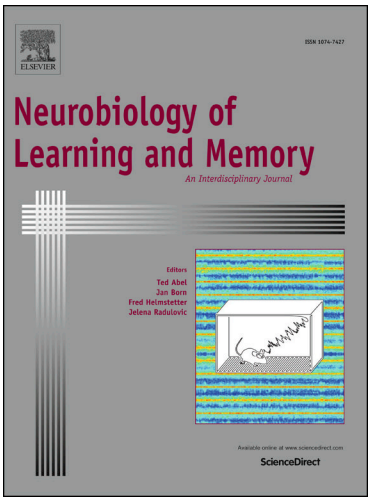

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The human pain system exhibits higher-order plasticity (metaplasticity)

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One sentence summary:

We demonstrate perceptual correlates of metaplasticity in the human pain system due to priming by high-frequency or very low-frequency stimulation.

Abstract

The human pain system can be bidirectionally modulated by high-frequency (HFS; 100Hz) and low-frequency (LFS; 1Hz) electrical stimulation of nociceptors leading to long-term potentiation or depression of pain perception (pain-LTP or pain-LTD). Here we show that priming a test site by very low-frequency stimulation (VLFS; 0.05Hz) prevented pain-LTP probably by elevating the threshold (set point) for pain-LTP induction. Conversely, prior HFS-induced pain-LTP was substantially reversed by subsequent VLFS, suggesting that preceding HFS had primed the human nociceptive system for pain-LTD induction by VLFS. In contrast, the pain elicited by the pain-LTP-precipitating conditioning HFS stimulation remained unaffected.

In aggregate these experiments demonstrate that the human pain system expresses two forms of higher-order plasticity (metaplasticity) acting in either direction along the pain-LTD to pain-LTP continuum with similar shifts in thresholds for LTD and LTP as in synaptic plasticity, indicating intriguing new mechanisms for the prevention of pain memory and the erasure of hyperalgesia related to an already established pain memory trace. There were no apparent gender differences in either pain-LTP or metaplasticity of pain-LTP. However, individual subjects appeared to present with an individual balance of pain-LTD to pain-LTP (a pain plasticity "fingerprint").

INTRODUCTION

Use-dependent long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission are established mechanisms of learning and memory formation in the hippocampus and neocortex (Artola & Singer, 1993; Bear & Malenka, 1994; Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Dudek & Bear, 1992). From the theory of LTP/LTD on a synaptic scale understanding of complex behaviors has emerged, which includes large scale phenomena like cognition, memory, fear or stress (e.g. Barco, Bailey & Kandel, 2006; Cooke & Bliss, 2006; Neves, Cooke & Bliss, 2008; Pape & Pare, 2010) and complex neurological and psychiatric pathophysiology as well as related treatments ranging from stimulus-based treatment regimens to drug discovery (Cooke & Bliss, 2005, 2006, 2011; Lynch, 2002; Marsden, 2013). The timeline of conceptual development concerning the relationship of neural plasticity and behavior has recently been comprehensively reviewed (Sweatt, 2016).

Although protective behaviors have been studied as readouts of LTP and LTD very early on (e.g. Kandel & Schwartz, 1982) it was not until the 1990s that it was discovered that LTP and LTD are also present at the first synapse of the nociceptive pathways in the mammalian spinal cord dorsal horn, where they control the sensitivity of the CNS to peripheral nociceptive inputs (Ikeda, Heinke, Ruscheweyh & Sandkühler, 2003; Randic, Jiang & Cerne, 1993; Svendsen et al., 1999). The pain system was the first in which the concepts of LTP and LTD-like responses were demonstrated to be relevant for plasticity of a human sensory system (Klein, Magerl, Hopf, Sandkühler & Treede, 2004). As predicted from *in vitro* and *in vivo* studies in animals (Ikeda, Heinke, Ruscheweyh & Sandkühler, 2003; Randic, Jiang & Cerne, 1993; Svendsen et al., 1999), high-frequency trains (100 Hz) from nociceptive primary afferents in humans elicited long-lasting LTP-like increases in pain perception (perceived as sustained hyperalgesia; 'pain-LTP'), lasting for hours or even days (Henrich, Magerl, Klein, Greffrath & Treede 2015; Klein, Magerl, Hopf, Sandkühler & Treede, 2004; Klein, Magerl & Treede, 2006; Pfau et al., 2001). Conversely, sustained low-frequency stimulation (1 Hz) led to LTD-like reduction in pain sensitivity (analgesia/hypoalgesia; 'pain-LTD') suggesting that spinal LTP and LTD are likely controlling pain sensitivity also at the behavioral level in humans (Klein, Magerl, Hopf, Sandkühler & Treede, 2004; Jung, Rottmann & Ellrich, 2009).

In the hippocampus and neocortex, the degree and direction of synaptic plasticity are modified by the state of synaptic efficacy and the recent activation history of a pathway precipitating a shift in the modification threshold (set point), which is defined either as the frequency, the level of postsynaptic depolarisation or the intracellular calcium concentration at which LTD crosses over to LTP (Artola & Singer, 1993; Bienenstock, Cooper & Munro, 1982; Cooper &

Bear, 2012; Dudek & Bear, 1992; Ngezahayo, Schachner & Artola, 2000; Yang & Faber, 1991) to the right (facilitating LTD and/or inhibiting LTP induction), or left (inhibiting LTD and/or facilitating LTP induction) (Coan, Irving & Collingridge, 1989; Fujii et al., 1996; Holland & Wagner, 1998; Huang, Colino, Selig & Malenka, 1992; Mayford, Wang, Kandel & O'Dell, 1995; Wang & Wagner, 1999; Wexler & Stanton, 1993). This activity-dependent higher-order modulation of synaptic plasticity [termed "metaplasticity" first in a paper from Roger Tsien's group (Deisseroth, Bito, Schulman & Tsien, 1995), but coining of the term fairly credited to W.C. Abraham (Abraham, 2008; Abraham & Bear, 1996; Abraham & Tate, 1997; Bear, 1995)], operates to "maintain the neural network on a standby mode to be able to relay new salient information upon entry*"* (Bear, 1995). Although metaplasticity was originally conceived as a sliding threshold for transition from LTD to LTP (Bear & Malenka, 1994; Stanton, 1996), recent metaanalysis suggests that it may operate by enhancing the separation of LTD and LTP, and expanding the "no man's land" at around the LTD-to-LTP transition threshold (a term coined by John Lisman; Artola, 2008; Lisman, 2001; Ngezahayo, Schachner & Artola, 2000). Although to date many hundred papers on metaplasticity have been published, the vast majority were related to hippocampal, cortical or amygdala plasticity, often in reduced preparations. Most recently, Yger and Gilson (2015) have reviewed all current models and concluded "that metaplasticity is an ubiquitous mechanism acting on top of classical Hebbian learning [i.e. as higher order plasticity] and promoting the stability of neural function over multiple timescales" and has thus to be conceived as a "key element in the framework of plasticity models" (Yger & Gilson, 2015, p.1).

Human studies are much rarer and have mostly related changes in motor cortical excitability to metaplasticity of the primary motor cortex (Bliem, Müller-Dahlhaus, Dinse & Ziemann, 2008; Delvendahl et al., 2010; Gentner, Wankerl, Reinsberger, Zeller & Classen, 2008; Hamada et al., 2009; Todd, Flavel & Ridding, 2009; Wankerl, Weise, Gentner, Rumpf & Classen, 2010). Only few human studies have related behavioral modification of e.g. motor evoked potential (Ni, Gunraj, Kailey, Cash & Chen, 2014) or the primary somatosensory evoked potential N20 (Bliem, Müller-Dahlhaus, Dinse & Ziemann, 2008) by paired associative stimulation, or impairment of tactile spatial acuity by theta burst stimulation (Jones et al., 2016) to metaplasticity. The spinal cord has only very recently been considered as a possible site of synaptic metaplasticity, notably using nociceptive transmission as an example, e.g. demonstrating that neonatal injury alters spinal spike-timing dependent plasticity (Li & Baccei 2016).

Evidence on a behavioral level for metaplasticity of spinal relays induced by training or inflammation have been shown (Grau et al., 2014). Notably, following spinal injury models they have related different signaling pathways to adaptive vs. maladaptive spinal mechanisms, the latter being a central concept in our understanding of development of persistent pain. In the present study we have used a well-established human model of LTP-like plasticity of pain perception (pain-LTP) to elucidate whether and how the recent activation history of a nociceptive pathway can induce activity-dependent metaplastic higher-order modulations of plasticity (i.e. plasticity of pain plasticity) in the human pain system on a behavioral levels, namely the conscious perception of pain.

MATERIALS AND METHODS

1. Subjects

This three-way cross-over study was performed in 12 healthy subjects (5 female, 22 - 37 years, mean age 25 years). Subjects were excluded from the study if their medical history or clinical examination provided evidence of neurological or dermatological disorders. Each subject was familiarized with the experimental procedure prior to the experiments but was naïve with respect to the scientific reasoning and hypotheses being tested.

The study was approved by the local ethics committee and subjects had given written informed consent according to the declaration of Helsinki. All subjects were comfortably seated in a reclining chair with their forearms on armrests.

2. Experimental design

Changes in pain perception to painful electrical test stimuli after high-frequency (HFS) and very low-frequency electrical stimulation (VLFS) were quantified by comparing pain ratings to single electrical test stimuli between the conditioned test site and an unconditioned contralateral control site.

2.1. Electrical stimuli

Conventional transcutaneous nerve stimulation with large surface electrodes recruits Aβ fibers at much lower current strengths than nociceptive Aδ- and C fibers. Thus it is difficult to obtain sufficient nociceptive input by this electrode configuration. The electrical threshold for nociceptive afferents decreases dramatically within their receptive field due to the superficial location of their axon terminals (Meyer, Davis, Cohen, Treede & Campbell, 1991). We exploited this property by applying all electrical stimuli through stainless steel punctate

electrodes (diameter: 250 µm). Because of the small diameter of the electrodes, a high current density was achieved already at low stimulus intensities, which favors activation of superficial epidermal nociceptive Aδ- and C fiber afferents (Inui, Tran, Hoshiyama & Kakigi, 2002). To achieve spatial summation within the receptive field of spinal cord neurons, 10 of these electrodes were arranged in a 6 mm circular array mounted in a small circular plastic frame, which was attached to the skin by double-adhesive tape. Cathodal electrical stimuli (pulse width: 2 ms) were applied via this array to the ventral forearm about 5 cm distal to the cubital fossa by a constant current stimulator (DS7H; Digitimer, Welwyn Garden City, UK). A large surface electrode on the ipsilateral upper arm served as an anode.

2.2. Individual electrical detection thresholds

Individual electrical detection thresholds (T) to single electrical test stimuli were determined by the geometric mean of five ascending and five descending series of single pulses [method of limits]. Mean electrical detection threshold across all experiments and test sites was 0.13 ± 0.09 mA (mean ± SD). Stimuli at the detection threshold were usually perceived as a slightly pricking and/or burning sensation (Hansen, Klein, Magerl & Treede, 2007), indicating sufficient activation of nociceptors (Ochoa & Torebjörk, 1989) already at detection threshold.

2.3. Single electrical test stimuli

Single electrical test stimuli were applied every 60 s (i.e. at 0.017 Hz) alternating between the conditioned skin site (test site) and the contralateral unconditioned control site. Stimulus intensity was adjusted at 10 x T. These pulses elicited a mild pain sensation in normal skin [c.f. (Klein, Magerl, Hopf, Sandkühler & Treede, 2004)]. The stimulus frequency we used did not prevent 'pain-LTP'-induction in previous studies.

2.4. High-frequency electrical stimulation (HFS)

High-frequency stimulation (HFS: 5 trains of 1s duration at 100 Hz, repeated at 10 s intervals) was applied to the left forearm through the same electrode as the test stimuli. The right forearm served as an unconditioned mirror-image control site. The stimulus intensity was set at 1.5 mA. HFS at this intensity has been shown to elicit moderate to strong pain, followed by long-term enhancement of perceived pain to the electrical test stimuli in normal naïve skin ["pain LTP"; c.f. (Hansen, Klein, Magerl & Treede, 2007; Klein, Magerl, Hopf, Sandkühler & Treede, 2004)].

2.5. Very-low frequency stimulation (VLFS)

The frequency of single electrical test stimuli was tripled compared to 'normal' pain testing (see 2.3.) by intercalating 120 additional electrical stimuli at 2.5x, 5x, 20x and 40xT between two electrical test stimuli at 10 x T. The sequence of electrical stimuli was: 2.5 x T, 5 x T, 10 x T, 20 x T, 40 x T, 10 x T (repeated 30 times), Altogether 180 single pulses were delivered at each test site at a frequency of 0.05 Hz (interstimulus interval of 20 s). Because the term 'low-frequency stimulation' usually refers to frequencies of about 1 Hz, we termed this protocol very-low frequency stimulation (VLFS). VLFS was applied either before HFS (experiment 2) or 1 h after HFS (experiment 3) in the test and control areas.

2.6. Experimental protocol

Each subject participated in the following three experiments. Their sequence was balanced across subjects:

Experiment 1 (Exp.1): HFS after normal baseline testing (original pain LTP protocol)

Baseline testing by single electrical test stimuli for one hour was followed by HFS five minutes after termination of the baseline testing. Changes in pain perception after HFS were followed up for another 3.5 h.

Experiment 2 (Exp.2): VLFS prior to HFS

VLFS was delivered during the baseline period over one hour. Five minutes after the last stimulus, HFS was given and testing at 0.017 Hz continued for another 3.5 h afterwards.

Experiment 3 (Exp.3): HFS prior to VLFS

Baseline testing (for one hour) was followed by HFS, and testing at 0.017 Hz continued for another hour (protocol like Exp. 1 for the first 2 hours). Then, very low frequency stimulation (VLFS) was delivered during the second hour after HFS, and testing at 0.017 Hz continued for another 1.5 h afterwards.

2.7. Pain ratings

Subjects rated the magnitude of pain to single electrical test stimuli as well as to HFS-trains on a numerical rating scale (NRS) ranging from 0 (non-painful) 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.

2.8. Data evaluation and statistics

Pain ratings to single electrical test stimuli at 10 x detection threshold were analyzed and transformed into decadic logarithmic values in order to achieve a secondary normal distribution of pain ratings. To avoid loss of zero-values, a small constant (0.1) was added to all ratings prior to logarithmic transformation (Magerl, Wilk & Treede, 1998). All pain ratings were referenced to the mean value of the one-hour baseline period, separately for test and control sites, by building the difference in log-transformed pain ratings. This procedure is equivalent to building a ratio of original pain ratings, but it avoids the skewed non-normal distribution of ratio data. In a second step, pain ratings referenced to baseline at the conditioned skin site were normalized to those at the control site. Baseline-referenced pain rating data were analyzed by a two-way repeated measures ANOVA (factors: test site and time) and least significant difference (LSD) post hoc tests to determine differences between conditioned and control skin sites over time. Pain ratings were analyzed for significant correlation between different experiments and states of potentiation (single-tailed probability calculations since only positive correlations were expected). To evaluate the habituation of pain ratings, we carried out a two-way ANOVA (factors: test site and first vs. last stimulus in baseline series) and least significant differences (LSD) post hoc tests.

RESULTS

Pain-LTP-inducing high-frequency electrical stimulation (HFS)

High-frequency electrical stimulation (HFS) elicited a strong pain in unconditioned skin (control experiment 1) rated on average as 53/100 on a 0-100 numerical rating scale (NRS) and gradually increasing to 63/100 NRS (Fig.1A). Average HFS-induced pain across all five trains was 59.5/100 (log10: 1.775 ± 0.041; Fig.1B). In experiment 2, HFS followed after a one hour baseline period of very low-frequency stimulation (VLFS). Pain ratings to HFS exhibited a similar slow increase and were of similar magnitude (51.6/100; log10: 1.712 ± 0.052, p=0.35 vs. exp.1). In experiment 3, HFS was also executed in unconditioned skin to be followed after one hour by a one hour period of very low-frequency stimulation (VLFS). Pain ratings in experiment 3 also exhibited the same slow increase and were of similar magnitude as both other experiment (51.6/100; log 10: 1.739 ± 0.052, p=0.53 vs. exp.1 and p=0.69 vs. exp.2).

Induction of pain-LTP in the naïve state of the nociceptive pathway (HFS alone; experiment 1)

To address these questions we first established the reference condition in a 4.5-hour experiment inducing an abrupt increase of pain sensitivity ['pain-LTP']. Single electrical test pulses at 10 x detection threshold (T), which were used to test sensitivity of the conditioned and control pathways elicited a very weak pain sensation (average pain rating 4.4/100 numerical rating scale, NRS) when given for the very first time in naïve skin (Fig. 2A). Stimulus repetition of the test pulses at a rate of 1 per minute resulted in an exponential decrease of perceived pain intensity over a one-hour baseline period at both the test site and unconditioned contralateral control site due to habituation (Harris, 1943; Rankin et al., 2009; Thompson & Spencer, 1966). However, habituation and dishabituation were completely uncorrelated to the magnitude of the subsequent pain-LTP (r = -0.11, n.s.) or decrement of the pain-LTP response (see accompanying Data in Brief article for details on distinction of the pain-LTP response from dishabituation; Magerl, Hansen, Treede & Klein 2018). Five trains of high frequency electrical stimulation (HFS; 100 Hz for 1 s at 10 x T, given every 10s at the test site) evoked intense pain (average NRS 59.5/100). Single test pulses after HFS revealed that HFS elicited a marked increase in pain sensitivity as compared to the control site (Fig.2A). When ratings at the test site were normalized to the corresponding ratings at the control site, the HFS-related pain increase became apparent as an LTP-like step increase in pain sensitivity (pain-LTP; Fig.2B). Average pain increase during the first hour after HFS was +57% above the unconditioned contralateral control site (log10 value: +0.195 ± 0.046, $p<0.002$), and pain remained significantly increased throughout the full 3.5-hour observation period (+45%, and +38% above the control site in the second and third hours after HFS, see also Fig.3A).

Prevention of HFS-induced pain-LTP by very low-frequency priming (VLFS preceding HFS; experiment 2)

It has been shown in the hippocampus (Coan, Irving & Collingridge, 1989; Fujii, Saito, Miyakawa, Ito & Kato, 1991; Fujii et al., 1996; Gisabella, Rowan & Anwyl, 2003; Holland & Wagner, 1998; Huang, Colino, Selig & Malenka, 1992; Mayford, Wang, Kandel & O'Dell, 1995; Wang & Wagner, 1999; Wexler & Stanton, 1993) and spinal dorsal horn (Miletic & Miletic, 2001) that induction of LTP can be prevented by priming a pathway with low-frequency stimulation (LFS). Therefore we tripled the frequency of test stimuli during the one hour baseline period preceding HFS (from 1 to 3 min^{-1}) by intercalating additional stimuli with varying stimulus intensities (2.5 – 40 x T @ 0.05 Hz, VLFS; very low-frequency stimulation protocol), which was still below the frequency (1 Hz) that we have previously used to induce pain-LTD (Klein, Magerl, Hopf, Sandkühler & Treede, 2004; see accompanying Data in Brief

article for details on distinction of the pain-LTD response from habituation; Magerl, Hansen, Treede & Klein 2018). As shown in Fig.2C, habituation was more pronounced during VLFS (experiment 2) compared to experiments 1 and 3, namely -78.0% vs. 42.4% (log10 mean: 0.657 ± 0.078 vs. 0.239 ± 0.035; $p < 0.001$), which is fully explained by the larger number of stimuli given (habituation rate/stimulus: experiment 2 (VLFS): 0.93 ± 0.15 % vs. 0.91 ± 0.25 % in experiments 1 and 3; $p = 0.95$). Under this condition, HFS applied at the test site five minutes after the last baseline measurement induced no separation between mean pain ratings at the test and control sites in raw data (Fig.2C) as well as normalized data (Fig2D and Fig.3B). Thus, in the group average pain-LTP was completely absent after HFS preceded by priming VLFS.

Reversal of HFS-induced pain-LTP by very low frequency stimulation (HFS preceding VLFS; experiment 3)

Numerous studies have demonstrated that LTP induced by HFS can be reversed by subsequent LFS protocols (Bashir & Collingridge, 1994; O'Dell & Kandel, 1994; Fujii, Saito, Miyakawa, Ito & Kato, 1991; Liu, Morton, Azkue, Zimmermann & Sandkühler, 1998), which without prior application of priming stimulation do not necessarily induce any synaptic plasticity themselves (Miletic & Miletic, 2001). We and others have shown that LFS at 1 Hz led to an LTD-like decrease in pain perception in naïve humans [pain-LTD; (Jung, Rottmann & Ellrich, 2009; Klein, Magerl, Hopf, Sandkühler & Treede, 2004)] and induces nociceptive LTD and reversal of nociceptive LTP in the rat spinal dorsal horn (Liu, Morton, Azkue, Zimmermann & Sandkühler, 1998).

To test whether VLFS with 0.05 Hz reverses pain-LTP, we applied VLFS during the time interval of 60 to 120 min after the induction of pain-LTP (+44% in the first hour after HFS; log10 value: +0.158±0.056, $p<0.02$ vs. control site). As shown in Fig.2E (raw data) and Fig.2F (normalized data), VLFS elicited a rapid decrease of pain ratings in the potentiated pathway, whereas it resulted in a transient increase in pain ratings at the control site (due to dishabituation, correlation with preceding habituation: $r=-0.85$, $p<0.001$). After the end of VLFS, pain ratings to test stimuli at 1 min^{-1} at the test and control sites were indistinguishable (-10%; log10: -0.046 ± 0.030, $p=0.15$ vs. control site), demonstrating in the group average that VLFS fully reversed a previously established pain-LTP (Fig.2E and F, and Fig.3C).

Threshold shifts for the induction of pain-LTP and pain-LTD highlight individual threshold signatures

The absence of pain-LTP after preceding VLFS may be due to a loss of synaptic plasticity in the pain pathways ("prevention" of LTP) or it may indicate a rightward shift of the thresholds for LTP induction towards higher frequencies. Analysis of individual changes in pain sensitivity after HFS across all three experiments provides evidence for such threshold shifts of human pain-LTP. Fig. 4A shows individual changes in pain sensitivity after HFS with (experiment 2) or without priming by preceding VLFS (Exp. 1). Prevention of pain-LTP in the primed condition would predict that after preceding VLFS, the subjects would not develop pain-LTP regardless of response magnitude in the non-primed condition (i.e. the dashed lines of individual subjects in Fig.4A would converge and pain ratings at the HFS site would not differ from the contralateral control site). However, subjects with strong pain-LTP in the naïve state (experiment 1) still showed some LTP after HFS with preceding VLFS (experiment 2), while 3 subjects with little pain-LTP in the naïve state even exhibited LTD after HFS when primed by preceding VLFS. Thus, relative individual susceptibility of developing synaptic plasticity in response to HFS was maintained after VLFS ($r=0.58$, $p<0.05$; illustrated by parallel dashed lines in Fig 4A). According to the concept of metaplasticity, these data suggest that priming VLFS had shifted the threshold for the induction of pain-LTP to the right (Ngezahayo, Schachner & Artola, 2000; Bienenstock, Cooper & Munro, 1982; Cooper & Bear, 2012; Yang & Faber, 1991).

Reversal of pain-LTP by subsequent VLFS, which itself does not lead to long-lasting synaptic modulation in the non-primed normal state, may either indicate the depotentiation of previously facilitated synapses or it may also be due to a shift in threshold. The latter would imply that preceding HFS may have primed the pathway to subsequent LTD induction by VLFS, as has been suggested by Artola and colleagues (Artola, 2008; Artola, Bröcher & Singer, 1990; Ngezahayo, Schachner & Artola, 2000). Both phenomena involve at least partly discriminable mechanisms (Lee, Barbarosie, Kameyama, Bear & Huganir, 2000). Fig.4B shows changes in individual pain sensitivity after HFS with or without subsequent VLFS (Exp. 3; before vs. after VLFS). True depotentiation of pain-LTP would imply that after VLFS pain-LTP estimates regardless of their magnitude after HFS have to converge to that of the unconditioned control site (i.e. as converging dashed lines of single subjects in Fig.4B). However, in the present study, the degree of pain decrease after VLFS was not proportional to the sensitizing effect of the preceding HFS, as demonstrated by the parallel dashed lines in Fig.4B and the significant correlation between the magnitude of pain perception before and after VLFS ($r=0.65$, $p<0.05$). In subjects in whom HFS elicited little or no pain-LTP, VLFS shifted responses into the pain-LTD domain, whereas in those developing strong pain-LTP,

pain-LTP was partially retained, but reduced. This is a strong argument against simple depotentiation of facilitated synapses (Bashir & Collingridge, 1994; Fujii, Saito, Miyakawa, Ito & Kato, 1991; Gisabella, Rowan & Anwyl, 2003). Instead, these data suggest that priming by HFS facilitated pain-LTD induction by VLFS by shifting the threshold for LTD-induction in the nociceptive pathways to the left (towards lower frequencies) consistent with the concept of metaplasticity (Artola, 2008). Conspicuously, subjects usually retained their position in the cohort in the normal compared to the primed states suggesting that every subject exhibited an individual set point of pain-LTP/pain-LTD balance (a pain plasticity "fingerprint").

No gender differences in the magnitude of pain-LTP and metaplasticity

Although it is obvious that the present study (encompassing two experiments with 12 subjects, each) was underpowered to study differences of subgroups it may nevertheless be of interest to compare the responses of male (n=7) and female subjects (n=5), since there is a pronounced preponderance of female pain patients in many chronic pain diseases (Greenspan et al. 2007). However, pain to single test stimuli at baseline (3.0/100 NRS, each; log10 mean: 0.475±0.095 vs. 0.475±181, p=0.94) and pain elicited by HFS (56/100 vs. 58/100 NRS; log10 mean: 1.748±0.051 vs. 1.763±0.059, p=0.85) were almost the same. Although not statistically significant, female subjects tended to exhibit a smaller magnitude of pain-LTP (+30% vs. +73%; log10 mean: 0.113±0.057 vs. 0.239±0.088, p=0.25). However, this is likely not representative for a gender difference, since in a previous sufficiently powered study (n=55) we could show that male and female pain-LTP were of the same magnitude (Pfau et al. 2011). Moreover, metaplasticity as reflected by the prevention of pain-LTP by preceding VLFS or reversal of pain-LTP by subsequent VLFS was undistinguishable between male and female subjects (Fig.4).

DISCUSSION

Overall, these data suggest that the activation history of nociceptive afferents in a human experimental pain model using epicutaneous electrical stimulation (Henrich, Magerl, Klein, Greffrath & Treede 2015; Inui, Tran, Hoshiyama & Kakigi, 2002; Jung, Rottmann & Ellrich, 2009; Klein, Magerl, Hopf, Sandkühler & Treede, 2004) strongly influences the extent and direction of pain plasticity induced by subsequent stimulation with different frequencies (i.e. inhibition of pain LTP in response to subsequent HFS, facilitation of pain-LTD in response to subsequent VLFS). This demonstrates that plasticity of signal transmission in the nociceptive system, which is mirrored in frequency-dependent pain-LTD and pain-LTP on the perceptual level, exhibits modifiable induction thresholds. In other words, this example of experimental

modification of pain plasticity is the first demonstration of higher-order plasticity of LTD/LTP ("metaplasticity") in a human sensory system having direct consequences for the magnitude of perceived pain [for comprehensive reviews on metaplasticity (Abraham, 2008; Hulme, Jones & Abraham, 2013; Yger & Gilson 2015)].

Metaplasticity serves to optimize the information storage capacity by flexibly adjusting the thresholds for LTP/LTD (Artola & Singer, 1993; Bienenstock, Cooper & Munro, 1982; Cooper & Bear, 2012) and increasing the stimulus-selectivity of the neuronal response (Abraham, 2008), whereas Hebbian plasticity such as LTP/LTD is believed to be the cellular mechanism for storing information associatively at individual synapses in an input-specific manner (Neves, Cooke & Bliss, 2008). Metaplasticity of nociceptive pathways has been demonstrated in the rat spinal cord *in vitro*. After increasing the frequency of a priming stimulus from 1/5min to 1/min, HFS induced spinal LTD instead of LTP (Miletic & Miletic, 2001), suggesting a shift in the LTP threshold to higher frequencies of the conditioning input and related intracellular calcium concentrations (Sandkühler, 2000; Sandkühler & Gruber-Schoffnegger, 2012). A series of studies in spinalized rat demonstrated suppression of nociceptive flexor reflex learning by non-contingent low-frequency noxious electrical shocks, which lasted for 2-4 days (Crown, Ferguson, Joynes & Grau, 2002; Ferguson et al., 2008; Huie et al., 2012). Amputation-induced loss of synaptic depression and facilitation of sensory activation in the anterior cingulate cortex has also been interpreted as metaplasticity in the nociceptive system (Kang et al., 2012; Wei & Zhuo, 2001; Wei, Li & Zhuo, 1999), and a mild preconditioning nerve lesion in the nociceptive system inhibited the induction of behavioral signs of neuropathic pain by a subsequent nerve injury (Moalem-Taylor, Li, Allbutt, Wu & Tracey, 2001). The restriction of the plasticity as well as metaplasticity effects we describe to the ipsilateral side suggests that both are likely predominantly an aspect of spinal nociceptive processing. Although the local restriction of the pain-LTP effect suggests spinal mechanisms the additional contribution of cortical areas is likely since pain-related potentiation of nociceptive processing has been shown in the insular and anterior cingulate cortex (Bliss, Collingridge, Kaang & Zhuo, 2016; Zhuo, 2007 & 2016).

For obvious reasons, the exact molecular mechanisms underlying the metaplasticity-like effects observed here cannot be comprehensively addressed in humans due to unavailability of many pharmacological tools for human studies. These mechanisms may reside in either spinal or supraspinal structures of the nociceptive pathways or both, and they likely involve a complex interaction of facilitatory and inhibitory mechanisms acting at AMPA and NMDA ionotropic glutamate receptors (Huang, Colino, Selig & Malenka, 1992; Lee, Barbarosie,

Kameyama, Bear & Huganir, 2000; Rebola, Carta, Lanore, Blanchet & Mulle, 2011), metabotropic glutamate receptors (Ferguson et al., 2008; Kang et al., 2012; Manahan-Vaughan, 1998), gamma-aminobutyric acid (GABA) receptors (Adermark & Lovinger, 2009; Miletic & Miletic 2001), μ-opioid receptors (Drdla-Schutting, Benrath, Wunderbaldinger & Sandkühler, 2012), or CB1 endocannabinoid receptors (Adermark & Lovinger, 2009; Kato et al. 2012; Yang, Lei, Xie, MacDonald & Jackson 2014). Noticably, we have shown recently that the mechanical pain sensitivity in an autoimmune neuropathic condition (Devic's disease) was strongly modulated by 2-AG switching from hyperalgesia to hypoalgesia with increasing plasma levels of 2-AG (Pellkofer et al. 2013).

Synaptic homeostasis of the nervous system, i.e. adjusting the neuronal excitability within a physiological range to promote network stability (Turrigiano, 2008, 2011), is a mechanism that has likely contributed to our findings. Metaplasticity is one mechanism of homeostatic regulation of synaptic strength (Desai, 2003; Hulme, Jones & Abraham, 2013) characterized by a set point modulation or by threshold shifts (Bienenstock, Cooper & Munro, 1982; Ngezahayo, Schachner & Artola, 2000) and has been reported in corticospinal pathways (Murakami, Müller-Dahlhaus, Lu & Ziemann, 2012) and modulation of somatosensory evoked potentials (Bliem, Müller-Dahlhaus, Dinse & Ziemann, 2008) in humans. Results of both priming protocols in our study are compatible with expansion of the range of LTD-inducing frequencies towards higher and lower frequencies. Consistent with the Bienenstock-Cooper-Munro (BCM) model of set-point modulation, VLFS on its own did not have any effect on synaptic efficacy (Bienenstock, Cooper & Munro, 1982). The set-point modulation may involve a moderate activation of NMDA receptors by the priming protocols, which then prevents the subsequent induction of LTP. We have previously shown that our stimulation protocol engages NMDA-receptors in humans (Klein et al., 2007). The engagement of NMDA receptors by priming regimens effectively reducing subsequent LTP induction in animals has consistently been shown for several brain structures (Coan, Irving & Collingridge, 1989; Fujii et al., 1996; Gisabella, Rowan & Anwyl, 2003; Huang, Colino, Selig & Malenka, 1992; Mockett, Coussens & Abraham, 2002; Rebola, Carta, Lanore, Blanchet & Mulle, 2011; Youssef, Addae & Stone, 2006). There is also a contribution of intracellular calcium through either voltage-dependent calcium channels or calcium mobilization from intracellular stores (Hulme, Jones, Ireland & Abraham, 2012; Wankerl, Weise, Gentner, Rumpf & Classen, 2010). In the human motor cortex, low frequency stimulation increased intracortical inhibition and occluded LTP- and LTD-like plasticity (Delvendahl et al., 2010; Hamada et al., 2009).

Metaplasticity, the plasticity of synaptic plasticity has a pivotal role in activity-dependent modulation of synaptic connectivity underlying learning and memory (Hulme, Jones & Abraham, 2013; Eckert & Abraham, 2013). Notably, prior or ongoing natural experience triggers metaplasticity in sensory neocortex occluding subsequent induction of LTP and enhancing LTD of synaptic responses (Clem, Celikel & Barth, 2008). Conversely, exposure to enriched environments even weeks after induction of stable hippocampal LTP interfered with LTP consolidation and triggered a rapid decay of LTP, which would otherwise be sustained for more than one year (Abraham, Logan, Greenwood & Dragunow, 2002). While metaplasticity renders synaptic plasticity flexible and apt to adapt to varying conditions rigidity of the LTP/LTD response, i.e. reduced or absent metaplasticity is associated with uncontrollable stress (Foy, Stanton, Levine & Thompson, 1987; Shors, Seib, Levine & Thompson, 1989; Inoue et al., 2013; Schmidt, Abraham, Maroun, Stork & Richter-Levin, 2013) and with behavioral or metabolic diseases like depression, diabetes, and even normal aging (Artola, 2008; Hulme, Jones & Abraham, 2013; Marsden, 2013; Zorumski & Izumi, 2012).

Taken together, we suggest that understanding the mechanisms and prerequisites of metaplasticity in the human pain system may bear important implications for the prevention and erasure of pain memory, since nociceptive LTP is likely an important process in the generation of chronic pain (Ruscheweyh, Wilder-Smith, Drdla, Liu & Sandkühler, 2011). Accordingly, dysregulation of CNS plasticity has been suggested to be involved in the pathophysiology of neuropathic pain (Sandkühler, 2000; Sandkühler & Gruber-Schoffnegger, 2012). Loss of metaplasticity precipitated by stress and other comorbid pathological conditions (Artola, 2008; Foy, Stanton, Levine & Thompson, 1987; Grau et al. 2014, 2017; Inoue et al., 2013; Hulme, Jones & Abraham, 2013; Marsden, 2013; Schmidt, Abraham, Maroun, Stork & Richter-Levin, 2013; Shors, Seib, Levine & Thompson, 1989; Zorumski & Izumi, 2012) may lead to maladaptive response rigidity of the nociceptive system offering a possible explanation for the fact that stress, anxiety, or depression are frequent comorbidities of chronic pain, and vice versa (Dickens, McGowan & Dale, 2003; Marsden 2011, 2013). Understanding the processes of plasticity and metaplasticity that either prevent or abolish experimentally-induced pain-LTP or pain-LTD may guide the development of activity-dependent treatment strategies for chronic pain, e.g. it may lead to rational strategies for tailoring transcutaneous nerve stimulation or deep brain stimulation. The data described here may also provide a translational perspective beyond nociception and pain, by offering a

window into a more general study of synaptic flexibility in conscious humans using metaplasticity of pain-LTP as a model system.

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FIGURES

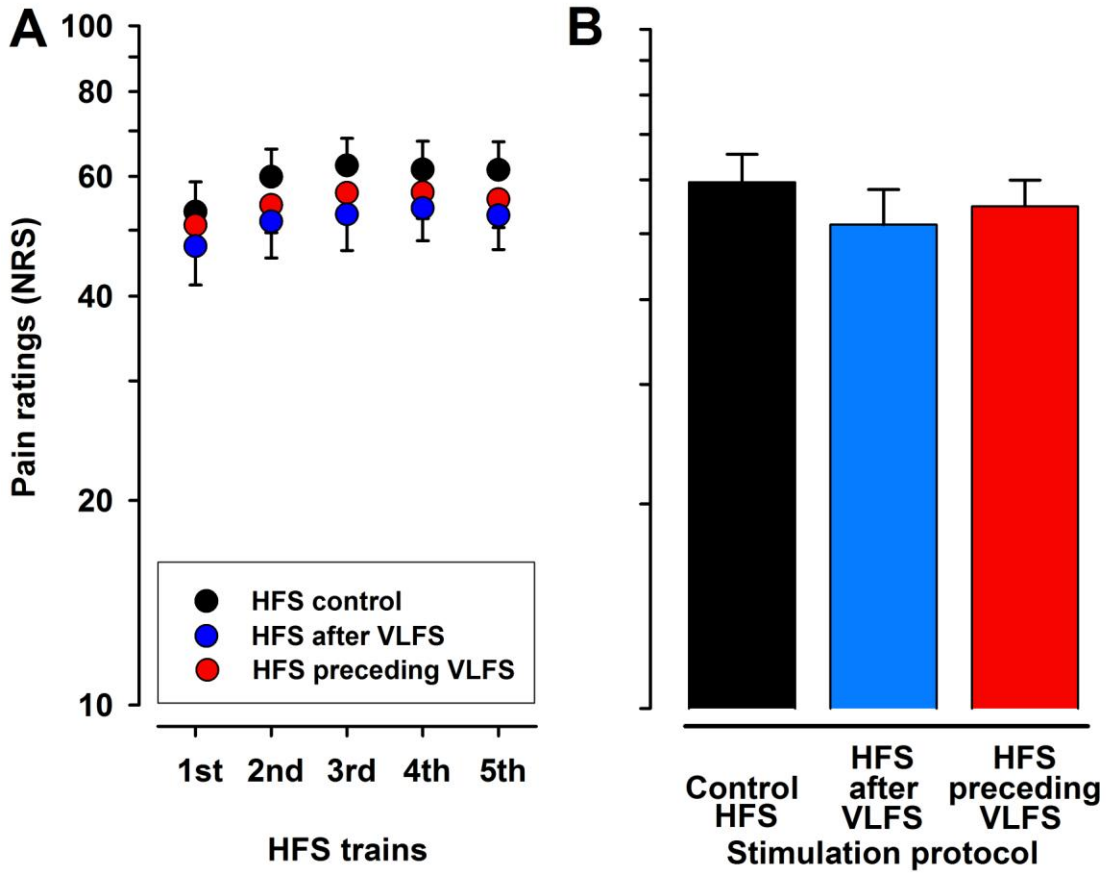

Fig.1 (A) Pain ratings to five trains of 1 s high-frequency stimulation (HFS) at 10 x detection threshold. **(B)** Average pain ratings across all five trains of 1 s high-frequency stimulation. Mean ± SEM.

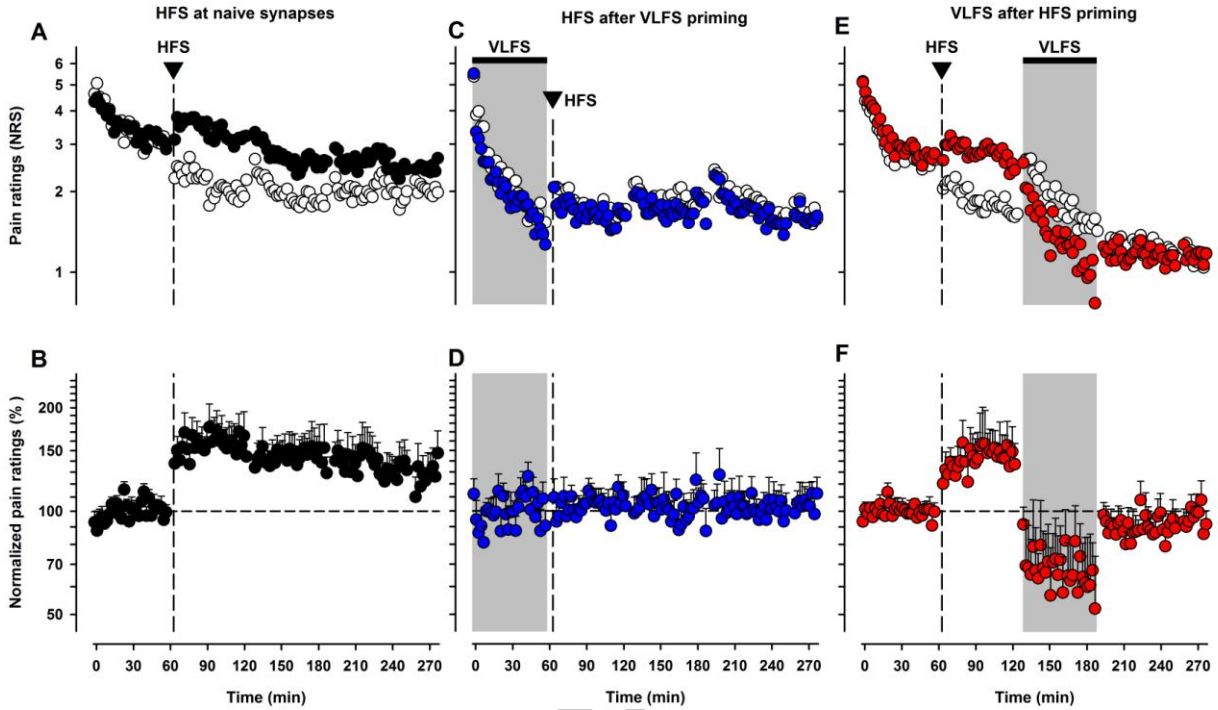

Fig.2 (A) High-frequency electrical stimulation (HFS) induced LTP of human pain sensation (pain-LTP). HFS through the same surface electrode used for the test stimuli led to a relative increase in pain sensation compared to the control site that outlasted HFS during the entire 3.5 h observation period (black dots = test site, white dots = control site).

(C) Prevention experiment with VLFS preceding HFS (VLFS - HFS). Priming by tripling the frequency of test stimuli (very low-frequency stimulation; VLFS) preceding HFS prevented pain-LTP induction (blue dots = test site, white dots = control site).

(E) Reversal experiment with VLFS following on previous HFS-induced LTP (HFS - VLFS). VLFS applied at 1 h after pain-LTP was established by HFS apparently reversed pain-LTP in the conditioned pathway (red dots = test site, white dots = control site). Pain ratings remained decreased when the VLFS was discontinued and test stimuli returned to the initial rate of 1 per minute.

Each dot in **A, C, E** represents the average of 2 pain ratings per single electrical test pulses at 10x detection threshold (T) at a rate of 1 stimulus per minute (i.e. a 2 min time window).

(B, D, F) Same data as in **A, C and E** with each dot representing pain ratings normalized to the unconditioned contralateral control side (see methods). Data in B, D and F are expressed as mean ± SEM.

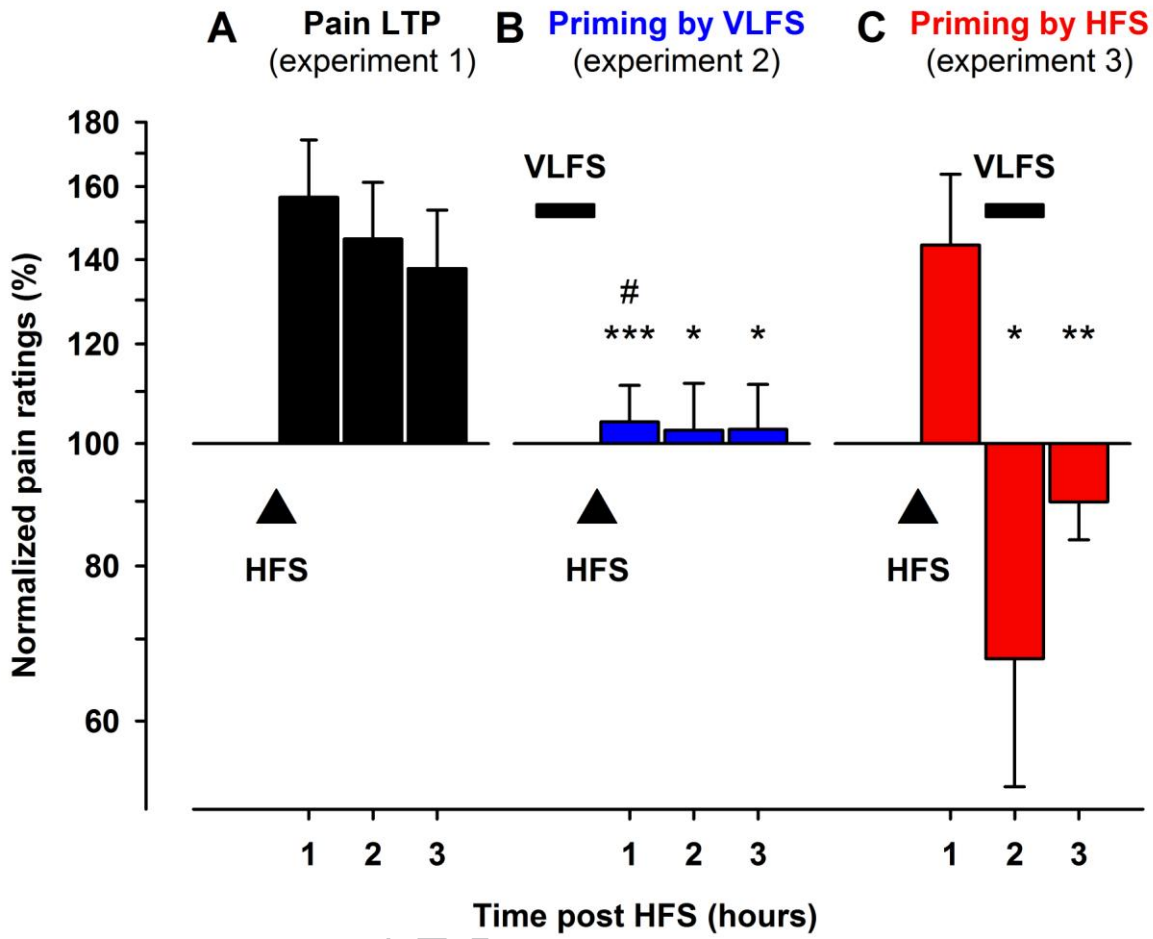

Fig.3. Summary of pain-LTP elicited by HFS, and interactions with VLFS. **(A)** HFS induced significant pain-LTP: +57%, +45%, and +38% compared to the unconditioned contralateral control site in the first, second, and third hours after HFS (Exp.1; black bars). Each bar represents normalized pain ratings to single electrical test stimuli at 10 x T averaged over a one-hour time window. **(B)** Priming by VLFS during the baseline period prevented HFS-induced pain-LTP (+4%, +2%, and +3% above the control site in the first, second, and third hours, n.s.; Exp.2; blue bars). **(C)** Significant HFS-induced pain-LTP (+44% in the first hour) was reversed overall by the VLFS (-10% vs. baseline after the VLFS; Exp.3; red bars). Mean ± SEM across twelve subjects.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. respective time window of original pain-LTP protocol (Exp. 1). # $p<0.05$ vs. respective time window of Exp.3.

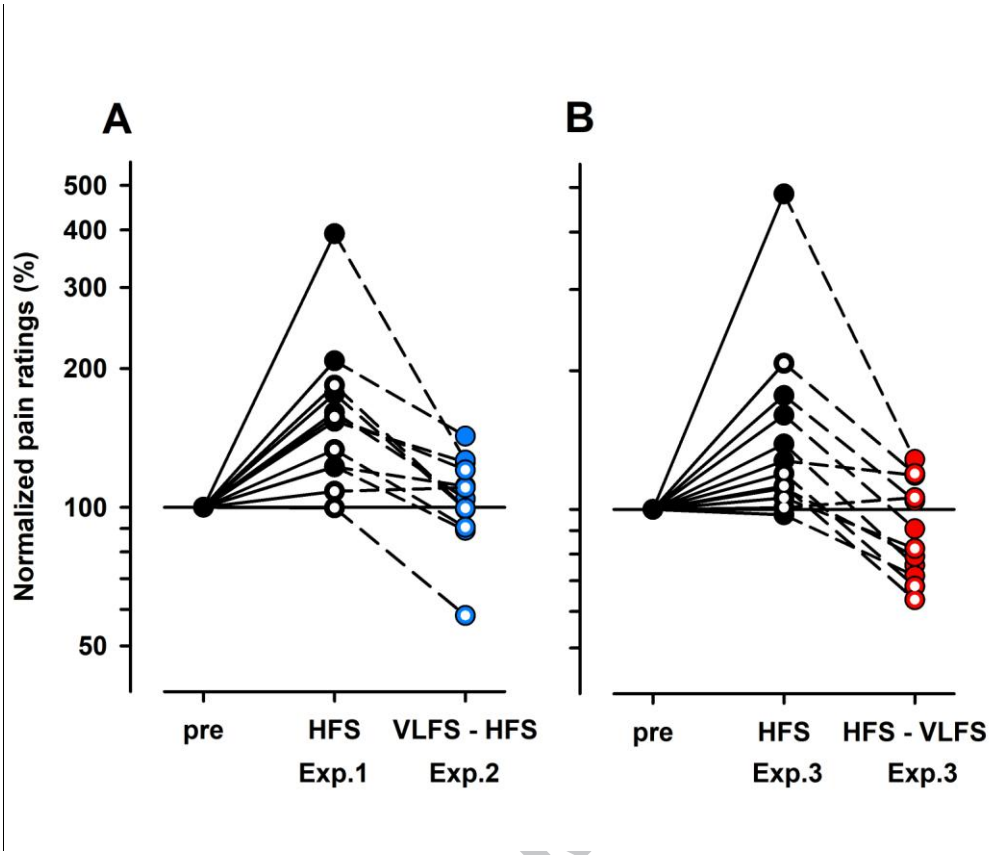

Fig.4. Threshold modulations of human pain sensitivity by HFS and VLFS. Every line connects the value for an individual subject in three different states (normalized to baseline pain sensitivity)

(A) HFS-induced changes in pain ratings after priming with VLFS [VLFS - HFS = second hour of Exp.2 (blue circles)] correlate to the changes in the naïve state (HFS = second hour of Exp.1) ($r=0.58$, $p<0.05$). The response magnitude shifted downward in parallel fashion towards and sometimes into the LTD range. (**B)** VLFS-induced changes in pain ratings after priming with HFS [HFS - VLFS = third hour of Exp.3 (red circles)] also correlated to the changes in the naïve state (HFS = second hour of Exp.3, i.e. pain-LTP prior to VLFS) ($r=0.65$, $p<0.05$). Again, the response magnitude shifted downwards in parallel fashion towards or into the LTD range. The dashed line connects each subject's value in a naïve HFS-alone condition (Exp.1) and in a primed state either VLFS preceding HFS [VLFS - HFS] (Exp.2) or either HFS preceding VLFS [HFS - VLFS] (Exp.3). Female subjects are marked by a white core of the circle representing their individual response magnitudes.

- High frequency stimulation protocols are valid tools to induce long-term potentiation of pain (pain-LTP)
- A specify conditioning protocol (very low frequency stimulation) modifies the expression of pain-LTP in two ways:
  - VLFS prevents the development of pain-LTP
  - VLFS reverses an already established pain-LTP
- These findings may pave the way for effective prevention of hyperalgesia development in a clinical context, or for the amelioration of hyperalgesia by adequate stimulation protocols