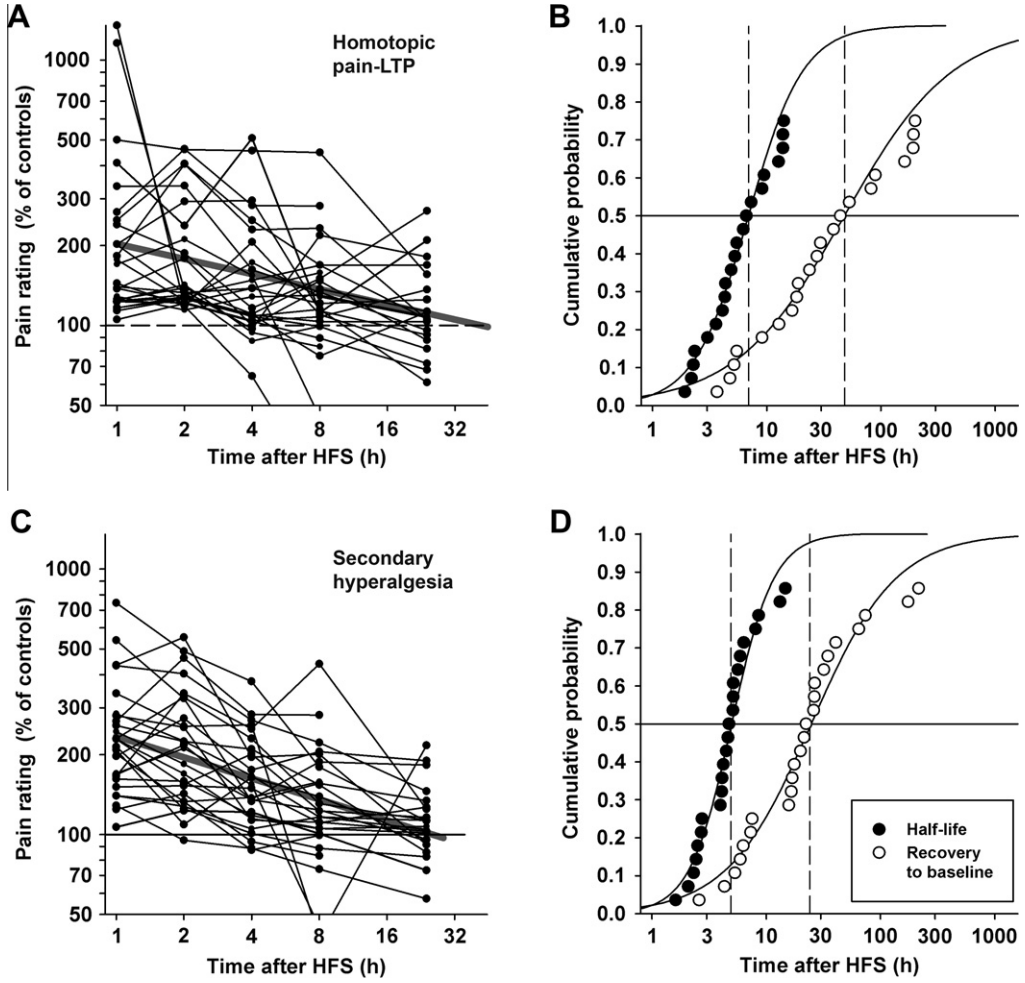

Fig. 3. Individual time courses of recovery from homotopic pain LTP and secondary hyperalgesia (A and C). Circles represent the individual $\log_{10}$ means of pain ratings of 28 subjects normalized to baseline and the contralateral control area averaged across a 20-min interval. The thick grey lines mark regression lines across the means of those 28 subjects. Cumulative normal distribution of the individual half-lives (solid circles) and predicted recovery time to baseline (open circles) for homotopic pain LTP (B) and secondary hyperalgesia (D). Mean half life ($t_{½}$) and predicted full recovery time ($t_{full\ recovery}$; see also Table 2) were estimated by Gaussian fit of the cumulative normal distribution functions (mean at intersection of 50% probability marked by the horizontal black line with the cumulative probability function). The time courses of 7 subjects for homotopic pain LTP (B) and of 4 subjects for secondary hyperalgesia (D) are not shown in these graphs because they did not fit the same unimodal normal distribution as a result of their very long half-lives, which suggested that they may be affiliated to a different subpopulation (for details, see text and Fig. 4).

1.679 ± 0.790) and 23.6 h ($\log_{10}$ mean ± SD 1.373 ± 0.611), respectively. There were no significant differences between the time course parameters of homotopic pain LTP and secondary hyperalgesia for this subgroup of subjects (both $P = .45$; Friedman ANOVA). Thus, all the above estimates of time-course parameters, either from individual or population functions, converged to similar results, regardless of whether they were estimated from the whole group or the subgroup exhibiting a higher magnitude of plasticity, and suggested the presence of early LTP1-like pain plasticity.

Notably, some subjects (10 of 28) deviated substantially from these LTP1-like psychometric functions in that they displayed very little or even no decrement of hyperalgesia within the 24-h observation period (Fig. 4), suggesting the additional induction of LTP2-like pain plasticity (LTP1 + LTP2 subgroup). In these subjects, extended time courses were usually observed for homotopic pain LTP or secondary hyperalgesia (7 of 28 for homotopic pain LTP and 4 of 28 subjects for secondary hyperalgesia); only 1 subject exhibited both. Interestingly, both subgroups did not differ significantly in their initial magnitude of homotopic pain LTP (160% vs 286% for LTP1 vs LTP1 + LTP2; $\log_{10}$ mean ± SEM 0.203 ± 0.039 vs 0.457 ± 0.129, $P = .09$) or secondary hyperalgesia (223% vs 223% for LTP1 vs LTP1+LTP2; $\log_{10}$ mean ± SEM 0.349 ± 0.043 vs 0.349 ± 0.054, $P = 1.00$). Precise determination of time courses in the LTP1 + LTP2 subgroup was impossible because the 24-h observation time window of was short relative to the estimated half-lives (>10 days to infinite; Fig. 4). Future studies should include measurements at later time points (over several days or weeks).

3.3. Correlation between the magnitudes and time courses of homotopic pain LTP, secondary hyperalgesia, and DMA

The magnitudes of homotopic pain LTP and secondary hyperalgesia were not correlated at any time in the whole study population (eg, $r = 0.10$ at 1 h after HFS, $P = .46$; Fig. 1C) as well as in the subgroup expressing substantial hyperalgesia (LTP+: $r = -0.19$, $P = .33$, data not shown). Moreover, there was no significant correlation between the magnitude and half-lives for both homotopic pain LTP and secondary hyperalgesia ($r = 0.29$ and $r = 0.31$, both $P > .10$, respectively). This lack of correlation was not due to lack of reliability because the magnitudes of homotopic pain LTP were highly correlated between the various time points of assessment until 8 h after HFS (mean correlation coefficient $r = 0.72$). The same held true for secondary hyperalgesia (mean correlation coefficient $r = 0.66$; both $P < .001$; Table 3).

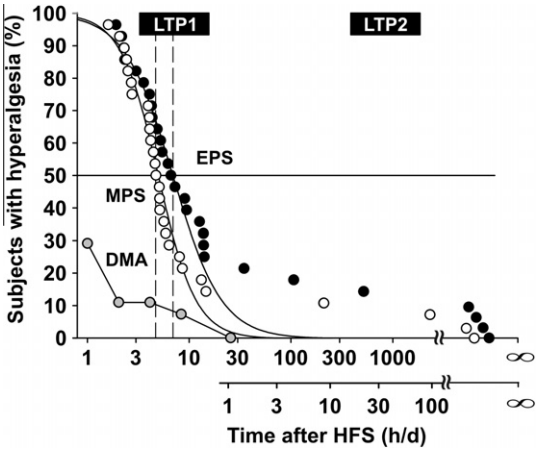

Fig. 4. Survival function of half-lives for individual time courses of homotopic pain LTP (EPS, solid circles) and secondary hyperalgesia (MPS, open circles). Circles represent the individual half-lives superimposed on a Gaussian fit. At the lower end, some subjects' half-lives (7 of 28 for EPS, 4 of 28 for MPS) deviate clearly from that function towards much longer half-lives (1 day to many days) characterized as LTP2-like pain plasticity. For comparison, the presence and time course of DMA are also shown (grey circles).

Table 3
Correlation coefficients of magnitudes of homotopic pain LTP and secondary hyperalgesia at different measuring times after HFS ($n = 55$).

Characteristic	1 h vs 2 h	2 h vs 4 h	4 h vs 8 h	Mean correlation
Homotopic pain LTP	0.58***	0.81***	0.74***	0.72***
Secondary hyperalgesia	0.74***	0.74***	0.43**	0.66***

** $P < .01$.
*** $P < .001$, Pearson's correlation.

Likewise, allodynia was not significantly correlated to either homotopic pain LTP or secondary hyperalgesia ($r = +0.04$ and $r = -0.13$, respectively, for the subgroup of $n = 16$; and $r = -0.05$ and $r = 0.10$, respectively, for the whole group of $n = 55$). The subgroups with our without DMA did also not differ in the expression of homotopic pain LTP (+34% vs +50% pain increase, respectively; $\log_{10}$ mean ± SEM 0.128 ± 0.055 vs 0.177 ± 0.045, $P = .50$). However, they did differ significantly in the expression of secondary hyperalgesia (+131% vs +75% pain increase, respectively; $\log_{10}$ mean ± SEM 0.363 ± 0.044 vs 0.243 ± 0.034, $P < .05$).

4. Discussion

In the present study, we demonstrated that both the time courses of homotopic pain LTP tested by electrical stimuli through the conditioning electrode (EPS) and secondary hyperalgesia to punctate mechanical stimuli (MPS) adjacent to conditioning HFS resemble early LTP (LTP1). This suggests the involvement of early-onset, transcription-independent mechanisms of long-term synaptic plasticity in both components of hyperalgesia. Although triggered simultaneously by HFS, homotopic pain LTP and secondary hyperalgesia were different and uncorrelated in individual magnitudes and time courses, pointing to differences in their post-translational mechanisms of plasticity. Some subjects (10 of 55) exhibited prolonged time courses of hyperalgesia, suggesting the additional induction of a more sustained hyperalgesia interpreted as LTP2-like pain plasticity. DMA was also induced in a smaller subgroup but exhibited a far shorter duration.

4.1. Homotopic pain LTP and secondary hyperalgesia share a LTP1-like time course

Animal studies on C-fibre-evoked field potentials in the spinal dorsal horn suggest that mechanisms of early LTP (LTP1)-like phosphorylation of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors by different protein kinases [46] as well as AMPA receptor insertion from a preformed pool into the postsynaptic membrane also occur in the nociceptive system [16,44]. We now show that electrically evoked homotopic pain LTP shares the time course of LTP1 for most subjects, suggesting that this component of hyperalgesia depends mainly on early LTP of synaptic transmission in spinal nociceptive neurons. Similarly, the decay of secondary hyperalgesia to pinprick stimuli is consistent with early-LTP1, which confirms a previous study on HFS-induced mechanical hyperalgesia in humans [16] and a previous study on pinprick hyperalgesia induced by intradermal capsaicin injection [23]. Thus, central sensitization of spinal nociceptive neurons underlying neurogenic secondary hyperalgesia to punctate mechanical stimuli also shares mechanisms of early LTP1 of synaptic transmission. This use-dependent type of central sensitization, however, differs from homosynaptic LTP in the hippocampus, neocortex, and also the spinal cord in that it involves heterosynaptic mechanisms (reviewed in [24]). As found previously, the second form of heterosynaptic facilitation, DMA, was much shorter lived (half-life about 2 h), and it was dynamically modulated by modulation of inciting ongoing pain, suggesting that short-term potentiation plays a more prominent role [21,22,27].

4.2. Dissimilarities between homotopic pain LTP and secondary hyperalgesia

Although the similar overall time courses suggested that early LTP1-like mechanisms largely govern both phenomena, the lack of correlations between the magnitude of homotopic pain LTP and secondary hyperalgesia, as well as between the individual time courses, point to some mechanistic differences. These findings add to differences shown in previous reports. First, low doses of the NMDA (N-methyl-D-aspartate) receptor antagonist ketamine just before conditioning HFS prevented homotopic pain LTP, but not secondary hyperalgesia [20]. Second, low-frequency stimulation at 1 Hz elicited long-term depression (LTD) in the facilitating pathway (homotopic analgesia [15,19], while at the same time pain to pinprick stimuli in adjacent skin was slightly enhanced (secondary hyperalgesia [19]). These differences suggest that the mechanisms of homotopic pain LTP is at least partly from those of secondary hyperalgesia.

4.3. Mechanistic implication

Compared to homotopic pain LTP, secondary hyperalgesia has been extensively studied [17,23,24,26,28,49] and may represent the behaviourally more relevant perceptual consequence of nociceptive conditioning as a result of its greater magnitude and much larger skin area involved (spread to uninjured skin sites). These heterosynaptic facilitation processes are driven not only by synaptic plasticity, but also by changes of segmental or descending inhibition or facilitation [5,34]. This may explain the different pharmacology and frequency dependence (see above). Any of these potential mechanisms will result in facilitation of the response. In contrast to homotopic long-term depression with the perceptual correlate of a decreased pain perception [19], heterotopic long-term depression of nociceptive transmission has not been shown so far.

Homotopic pain LTP to electrical stimuli is likely a far more complex phenomenon. It is tempting to conceive it as a perceptual

correlate of homosynaptic LTP [19] in the facilitating C-fibre pathway, and there is psychophysical evidence for a small component of facilitated C-fibre input [11]. However, homosynaptic plasticity may not only involve LTP, but also the opponent process LTD. Indeed, in the rat spinal cord, selective HFS of Aδ nociceptors induced LTD of nociceptive synaptic transmission rather than LTP [25,38,48]. Because LTP and LTD depend on the activation of different second messenger pathways (Ca^{2+}-dependent activation of protein kinases vs phosphatases) and/or the activation of different NMDA receptor subunits, namely NR2A and NR2B [7,30,47], conditioning HFS may drive partially opposing processes in parallel. Because the conditioning electrical stimulation inevitably activates both Aδ and C fibres, a homosynaptic facilitation (induced by C fibres) and homosynaptic depression (induced by Aδ-fibre input) may occur simultaneously explaining the significantly lower magnitude of homotopic pain LTP compared to secondary hyperalgesia.

An additional cause for differences in magnitude of homotopic pain LTP and secondary hyperalgesia may be the different test stimuli used (EPS, MPS). Mechanonociceptive Aδ-fibre input tested by pinprick is the sensory channel that is specifically gated in secondary hyperalgesia [28,49]. Other nociceptive sensory channels are not gated (eg, heat-sensitive nociceptors). Thus, the less specific electrical testing [13,32] may simply dilute the test signal, mixing facilitated and nonfacilitated inputs, leading to apparent weaker pain plasticity (see also [19]).

In this study, we found only a marginal correlation between homotopic pain LTP tested by electrical stimuli and secondary hyperalgesia tested by pinprick stimuli for both the magnitude and time course of the responses. We have met this limited correlation in all previous studies. Regardless of how high the correlation was in this and other data (ranking from $r = 0.10$ in this study to $r = 0.49$ in [18]), it was always low (always <25% of common variance), suggesting that these response were governed by partially different mechanisms. We propose that heterosynaptic mechanisms underlying secondary hyperalgesia, which are signalled by capsaicin-insensitive Aδ nociceptors, may also have partially contributed to homotopic pain LTP, leading to this limited correlation (see above).

4.4. Transition of early LTP1 to late LTP2 in human homotopic pain LTP and secondary hyperalgesia and possible relevance for the development of chronic pain disorders

Interestingly, some subjects' hyperalgesia time courses with half-lives beyond 30 h or nonadapting time courses did not fit the pattern of LTP1-like pain plasticity. This may possibly indicate the transition of pain plasticity into the domain of late LTP. Thus, even with this brief, intense conditioning input (5 × 1 s of HFS), longer-lasting LTP2/3 may have been initiated for which transcriptional processes and de novo protein synthesis are necessary. There is some evidence from in vivo and in vitro hippocampus electrophysiology that a single episode of HFS may lead to LTP1 lasting hours, but also to LTP2 lasting days or weeks [2,8,36] The molecular mechanisms of LTP can be divided into 2 phases: induction, ie, triggering the potentiation; and maintenance, ie, sustaining the potentiation over time. Although a number of factors are involved in the transition of early to late LTP (eg, [1,9,31,35]), only a single molecule, the brain-specific atypical protein kinase C isoform Mzeta (PKMzeta), has been found both necessary and sufficient for maintaining LTP [33,39]. Thus, sustained facilitation of pain perception, as it occurred in subjects with extended hyperalgesia responses, may involve induction of late LTP and induction of a maintenance mechanism.

From the current data, it is impossible to estimate the temporal properties of the LTP2 component precisely because more than half of the recovery times are extrapolated beyond 24-h observation. Thus, the precise analysis of this potentially important finding warrants future extended assessment protocols to confirm these results. Although the precise estimation of time constants in human pain plasticity awaits such more extended experimental protocols to delineate their precise duration, they may nevertheless indicate that a transition of pain plasticity into the LTP2 domain may occur even with very short-lived, intense pain stimuli. Subjects with facilitated transition from early to late LTP may be predisposed to LTP2-like lasting hyperalgesia responses and the development of chronic pain states.

4.5. Conclusion

We demonstrated that homotopic hyperalgesia to electrical stimuli induced by HFS shows decay characteristics of homosynaptic early LTP (LTP1) similar to that occurring in the hippocampus and other brain areas. HFS also induced heterosynaptically mediated secondary hyperalgesia that decayed with a similar time course, suggesting that posttranslational modification of synaptic transmission is the predominant neurobiological mechanism underlying both components of central sensitization in this brief and intense conditioning paradigm (5 × 1 s 100 Hz HFS). The lack of a substantial amount of common variance for both magnitude and time course of homotopic pain LTP and secondary hyperalgesia suggests that the central sensitization process within the facilitating and facilitated nociceptive pathways may recruit at least partially different mechanisms. There was a wide variation in magnitude and duration of pain plasticity responses. Some subjects (roughly 20%) developed long-lasting pain plasticity (days to weeks), which possibly indicated a transition from early LTP1 into late LTP2 of pain. This pronounced variability of LTP-like pain plasticity suggests individual differences in the propensity to develop long-lasting central sensitization and possibly chronic pain syndromes.

Conflict of interest

The authors declare that they have no conflict of interest.

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