doi:10.1093/brain/awv108 BRAIN 2015: 138; 2505–2520 | 2505

BRAIN
A JOURNAL OF NEUROLOGY

Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans

Florian Henrich,* Walter Magerl,* Thomas Klein,† Wolfgang Greffrath and Rolf-Detlef Treede

*These authors contributed equally to this work.

See Sandkühler (doi:10.1093/brain/awv193) for a scientific commentary on this article.

Long-term potentiation in the spinal dorsal horn requires peptidergic C-fibre activation in animals. Perceptual correlates of long-term potentiation following high-frequency electrical stimulation in humans include increased sensitivity to electrical stimuli at the high frequency stimulation site (homotopic pain–long-term potentiation) and increased sensitivity to pinprick surrounding the high frequency stimulation site (heterotopic pain–long-term potentiation, equivalent to secondary hyperalgaesia). To characterize the peripheral fibre populations involved in induction of pain–long-term potentiation, we performed two selective nerve block experiments in 30 healthy male volunteers. Functional blockade of TRPV1-positive nociceptors by high-concentration capsaicin (verified by loss of heat pain) significantly reduced pain ratings to high frequency stimulation by 47% ($P < 0.001$), homotopic pain–long-term potentiation by 71% ($P < 0.01$), heterotopic pain–long-term potentiation by 92% ($P < 0.001$) and the area of secondary hyperalgesia by 76% ($P < 0.001$). The selective blockade of A-fibre conduction by nerve compression (verified by loss of first pain to pinprick) significantly reduced pain ratings to high frequency stimulation by 37% ($P < 0.01$), but not homotopic pain–long-term potentiation (−5%). It had a marginal effect on heterotopic pain–long-term potentiation (−35%, $P = 0.059$), while the area of secondary hyperalgesia remained unchanged (−2%, $P = 0.88$). In conclusion, all nociceptor subclasses contribute to high frequency stimulation-induced pain (with a relative contribution of C > Aδ fibres, and an equal contribution of TRPV1-positive and TRPV1-negative fibres). TRPV1-positive C-fibres are the main inducers of both homotopic and heterotopic pain–long-term potentiation. TRPV1-positive A-fibres contribute substantially to the induction of heterotopic pain–long-term potentiation. TRPV1-negative C-fibres induce a component of homotopic self-facilitation but not heterotopic pain–long-term potentiation. TRPV1-negative A-fibres are the main afferents mediating pinprick pain and hyperalgesia, however, they do not appear to contribute to the induction of pain–long-term potentiation. These findings show that distinct peripheral fibre classes mediate induction of long-term potentiation-like pain amplification, its spatial spread to adjacent skin (i.e. secondary hyperalgesia), and the resulting enhanced sensitivity to pinprick in humans. Nociceptive afferents that induce pain amplification can be readily dissociated from those mediating pain. These findings add substantially to our understanding of the mechanisms of pain amplification, that form the basis for understanding the mechanisms of hyperalgesia encountered in patients.

Centre for Biomedicine and Medical Technology Mannheim (CBTM), Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany
†Present address: Mundipharma Research, Höhenstraße 10, 65549 Limburg (Lahn), Germany

Correspondence to: Walter Magerl,
Chair of Neurophysiology,
Centre for Biomedicine and Medical Technology Mannheim (CBTM),

Received August 22, 2014. Revised February 9, 2015. Accepted February 24, 2015

Medical Faculty Mannheim of Heidelberg University,
Ludolf-Krehl-Str. 13 - 17,
68167 Mannheim,
Germany
E-mail: walter.magerl@medma.uni-heidelberg.de

Keywords: hyperalgesia; dynamic mechanical allodynia; central sensitisation; transient receptor potential channels (TRPV1); peptidergic nociceptors

Abbreviations: DMA = dynamical mechanical allodynia; DRG = dorsal root ganglion; EDT = electrical detection threshold; HFS = high frequency stimulation; LTP = long-term potentation; NRS = numerical rating scale

Introduction

Persistent pain is a pathology affecting numerous patients. Traditionally chronic pains have been viewed as pathologies directly related to the precipitating diseases. Around the turn of the century, however, it has been pinpointed that a particular disease can yield different types of sensory changes including pain, and conversely, that the same patterns of changes are identified in different painful diseases. This concept implies that pain mechanisms may be independent of aetiology (Woolf *et al.*, 1998; Finnerup and Jensen, 2006; Truini *et al.*, 2013), and that mechanism-based treatment may allow individualized treatment rationales both within and across diseases (Woodcock *et al.*, 2007). Amplification of the synaptic transmission from peripheral to central nociceptive neurons has been identified as one important aspect of persistent pain mechanisms in animals and humans, which affects a major proportion of pain patients (Maier *et al.*, 2010; Schliessbach *et al.*, 2013). Relevant aspects of this pathology can be studied using human surrogate models of pain (Klein *et al.*, 2005).

Pain amplification at the spinal cord level encompasses several potential mechanisms such as increased synaptic strength, reduced intraspinal segmental or descending inhibition, and increased descending facilitation, which were filed under the umbrella term 'central sensitization' (Box 1) (Woolf and Salter, 2000), although the utility of this label has been debated (Sandkühler 2009; Woolf 2014). Increased synaptic efficacy of spinal nociceptive transmission can be reconceptualized as a spinal cord variety of the ubiquitous mechanisms of long-term potentiation (LTP) of synaptic transmission (Ji *et al.*, 2003; Zeilhofer, 2005; Cooke and Bliss, 2006; Klein *et al.*, 2008; Ruscheweyh *et al.*, 2011). The primary afferent fibre types involved in induction and signalling of human LTP-like increases in pain perception are currently unknown, but important for translating cellular neurobiological findings to their presumed clinical application (Woolf and Salter, 2000; Zeilhofer, 2005; Sandkühler, 2009). We will use the term pain-LTP to describe long-lasting pain amplification induced by afferent input but are aware that additional mechanisms may contribute.

High-frequency electrical stimulation (HFS) of C-nociceptors elicits LTP of synaptic transmission in rat spinal dorsal horn (Randic *et al.*, 1993; Lozier and Kendig, 1995; Vikman *et al.*, 2001; Ikeda *et al.*, 2003; Ji *et al.*, 2003; Cooke and Bliss, 2006; Tan and Waxman, 2012), and enhanced pain sensitivity in human skin at the HFS-conditioned site (homotopic pain-LTP) and in adjacent skin (heterotopic pain-LTP, which is equivalent to secondary hyperalgesia; Klein *et al.*, 2004, 2008; Lang *et al.*, 2007; van den Broeke *et al.*, 2011). Pain-LTP lasts for several hours consistent with early LTP (LTP1), but may in some subjects transit to late LTP (LTP2), making it a candidate for persistent hyperalgesia in patients with chronic pain (Rygh *et al.*, 2005; Klein *et al.*, 2006; Wilder-Smith and Arendt-Nielsen, 2006; Pfau *et al.*, 2011; Ruscheweyh *et al.*, 2011).

We previously identified the mechanisms of human secondary hyperalgesia following capsaicin injection, which involved two mutually exclusive primary afferent pathways, namely capsaicin-sensitive (i.e. TRPV1-positive) C-nociceptors for induction and capsaicin-insensitive (i.e. TRPV1-negative) Aδ-mechanonociceptors for signalling this state of pain amplification (Ziegler *et al.*, 1999; Magerl *et al.*, 2001). This model of pain amplification has previously been shown to be due to central sensitization of spinal nociceptive neurons (Simone *et al.*, 1991) to their unchanged peripheral input (Baumann *et al.*, 1991). However, this model had shortcomings limiting its generalization to other types of hyperalgesia related to central sensitization. First, due to the TRPV1-selective agonist capsaicin it excluded hyperalgesia induction by TRPV1-negative nociceptors (Brenneis *et al.*, 2013), such as MRGPRD-positive nociceptors mediating mechanical pain (Zylka *et al.*, 2005; Cavanaugh *et al.*, 2009; Zhang *et al.*, 2013), MRGPRA3-positive nociceptors mediating itch (Qu *et al.*, 2014), or MRGPRB4-positive fibres mediating pleasant touch (Vrontou *et al.*, 2013). Second, our model did not address homotopic pain-LTP, which may include a component of C-fibre-induced self-facilitation (Randic *et al.*, 1993; Klein *et al.*, 2004; Ikeda *et al.*, 2006; Hansen *et al.*, 2007). Third, differential sensitivity of C- and Aδ-nociceptors to capsaicin (Szolcsanyi, 1987; Lawson *et al.*, 1997; Ringkamp *et al.*, 2001; Lawson *et al.*, 2002; Bachy *et al.*, 2011) and to pinprick stimuli

(Garell *et al.*, 1996; Andrew and Greenspan, 1999; Slugg *et al.*, 2000) may have biased our analysis.

The present study aimed at an unbiased view for both induction and testing of hyperalgesia by using epicutaneous electrical stimuli exciting Aδ and C-nociceptors equally with no preference (Kress *et al.*, 1992; Messlinger *et al.*, 1995; Peng *et al.*, 1999) combined with selective elimination protocols for TRPV1-positive nociceptors by sustained exposure to high concentration capsaicin (Nolano *et al.*, 1999; Magerl *et al.*, 2001; Rage *et al.*, 2010; Anand and Bley, 2011; Cavanaugh *et al.*, 2011), and A-fibre-selective conduction blockade by nerve compression (Ziegler *et al.*, 1999; Magerl *et al.*, 2001). We hypothesized to corroborate the predominant role of TRPV1-positive C-nociceptors for induction of secondary hyperalgesia, and to disclose the potential contribution of other nociceptive afferents to homotopic and heterotopic pain-LTP, and hence close the gap between animal models of LTP and changes in human pain sensitivity.

Box 1 Central sensitization

The term 'central sensitization' is agreed terminology as defined by the International Association for the Study of Pain (IASP): 'Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input.' (*c.f.* http://www.iasp-pain.org/Taxonomy). From the same website: 'This may include increased responsiveness due to dysfunction of endogenous pain control systems. Peripheral neurons are functioning normally; changes in function occur in central neurons only. This is a neurophysiological term that can only be applied when both input and output of the neural system under study are known, e.g. by controlling the stimulus and measuring the neural event. Clinically, sensitization may only be inferred indirectly from phenomena such as hyperalgesia or allodynia.'

Materials and methods

Volunteers

Thirty-five healthy male volunteers (24 ± 3 years) participated in the study. Restriction to male subjects was chosen to avoid interference with female hormonal cycle, which may be an uncontrolled source of response variability (for reviews see Greenspan *et al.*, 2007; Martin 2009). Our preliminary data suggest that females differ from males in the decay but not the induction of pain-LTP (Pfau *et al.*, 2011 and unpublished data). Further exclusion criteria were current medical treatment, chronic pain, psychological disorders, or past drug abuse. Local ethics committee approval had been obtained.

Twenty volunteers participated in the first experiment with LTP induction in capsaicin-desensitized skin. Fifteen volunteers were enrolled in screening for the second experiment with LTP induction under A-fibre conduction-blockade, three of which were excluded because of lack of an autonomous innervation territory of the superficial radial nerve. Two volunteers participated in both experiments.

LTP induction with high frequency electrical stimulation

Trains of electrical stimuli applied through 10 punctate stainless steel electrodes (diameter: 250 µm each) were used to induce pain-LTP (Klein *et al.*, 2004) using a constant current stimulator (DS7H, Digitimer^{TM}). In the first experiment the electrical detection threshold (EDT) of the first day before capsaicin treatment was used because capsaicin desensitization increased EDTs. In the second experiment the EDT was determined at the beginning of the experiment before induction of the compression block.

Pain-LTP was induced by HFS with five 1-s trains of 100 Hz stimuli of 2 ms duration at 10 × the EDT. HFS was expected to mimic injury-induced high-frequency discharge and hence to induce changes in synaptic efficacy similar to a real injury. In each experiment, two skin sites were stimulated by HFS (one within the selective nerve block region, one outside); a third skin site served as unconditioned control without HFS. Test areas in the first experiment (LTP induction in capsaicin-desensitized skin) were the upper forearm for capsaicin treatment, the contralateral forearm for vehicle treatment and the distal forearm near the wrist as unconditioned control without HFS (Fig. 1). In the second experiment (LTP induction under A-fibre conduction-blockade of the superficial radial nerve) the test areas were on the hand dorsum (Fig. 2). Experiments were separated by at least 4 months to allow for regeneration of capsaicin-treated skin (Nolano *et al.*, 1999; Rage *et al.*, 2010). The starting site for HFS was balanced over the study. Volunteers were instructed to rate perceived pain for every HFS train on a numerical rating scale (NRS, anchored at 0 = no pain, 100 = most intense pain imaginable). Volunteers were trained in the rating procedure for 5 min in a fourth skin site before EDT.

Electrical and mechanical test stimuli

Homotopic pain-LTP was tested with single electrical stimuli (2 ms duration, 10 × EDT) through the electrode used for HFS, and rated on the 0–100 NRS to estimate electrical pain sensitivity. Heterotopic pain-LTP was tested with mechanical stimuli applied by calibrated weighted pins of 250 µm diameter and seven forces ranging from 8–512 mN (MRC Systems); mean NRS rating across these stimuli indicated mechanical pain sensitivity. The presence of pain to stroking light touch, i.e. dynamic mechanical allodynia (DMA) was tested with cotton wool, a calibrated Q-Tip, and a soft brush that were moved across the skin in 1–2 cm strokes, and average NRS to tactile stimuli quantified DMA. Homo- and heterotopic test stimuli were applied at the three test sites every 5 min, eight times before HFS (baseline) and 16 times after HFS. One area was treated with nerve block and HFS, one area with HFS only and the third area was an unconditioned control without HFS to monitor habituation or other fluctuations of pain sensitivity.

Areas of secondary hyperalgesia to a pinprick stimulus of 128 mN were mapped along eight tracks (all separated at a 45° angle) at 45 and 90 min after HFS, by moving the test site in small steps of a few millimetres. Testing started from far outside the hyperalgesic skin and slowly moved towards the HFS site. The border of the secondary hyperalgesia area was identified by an abruptly increased painfulness of the pinprick

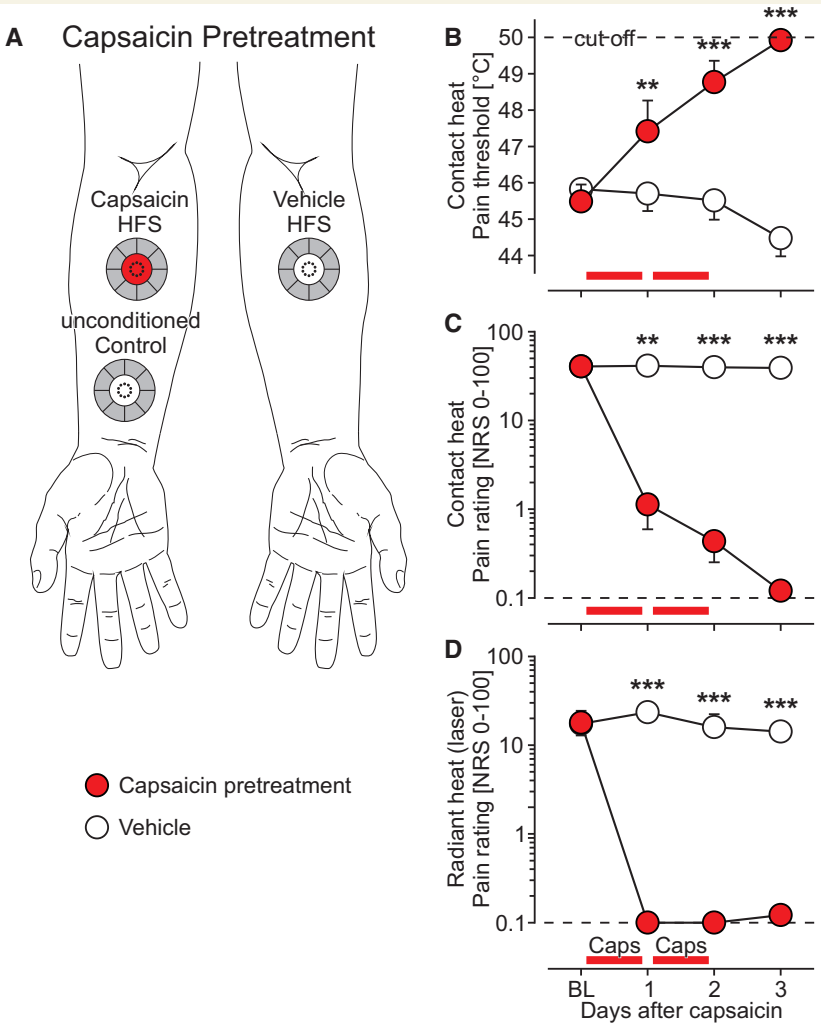

Figure 1 Methods of the first experiment in 20 volunteers (LTP induction in capsaicin-desensitized skin). (**A**) Position of capsaicin patch and vehicle patch where HFS electrodes were placed. Unconditioned control area on distal forearm. Shaded area around the electrode: test area for pinprick. (**B–D**) Monitoring the loss of TRPV1-positive afferents in the forearm by heat pain thresholds to contact heat with a Peltier thermode (**B**), pain to supra-threshold heat stimuli by Peltier thermode (50°C, 4-s plateau) (**C**), and pain to supra-threshold heat stimuli by radiant heat (thulium laser, 470 mJ, 3 ms, 20 mm^2 (**D**). Baseline before patch application (BL), after 22 h of capsaicin patch (1), after a second 22 h of capsaicin patch (2), and following additional 24 h of rest after the second capsaicin patch (3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; paired *t*-test capsaicin-treated versus vehicle-treated skin.

stimulus and mapped to the precision of 1 mm by moving the test stimulus inside and outside of the hyperalgesia border.

Induction and monitoring of desensitization of TRPV1-positive afferents by topical capsaicin

For the first experiment, a 4.5 cm^2 area on one proximal forearm was pretreated with an 8% capsaicin patch (Qutenza®) for 2 × 22 h on two consecutive days (Fig. 1). We did not use the clinical routine of pretreatment with local anaesthetics for three reasons: (i) the capsaicin-treated area was small, and thus spatial summation effects of pain very weak; (ii) accordingly, we expected that capsaicin-induced pain would be moderate as found in previous experiments (Magerl *et al.*, 2001); and (iii) local anaesthetics only effectively control capsaicin-induced pain at low concentration (0.075%; Yosipovitch *et al.*, 1999), but not at higher concentrations of capsaicin (1%; Fuchs *et al.*, 1999). As expected capsaicin-induced pain upon application of the first Qutenza® patch was mild with an average pain rating across the 22 h period of 17.9 (on a 0–100 NRS; $\log_{10}$: 1.256 ± 0.080). Pain dropped significantly to less than half upon application of the second Qutenza® patch, to 6.8 ($\log_{10}$: 0.841 ± 0.141, $P < 0.001$), which indicated substantial reduction of capsaicin sensitivity already after the first patch. Moreover, maximal pain ratings during the 22 h period for the first and second patch were also mild to moderate (on average 32.4 and 18.9). LTP induction was performed 24 h after removal of the second patch, to allow for a decline of primary and secondary hyperalgesia. The contralateral forearm was treated with a vehicle patch.

Three tests were done on both sites to verify the desensitization of TRPV1 positive fibres: (i) heat pain threshold, measured with a Thermal Sensory Analyzer (TSA, Medoc Ltd) and a small Peltier thermode (1.6 × 1.6 cm^2) by 1°C/s heat

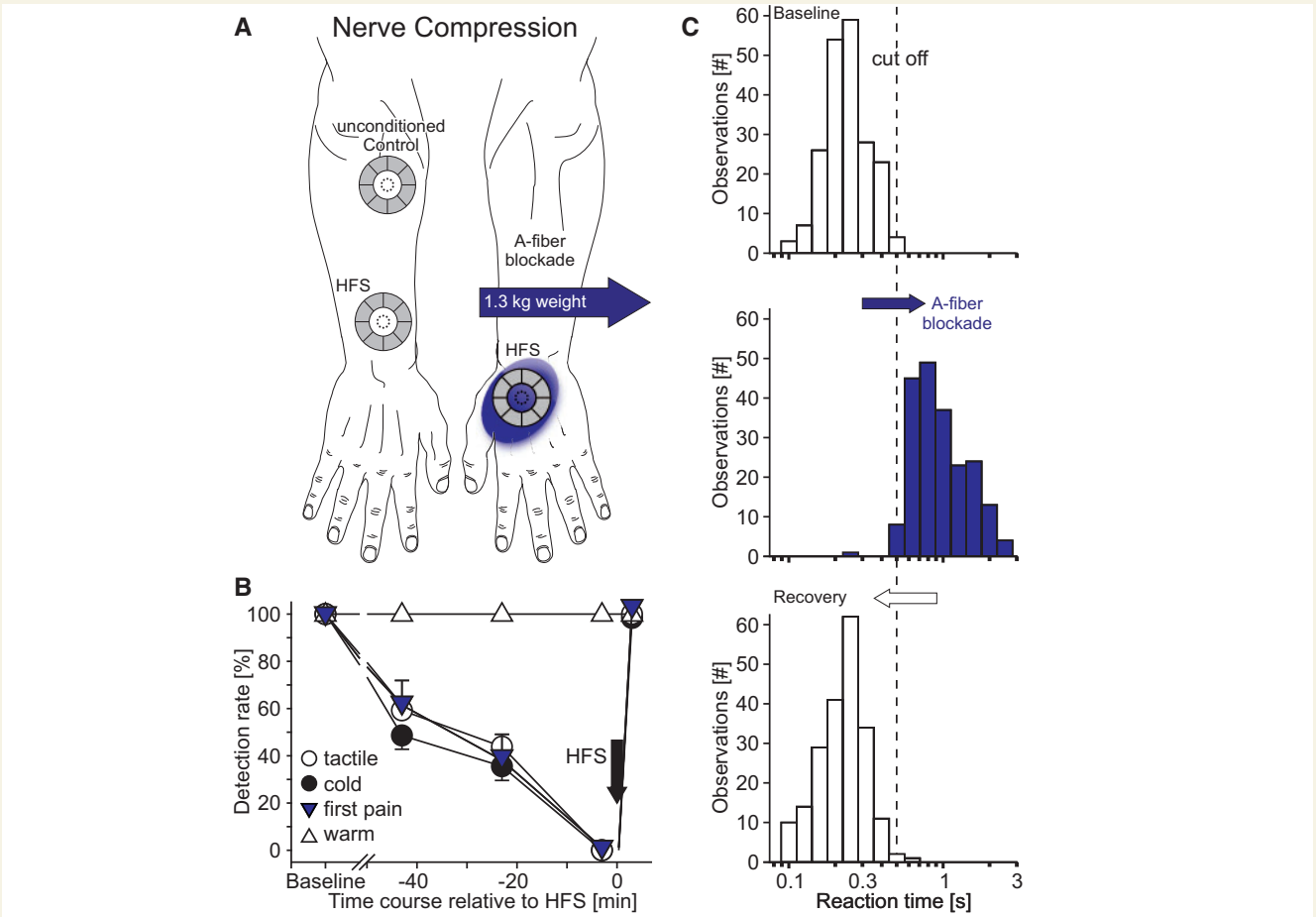

Figure 2 Methods of the second experiment in 12 volunteers (LTP induction under A-fibre-conduction-blockade). (**A**) Weight (1.3 kg) on the superficial radial nerve to induce a territory (grey cloud) of loss of A-fibre-related sensation including loss of first pain to pinpricks. As naïve skin area the opposite hand was used, HFS electrodes were placed on both hands. Unconditioned control area on proximal forearm. Shaded area around the electrode: test area for pinprick. (**B**) Monitoring of A-fibre-related sensory parameters: tactile detection (open circle), cold detection (filled black circle), and first-pain detection (filled blue triangle), fast reaction time to pinprick (< 500 ms) and of C-fibre-related sensory parameter: warmth detection (open triangle). Error bars show standard error of the mean (SEM). Time scale of *x*-axis is related to time point of fully developed A-fibre blockade followed immediately by HFS. (**C**) Reaction times to pinprick before induction of the A-fibre blockade (baseline), at fully developed A-fibre blockade and 2 min after recovery from the block (recovery).

ramps; (ii) pain ratings to three suprathreshold heat pain stimuli (starting and ending at 34°C with an increase and decrease of 8°C/s and 4 s plateau at 50°C with the same thermode); and (iii) pain ratings to nine single thulium laser pulses (470 mJ, 1 ms, Themis®, Starmedtec) were applied in a 3 × 3 spatial pattern. These tests were done before the first patch was applied, after patch removals on Days 2 and 3, and after pain testing and HFS at Day 4. Indirect assessment of functionality, like nociceptor-dependent blood flow responses, was not chosen, since we have previously shown that a similar treatment protocol eliminated blood flow responses almost completely. Moreover, for sustained time courses of days we observed that psychophysical measures recovered faster than blood flow responses (Magerl *et al.*, 1987).

Induction and monitoring of compression block of myelinated A-fibres

For the second experiment, A-fibre signalling from the innervation territory of the superficial radial nerve in one hand was reversibly blocked as described previously (Ziegler *et al.*, 1999). The opposite hand served as untreated (naïve) control (Fig. 2). The non-dominant arm was placed into a custom-made groove and fixed with small cushions to avoid movements of the arm and hand. A 2.5-cm wide rubber band was placed on the wrist to compress the superficial radial nerve against the underlying bone. The band was loaded with a 1.3 kg hanging weight generating a slowly developing but rapidly reversible conduction block (LaMotte and Thalhammer, 1982; Bromm *et al.*, 1983; Ziegler *et al.*, 1999; Magerl *et al.*, 2001). The volunteers were advised to keep the hand still during block induction.

The development and recovery of the conduction block was monitored at 30, 50, 70, 90 and 110 min after compression onset, and a few minutes after block release. If the block was not complete after 110 min (see criteria below), the experiment was terminated for safety reasons and the volunteer was excluded from further investigation. After the block was established, areas with loss of tactile and cold perception (Sinclair *et al.*, 1952) and increase of reaction time were marked on the skin,

traced and photographed digitally. The monitoring sequence consisted of measuring tactile, warm and cold detection and the reaction time for mechanical pain stimuli (Sinclair and Stokes, 1964; Ziegler *et al.*, 1999). Tactile detection was tested 20 times in a 4 × 5 cm area by 4 mN von Frey hairs (1.1 mm diameter). Twenty warm and cold stimuli were randomly applied with water-filled glass tubes kept at 57°C and 7°C (effective skin temperatures 40° and 20°). Volunteers had to close their eyes and answer if stimuli felt either 'warm', 'cold', 'tactile' or 'unclear'. Mechanical pain stimuli were applied in the same area with 128 mN pinprick (MRC Systems) starting a stopwatch, which was stopped by the volunteers with the free dominant hand when perceived. To avoid misses when stimuli were perceived only weakly, each pinprick was preceded by an auditory cue.

The criteria for accepting a selective and complete A-fibre block were an 3 × 3 cm^2 area with loss of tactile detection, cold detection and an increase of reaction time to pinprick to >500 ms, but preservation of warm detection (Ziegler *et al.*, 1999). Loss of warm detection implied comprised C-fibre function and was a criterion for exclusion. The autonomous innervation area of the superficial radial nerve had been identified in a screening session to identify an area for electrode placement in the main experiment. The autonomous innervation area was stable over time (Campero *et al.*, 2005).

Data evaluation and statistics

Statistics were calculated with SPSS 20 (IBM™) and Excel 2010 (Microsoft™). To obtain secondary normal distribution of rating data, for all NRS ratings a constant of 0.1 was added to avoid loss of zero ratings and they then were $\log_{10}$-transformed (Magerl *et al.*, 1998). All $\log_{10}$-ratings were normalized to the baseline (first eight ratings before HFS). Mechanical and electrical pain sensitivity were additionally normalized to the unconditioned control site (by taking the difference of the log values). DMA rating data were not normalized as these tactile stimuli normally do not evoke pain.

Ratings of HFS-induced pain, laser pain and 50°C heat stimuli were also $\log_{10}$-transformed after adding 0.1. Geometric mean was also calculated for reaction times in A-fibre block monitoring and EDT. All statistical differences were assessed with ANOVA and paired *t*-tests. Data are shown either as arithmetic mean and standard error of the mean (SEM) for non-log-transformed data, or for reaction time data and pain ratings as geometric mean, and additionally mean and SEM of $\log_{10}$-transformed data.

Results

First experiment monitoring: capsaicin desensitization

Capsaicin desensitization for 2 × 22 h followed by a 24 h rest period raised heat pain thresholds and eliminated pain to brief suprathreshold heat stimuli (Fig. 1). The initial heat pain threshold was almost the same for both sides with 45.6°C in the test forearm versus 45.5°C in the control forearm ($P = 0.63$). In vehicle-treated skin, heat pain threshold remained constant for four consecutive days (paired *t*-test, $P > 0.71$, Fig. 1B). In capsaicin-treated skin, the heat pain threshold rose from 45.5 to 49.9 ($P < 0.001$) and was above the upper cut-off limit in most subjects (19/20). Pain to suprathreshold contact heat stimuli (50°C, 4 s plateau) was identical on Day 1 for both sides with 40/100 NRS. For the vehicle-treated skin it remained unchanged (39/100), whereas for the capsaicin site it decreased to 0.12/100 on Day 4 (paired *t*-test $P < 0.001$, Fig. 1C). Pain ratings to 470 mJ laser stimuli were similar on both sides before application of vehicle (28.6/100) and capsaicin patches (27.7/100). In vehicle-treated skin, laser-induced pain stayed nearly the same from the first to the last day ($P = 0.53$, Fig. 1D), but decreased in capsaicin-treated skin to 0.12/100 ($P < 0.001$, paired *t*-test).

Second experiment monitoring: A-fibre blockade

Monitoring of A-fibre conduction blockade is shown in Fig. 2. The median time to reach all criteria for full block (area of at least 3 × 3 cm with no cold and tactile perception, and increased reaction time to >500 ms for pinprick, but no loss of warm perception) was ~70 min (range: 30–110 min). Three volunteers did not reach the block criteria after 110 min in the screening and were excluded from the main experiment. Note that A-fibre blockade was released immediately after HFS, so both pre- and post-HFS sensory testing were done in skin with intact A-fibre conduction.

HFS-induced pain

HFS-pain ratings exhibited a smooth increase over the five trains under all experimental conditions (Fig. 3). In the first experiment (capsaicin desensitization), HFS-pain in vehicle-treated skin started with a mean NRS of 29.5/100 NRS and rose to 37.2/100 NRS. Pain ratings in capsaicin-treated skin were substantially reduced: they started at 14.5/100 NRS and rose to 18.6/100 NRS. Mean HFS-induced pain across all trains in normal skin was 35.2 NRS ($\log_{10}$: 1.547 ± 0.076) and was reduced by 47% in capsaicin-treated skin to 16.9 NRS ($\log_{10}$: 1.227 ± 0.101; $P < 0.001$). The increase of pain over HFS trains did not differ between vehicle and capsaicin-desensitized skin (ratio: 1.57 ± 0.35 versus 2.12 ± 0.76; $P = 0.46$).

In the second experiment (A-fibre conduction blockade), HFS-induced pain in naïve skin of the hand dorsum started at 32.4/100 and gradually increased to 47.5/100. After complete and selective blockade of A-fibre conduction, pain was reduced to 19.3/100 (first train) and 31.2/100 (fifth train). Mean HFS-induced pain across all trains in normal skin was 41.3 NRS ($\log_{10}$: 1.616 ± 0.066) and was reduced by 37% under A-fibre conduction blockade to 25.7 NRS ($\log_{10}$: 1.410 ± 0.086; $P < 0.001$). The increase of pain over HFS trains did not differ between naïve skin and skin with functional A-fibre blockade (ratio: 1.53 ± 0.10 versus 1.74 ± 0.15; $P = 0.23$).

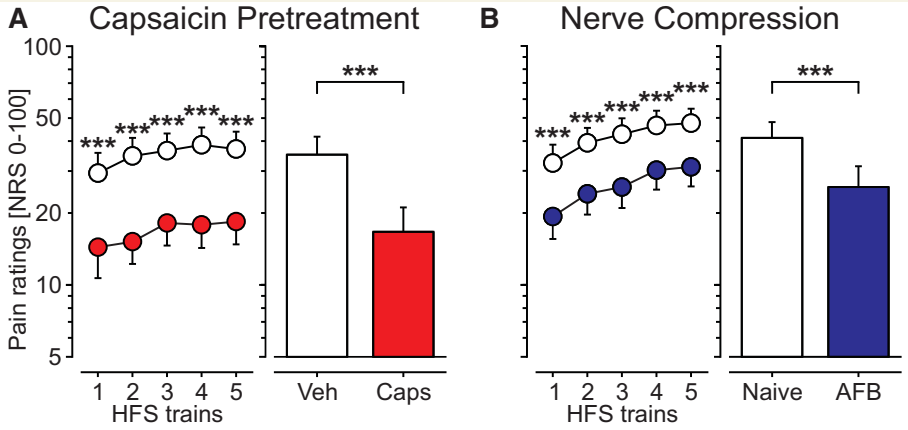

Figure 3 Pain ratings to HFS through punctate epicutaneous electrodes. (**A**) HFS pain in capsaicin-treated skin was significantly reduced by 47% compared to vehicle (Veh) skin ($P < 0.001$). (**B**) HFS pain in A-fibre-blocked skin was significantly reduced by 37% compared to naïve skin ($P < 0.001$). Geometric mean ± SEM. Note logarithmic *y*-axis. ***$P < 0.001$ for treatment versus vehicle-treated or naïve skin; paired *t*-test. Red circles/bars indicate capsaicin pretreatment (Caps); blue circles/bars indicate A-fibre blockade (AFB); open circles/bars indicate vehicle treated (Veh) or naïve.

Homotopic pain-LTP

Pain ratings to electrical stimuli at the HFS site (homotopic pain-LTP, Fig. 4) were increased abruptly by HFS in vehicle-treated skin to 2.29-fold of the control site (mean $\Delta\log_{10}$: 0.360 ± 0.114; $P < 0.01$). In skin pretreated with the high concentration capsaicin patch (Fig. 4A), the magnitude of homotopic pain-LTP was reduced by 71% compared to the vehicle site ($P < 0.01$). However, there was still a significant pain increase to 1.38-fold of control following HFS (mean $\Delta\log_{10}$: 0.141 ± 0.076; $P < 0.05$), but at no time during the baseline period (all at least $P > 0.20$ for capsaicin-treated or vehicle-treated versus unconditioned control, and for capsaicin-treated versus vehicle-treated skin).

In contrast to the capsaicin experiment, A-fibre conduction blockade left HFS-induced homotopic pain-LTP almost unaltered (Fig. 4B). Pain ratings to electrical stimuli after HFS in naïve skin increased to 1.91-fold of control (mean $\Delta\log_{10}$: 0.281 ± 0.048, $P < 0.001$). Pain ratings to electrical stimuli in A-fibre block skin increased to 1.86-fold of control (mean $\Delta\log_{10}$: 0.270 ± 0.059, $P < 0.001$). Differences of pain ratings to electrical stimuli after HFS (A-fibre blocked skin versus naïve) were only ~5% and were not significant ($P = 0.78$).

Heterotopic pain-LTP

Pain ratings to pinprick stimuli (Fig. 4C) surrounding the HFS site in vehicle-treated skin in the first experiment increased to 2.19-fold of control (mean $\Delta\log_{10}$: 0.342 ± 0.062, $P < 0.001$). Pain ratings to pinprick stimuli in capsaicin-desensitized skin (Fig. 4C) increased to 1.11-fold of control (mean $\Delta\log_{10}$: 0.050 ± 0.020, $P < 0.05$ versus control), but the magnitude was reduced by 92% compared to the vehicle site ($P < 0.01$). Moreover, the increase in pain ratings to pinprick stimuli in capsaicin-desensitized skin was significant only from the second to the fourth post-HFS point (1.18-fold, $P = 0.058$; 1.28-fold $P < 0.001$; 1.14-fold $P < 0.05$), suggesting short-term potentiation rather than long-term potentiation. Moreover, the area of secondary hyperalgesia to pinprick on the ventral forearm was reduced by 76% from 39.0 ± 8.9 cm^2 to 9.3 ± 2.4 cm^2 ($P < 0.001$; Fig. 4E).

Pain ratings to pinprick stimuli around the HFS site in naïve skin in the second experiment increased to 1.80-fold of control (mean $\Delta\log_{10}$: 0.255 ± 0.070, $P < 0.001$ versus baseline). Pain ratings to pinprick stimuli in A-fibre blocked skin (Fig. 4D) increased to 1.52-fold of control (mean $\Delta\log_{10}$: 0.180 ± 0.065, $P < 0.05$ versus baseline). The reduction in HFS-induced heterotopic pain-LTP by 35% was of marginal significance ($P = 0.059$, naïve versus A-fibre blocked skin). In contrast, the area of secondary hyperalgesia to pinprick, which was much smaller on the hand dorsum, remained undiminished (10.0 ± 1.9 cm^2 to 9.8 ± 2.1 cm^2; $P = 0.88$; Fig. 4F).

Dynamic mechanical allodynia

After HFS in normal skin, most subjects (16/20) perceived some of the light stroking stimuli adjacent to the HFS site on the forearm as slightly painful (dynamic mechanical allodynia, Fig. 5). Accordingly after HFS, pain to dynamic light tactile stimuli increased significantly compared to pain ratings in normal skin of the unconditioned control site (mean pain rating: 0.163; $\log_{10}$: −0.788 ± 0.076, $P < 0.05$ versus control, Fig. 5A). In capsaicin-desensitized skin (Fig. 5A) DMA was significantly reduced versus vehicle (−66%, $P < 0.01$) and not significant versus control.

In the second experiment on the hand dorsum only a minority of the subjects (2/12) experienced dynamic

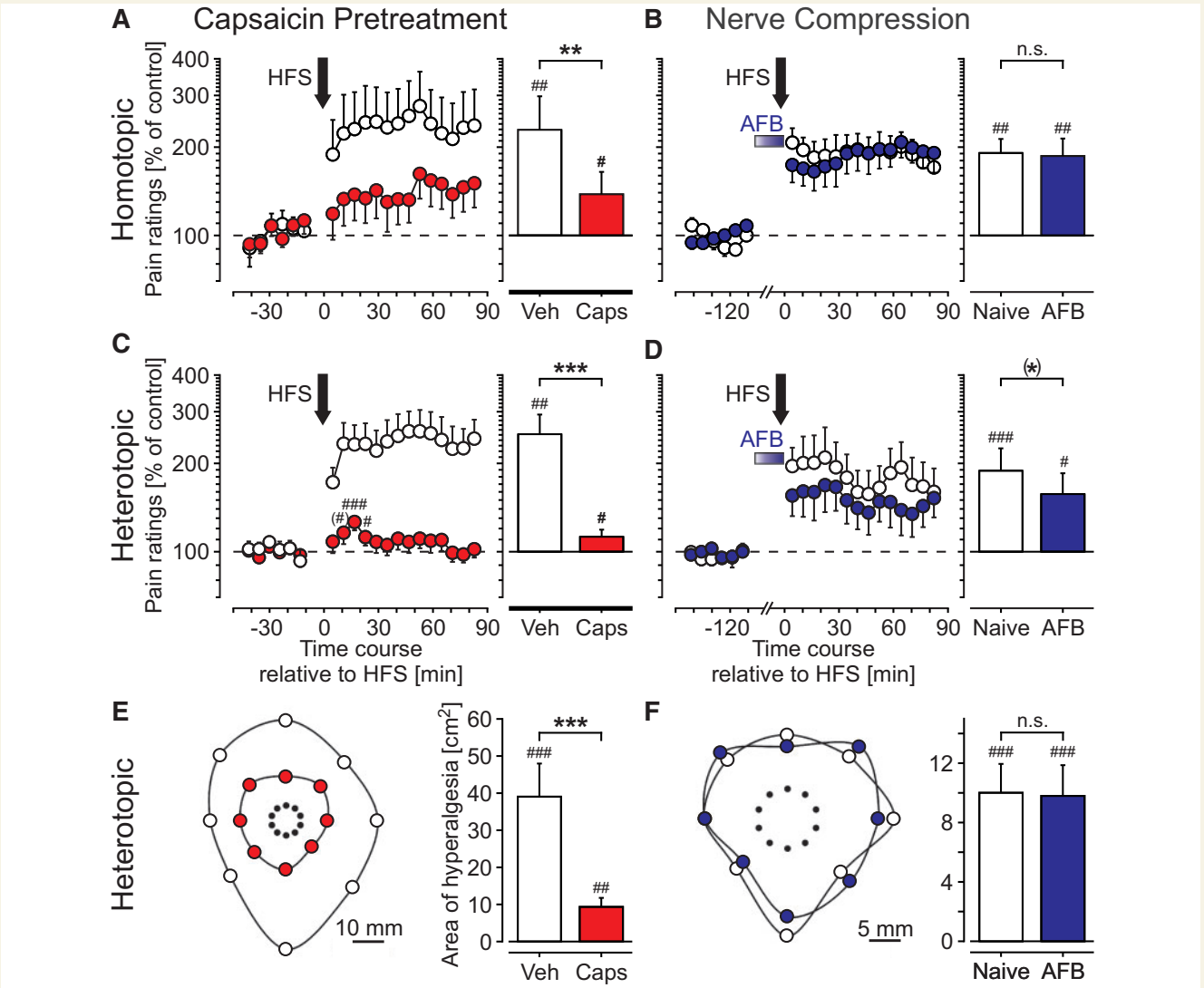

Figure 4 Homotopic pain-LTP to electrical stimuli and heterotopic pain-LTP to pinprick stimuli: time courses and average magnitudes. Areas of secondary hyperalgesia to pinprick stimuli (*lower panel*; **E** and **F**): shapes of secondary hyperalgesia areas (*left* panels) and average areas (*right* panels). (**A**) Homotopic pain-LTP in capsaicin-treated skin was significantly reduced by 71% versus vehicle-treated skin ($P < 0.01$). The remaining homotopic pain-LTP in capsaicin-treated skin was significant versus unconditioned control ($P < 0.05$). (**B**) Homotopic pain-LTP in A-fibre-blocked (AFB) skin was only slightly reduced by 5% versus naïve skin (n.s.). Homotopic pain-LTP in A-fibre-blocked skin was significant versus unconditioned control ($P < 0.01$). (**C**) Heterotopic pain amplification was almost impossible to establish with HFS in capsaicin-treated skin compared to vehicle-treated skin (−92%). The increase of pain in capsaicin-treated skin (13%) was significant versus control for only 30 min, consistent with TRPV1-negative fibre-mediated short-term potentiation. (**D**) Secondary hyperalgesia in A-fibre-blocked skin was reduced by 35% compared to naïve skin ($P = 0.059$). Secondary hyperalgesia was significant versus unconditioned control ($P < 0.001$) and A-fibre-blocked skin ($P < 0.05$). (**E**) Area of secondary hyperalgesia following HFS in capsaicin-treated skin of the ventral forearm was strongly diminished compared to HFS in vehicle-treated skin. **F)** Area of secondary hyperalgesia following HFS in A-fibre-blocked skin of the hand dorsum did not differ from naïve skin. Geometric mean ± SEM. Note logarithmic *y*-axis. Data normalized to both baseline and unconditioned control site; sliding averages across three data points. (*)$P = 0.059$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, paired *t*-test for capsaicin-treated skin or A-fibre-blocked skin versus vehicle treated or naïve skin. ($^{\#}$)$P = 0.058$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, paired *t*-test for capsaicin or A-fibre blockade versus unconditioned control site. Red circles/bars indicate capsaicin pretreatment (Caps); blue circles indicate A-fibre blockade (AFB); open circles/bars indicate vehicle treated (Veh) or naïve.

mechanical allodynia. Accordingly, mean ratings were low (mean pain rating: 0.116; $\log_{10}$: -0.934 ± 0.065) and did not differ significantly from unconditioned normal skin where allodynia was fully absent ($P = 0.17$). Under the condition of a fully developed compression block (Fig. 5B) HFS-induced dynamic mechanical allodynia in these two subjects remained completely unchanged (mean pain rating: 0.118 $\log_{10}$: -0.928 ± 0.066, $P = 0.15$ versus control and $P = 0.12$ versus HFS in normal skin, Fig. 5B).

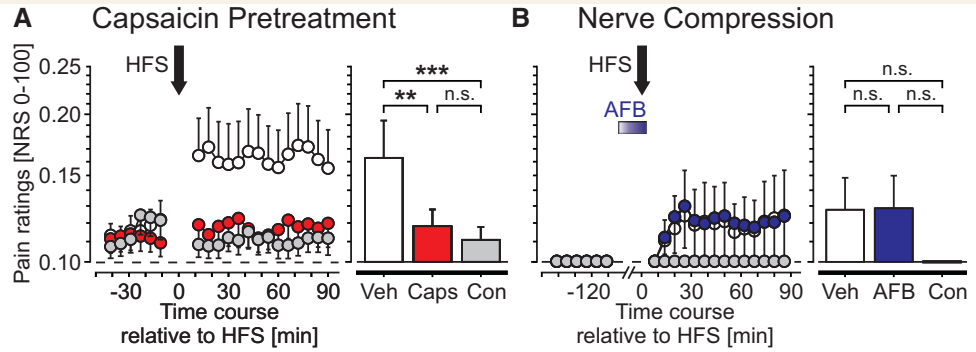

Figure 5 Dynamic mechanical allodynia to light touch: time courses and average magnitudes. (**A**) DMA in capsaicin-treated skin was significantly reduced by 66% versus vehicle-treated skin and remaining DMA was not significant versus unconditioned control. (**B**) DMA was identical in A-fibre-blocked and naïve skin (not significant since only 2/12 subjects exhibited dynamic mechanical allodynia). Geometric mean ± SEM. Note the logarithmic *y*-axis. Due to the non-linear data transformation a pain rating of 0.10 [$\log_{10}$: −1.00] represents a 0 pain rating in all subjects. All figures show sliding averages across three data points. All statistics are paired *t*-tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, paired *t*-test. Red circles/bars indicate capsaicin pretreatment; blue circles/bars indicate A-fibre blockade (AFB); grey circles/bars indicate control site (Con); open circles indicate vehicle treated (Veh) or naïve. n.s = not significant.

Estimation of primary afferent fibre types

The two nerve block experiments provide information on relative contributions of TRPV1-positive and TRPV1-negative fibres, and C- and A-fibres to the induction of HFS pain, homotopic pain-LTP and heterotopic pain-LTP. The observed percentage decreases can be conceived as percentage contribution by the fibre type that was selectively blocked, and the remaining percentage change versus control can be conceived as the contribution by the fibre types that were not blocked. These percentages are equivalent to row- or column sums in 2 × 2 tables (Table 1); however, these sums are consistent with many different combinations of individual percentages in these 2 × 2 tables. Table 1 shows two possible solutions, one with the maximum contribution by TRPV1-positive C-fibres (A) and another with their minimum contribution (B), leading to the range estimates at the bottom of the table (C). For HFS pain, these ranges are wide and all four fibre types may have contributed to this sensation. For homotopic pain-LTP we obtained evidence that only C-fibres (TRPV1-positive and TRPV1-negative) contributed to its induction. For heterotopic pain-LTP, our data suggest that only TRPV1-positive primary afferents (A- and C-fibres) contributed to its induction.

Discussion

Using electrical stimuli that non-specifically activate all peripheral nerve fibres and two selective nerve block procedures in humans allowed us to differentiate the relative contributions to the induction of pain-LTP by A-fibre versus C-fibre nociceptors and by TRPV1-positive versus TRPV1-negative nociceptor subpopulations. Our homotopic pain-LTP experiments confirmed the predicted self-facilitation of TRPV1-positive C-fibre input (Ikeda *et al.*, 2006; Hansen *et al.*, 2007), but also disclosed a significant self-facilitation of TRPV1-negative C-fibre input—a novel finding not yet described in dorsal horn electrophysiology. In addition, heterotopic pain-LTP experiments suggested that LTP of TRPV1-positive C-fibres caused secondary hyperalgesia to pinprick stimuli, which requires the interaction of at least two different pathways (Torsney, 2011), as pinprick pain is predominantly mediated by TRPV1-negative A-fibre nociceptors (Ziegler *et al.*, 1999; Magerl *et al.*, 2001). Moreover, not only TRPV1-positive C-fibre input but also TRPV1-positive A-fibre input contributed to induction of heterotopic pain-LTP, another novel finding of this study. No secondary hyperalgesia was seen after capsaicin desensitization, and accordingly TRPV1-negative A and C-fibre nociceptors do not contribute to its induction.

Fibre populations activated by HFS through punctate electrodes

Consistent with the non-specific activation of intraepidermal nerve fibres by epicutaneous electrical stimuli, all nociceptors appeared to contribute to HFS-induced pain. According to capsaicin desensitization, TRPV1-positive and TRPV1-negative fibres contributed about equally; according to selective A-fibre conduction blockade, C-fibres contributed more than A-fibres.

Whereas transcutaneous electrical stimulation of nerve trunks preferentially activates Aβ-fibres, intraepidermal electrodes have a more selective action on Aδ fibres at low intensities (i.e. 2× threshold; Mouraux *et al.*, 2010; Inui and Kakigi, 2012), when high current densities are restricted to the epidermis that does not contain any Aβ-fibres. Our epicutaneous 10-contact point electrode activates peptidergic C-fibre afferents in the skin, because it

Table 1 Estimated percentages of fibre type contributions to perceived pain

		HFS pain			Homotopic LTP			Heterotopic LTP		
	Fibre type	C	A	Sum	C	A	Sum	C	A	Sum
A	TRPV1-pos	47	0	47	71	0	71	65	27	92
	TRPV1-neg	16	37	53	24	5	29	0	8	8
	Sum	63	37	100	95	5	100	65	35	100
B	TRPV1-pos	10	37	47	66	5	71	57	35	92
	TRPV1-neg	53	0	53	29	0	29	8	0	8
	Sum	63	37	100	95	5	100	65	35	100
C	TRPV1-pos C		**10-47**			**66-71**			**57-65**	
	TRPV1-neg C		**16-53**			**24-29**			0-8	
	TRPV1-pos A		0-37			0-5			**27-35**	
	TRPV1-neg A		0-37			0-5			0-8	

(**A** and **B**) Mean percentages from Figs 3–5 led to the row sum percentages (from Experiment 1) and column sum percentages (from Experiment 2) in these 2 × 2 tables. Individual cell percentages are ambiguous, but as soon as one cell is known the others can be calculated precisely. HFS pain: lowest contribution (37%) is by A-fibres, which may be all TRPV1-negative (A) or all TRPV1-positive (B) or somewhere in between. Homotopic LTP: lowest contribution (5%) is by A-fibres, which may be all TRPV1-negative (A) or all TRPV1-positive (B) or in between. Heterotopic LTP: lowest contribution (8%) is by TRPV1-negative fibers, which may be all A-fibres (A) or all C-fibres (B) or in between. (**C**) Range estimates from parts A and B indicate which fibres make significant (bold) and which ones make insignificant contributions to the phenomena studied.

elicits neurogenic inflammation (Klein *et al.*, 2004) and a burning pain quality (Hansen *et al.*, 2007). The vast majority of fibres in any type of nerve are unmyelinated C-fibres (72–81%; Ochoa and Mair, 1969; Schmalbruch, 1986; Heppelmann *et al.*, 1988; Hines *et al.*, 1996). Amongst the remaining myelinated afferents ~70–80% are thinly myelinated Aδ-fibres (Ochoa and Mair, 1969; Heppelmann *et al.*, 1988). Thus in skin nerves the proportions of afferents are ~80:15:5% for C, Aδ, and Aβ-fibres. The major proportion of C and Aδ-fibres are nociceptive, a minor proportion are tactile afferents or innocuous thermoreceptors (Adriaensen *et al.*, 1983; Treede *et al.*, 1998; Vallbo *et al.*, 1999; Campero and Bostock, 2010). Thus, our findings that C-fibres predominate over Aδ-fibres in mediating HFS-induced pain may simply reflect the different population sizes of peripheral nociceptors. On the other hand, the stimulus frequency used in our experiments (100 Hz) may favour A-fibre activation due to their better ability to follow high frequency stimulation than C-nociceptors, which are more prone to conduction failure (Raymond *et al.*, 1990; Weidner *et al.*, 1999; Serra *et al.*, 2012). In our data, the ratio of C versus Aδ-nociceptor mediation of pain was ~2:1, whereas the fibre count ratios were 5:1. This difference supports the concept that A-fibres may contribute more to the magnitude of sensation than C-fibres (Bromm *et al.*, 1983; Magerl *et al.*, 1999; Cruccu *et al.*, 2003). However, the better frequency-following properties of A-fibre nociceptors may have moderately magnified the A-fibre component in relation to the C-fibre component, thereby favouring it's uncovering.

The population size of TRPV1-expressing dorsal root ganglion (DRG) neurons in cell cultures from avulsed human ganglia ranged from 35% to 71% (Anand *et al.*, 2006). In human microneurographical recordings, the vast majority of C-fibre nociceptors were excited by capsaicin, including all mechano-sensitive and numerous mechano-insensitive ones (Schmelz *et al.*, 2000). Similar results were obtained in primates (Baumann *et al.*, 1991; Ringkamp *et al.*, 2001). In rodents, TRPV1 receptors are expressed in both the peptidergic subgroup that contain substance P and/or calcitonin gene-related peptide (CGRP), and in the non-peptidergic subgroup that bind the lectin IB4 (Snider and McMahon, 1998). In rats, 85% of the substance P-expressing DRG neurons and 60–80% of the IB4-positive neurons co-express TRPV1 (Tominaga *et al.*, 1998; Guo *et al.*, 1999). In the mouse, TRPV1 expression is more restricted to peptidergic C-fibre nociceptors with about twice as much capsaicin-sensitivity than in IB4-binding DRG neurons (Dirajlal *et al.*, 2003; Hjerling-Leffler *et al.*, 2007; Lawson *et al.*, 2008; Cavanaugh *et al.*, 2009).

Acute ablation of capsaicin-sensitive mouse DRGs induced a complete and selective loss of acute heat-pain sensitivity without affecting mechanically-induced pain (Cavanaugh *et al.*, 2009). Furthermore, ablation of TRPV1-expressing neurons depleted the substance P-expressing population in mice (Hsieh *et al.*, 2012). More than 90% of the mouse IB4-positive cells also express the sensory neuron-specific G protein-coupled receptor Mrgprd (Cavanaugh *et al.*, 2009) and Mrgprd-expressing neurons account for essentially all non-peptidergic cutaneous afferents (Zylka *et al.*, 2005). This mainly capsaicin-negative subpopulation of nociceptors is essential for the detection of noxious mechanical stimuli, the development of mechanical hyperalgesia induced by inflammation but does not contribute to detection of painful cold and heat (Cavanaugh *et al.*, 2009). Thus, there is evidence for modality specificity in peripheral C-fibre nociceptors: TRPV1-expressing peptidergic nociceptors mediate heat-sensitivity, and Mrgprd-positive non-peptidergic neurons mediate mechano-sensitivity. Previous capsaicin-desensitization studies in humans are consistent with this concept (Nolano *et al.*, 1999; Magerl *et al.*, 2001). There may be an even finer functional distinction of mechanosensitive C-nociceptor subclasses as suggested recently (Wooten *et al.*, 2014).

In rodents, about one-third of TRPV1 expressing nociceptors were A-fibre nociceptors (Ma, 2002; McCoy *et al.*, 2012; Mitchell *et al.*, 2014); in turn, ~20% of the Aδ fibre neurons co-expressed TRPV1 (Bachy *et al.*, 2011; Cavanaugh *et al.*, 2011). In guinea pigs, about one-third of the A-fibre nociceptors also expressed substance P (Lawson *et al.*, 1997). In primates many A-fibre nociceptors respond to capsaicin (Baumann *et al.*, 1991; Meyer *et al.*, 1991; Ringkamp *et al.*, 2001) and about one-third of them exhibit a rapid response to heat that is likely mediated by TRPV1 (Treede *et al.*, 1998). Electrophysiological experiments directly investigating human Aδ fibre nociceptors are rare; capsaicin-sensitivity was not determined in those (Adriaensen *et al.*, 1983; Bromm *et al.*, 1984). Nonetheless, it is likely that also in humans there is a population of TRPV1-expressing A-fibre nociceptors that may also be able to release substance P, and that are blocked by capsaicin treatment.

In conclusion, across species there are TRPV1-expressing heat-sensitive and peptidergic A- and C-fibre nociceptors, TRPV1-negative mechano-sensitive and non-peptidergic nociceptors, and there may be a small population (~15–23%) of substance P-positive nociceptors that do not express TRPV1 (Tominaga *et al.*, 1998; Guo *et al.*, 1999; Valtschanoff *et al.*, 2001). Our next question is to what extent these populations may contribute to the conditioning and the facilitated pathways in human LTP.

Nociceptors contributing to homotopic pain-LTP

Homotopic pain-LTP in our study was unaffected by complete and selective A-fibre conduction blockade, demonstrating that high-frequency firing only in C-fibres was sufficient to induce this type of pain amplification. This is consistent with data from rat spinal cord slice preparations, where recruitment of C-fibres was obligatory for induction of nociceptive LTP (Sandkühler, 2007). LTP in lamina I neurons induced by HFS of C-fibres depends on co-activation of NMDA receptors by glutamate and neurokinin 1 receptors (NK1-R) by substance P, and resulting activation of T type calcium channels (Ikeda *et al.*, 2003; Heinke *et al.*, 2004; Naka *et al.*, 2013). Substance P acting at NK1-R in the superficial dorsal horn of rats is also essential for central sensitization induced by capsaicin through activation of TRPV1-positive afferents (Nichols *et al.*, 1999; Khasabov *et al.*, 2002; Vierck *et al.*, 2003; Rygh *et al.*, 2006). Because the majority of peptidergic nerve fibres express TRPV1 across species one can assume that in our study, those neurons were eliminated by capsaicin preincubation. Thus, as in the rat, the induction of spinal LTP in humans relies mostly on peptidergic neuron signalling in the dorsal horn, suggesting that TRPV1-positive afferents and spinal NK1-R significantly contribute to the induction of chronic pain states in humans.

Homotopic pain-LTP was also induced via fibres not expressing TRPV1 since after capsaicin treatment significant pain-LTP remained. Two explanations may account for this phenomenon: (i) substance P-containing TRPV1-negative fibres may induce LTP via the known neurokinin pathway. In rat, there may be a small population (15–23%) of substance P-positive nociceptors that do not express TRPV1 (Tominaga *et al.*, 1998; Guo *et al.*, 1999) and hence may be able to release substance P after capsaicin desensitization. However, these findings were not replicated recently, and the persistence of substance P and/or calcitonin gene-related peptide release in capsaicin-desensitized animals has not been shown functionally; (ii) induction of LTP is at least partly independent of NK1-receptor signalling. Brain-derived neurotrophic factor (BDNF) was detected in non-peptidergic C-fibre neurons in rats and contributes to spinal LTP of C-fibre-evoked field potentials (Zhou *et al.*, 2008, 2011). Thus BDNF release may be an alternative to substance P release for induction of homotopic spinal LTP (Ren and Dubner, 2007). Moreover, our psychophysical findings may in part also be explained by supraspinal rather than spinal mechanisms (descending facilitation or lowered descending inhibition; Pertovaara, 1998; Urban and Gebhart, 1999).

Nociceptors contributing to heterotopic pain-LTP

LTP of spinal nociceptive transmission and secondary hyperalgesia can be induced by HFS as well as by injection of the TRPV1 agonist capsaicin indicating that TRPV1-expressing nociceptors contribute, at least in part, to induction of these types of pain amplification (Simone *et al.*, 1989; LaMotte *et al.*, 1991; Ziegler *et al.*, 1999; Magerl *et al.*, 2001; Ikeda *et al.*, 2003, 2006). The absence of a significant heterotopic pain-LTP in capsaicin-treated skin suggests that TRPV1-expressing nociceptors are essential for this type of pain amplification. However, a very short-lived (<30 min) potentiation of pinprick pain remained after HFS in capsaicin-desensitized skin suggesting that TRPV1-negative fibres can induce short-term potentiation of pain (pain–STP; Shyu and Vogt, 2009; Rahn *et al.*, 2013).

In a previous study, the magnitude of secondary hyperalgesia remained unaltered when capsaicin was injected under selective A-fibre conduction blockade interpreted as absence or occlusion of a potential contribution of Aδ-fibre nociceptors to the induction of pain amplification at central nociceptive neurons (Ziegler *et al.*, 1999). However, the relative role of A-fibre nociceptors may have been underestimated in the capsaicin injection model by their lower sensitivity to capsaicin (Szolcsanyi, 1987; Lawson *et al.*, 1997, 2002; Ringkamp *et al.*, 2001). In the electrical HFS model the complete block by capsaicin desensitization and a moderate 35% decrease by A-fibre conduction blockade revealed that TRPV1-bearing Aδ-fibre nociceptors (AMH II

polymodal nociceptors) contribute substantially to induction of heterotopic pain-LTP. Thus, heterotopic pain-LTP (secondary hyperalgesia) is triggered collectively by TRPV1-positive A and C fibre nociceptors, but not by TRPV1-negative nociceptors.

Dynamic mechanical allodynia (pain to light touch), a companion symptom of secondary hyperalgesia, followed a similar pattern and was also completely absent when HFS was performed in capsaicin-desensitized skin and thus induction of allodynia also depended completely on TRPV1-positive nociceptors. The very mild level of dynamic mechanical allodynia as seen previously (Ziegler *et al.*, 1999; Klein *et al.*, 2004; Lang *et al.*, 2007) precluded conclusions on the potential contribution of Aδ-nociceptors to its induction.

Conclusions and outlook

Consistent with the concept that epicutaneous electrical pulses can activate all intradermal nerve endings, we found that all nociceptors contributed to HFS-induced pain (with a relative contribution of C > Aδ fibres, and an equal contribution of TRPV1-positive and TRPV1-negative fibres). As predicted from animal experiments, TRPV1-positive C-fibre nociceptors made the largest contribution to induction of both homotopic and heterotopic pain-LTP (secondary hyperalgesia). TRPV1 negative C-fibres induced homotopic self-facilitation but not heterotopic pain-LTP, likely related to MRGPRD-expressing afferents (Zhang *et al.*, 2013), which needs further study in spinal cord electrophysiology. TRPV1-positive A-fibres made a minor contribution to induction of heterotopic pain-LTP not seen with capsaicin-induced secondary hyperalgesia (Magerl *et al.*, 2001), hence it may require HFS-induced high discharge frequency that is not reached by chemical stimulation. Although TRPV1-negative A-fibres are the main primary afferents mediating pinprick pain that was strongly facilitated in heterotopic pain-LTP, they do not appear to contribute to the induction of pain-LTP. These findings highlight that distinct peripheral fibre classes mediate induction of pain-LTP, its expansion to adjacent skin, and the resulting enhanced sensitivity to pinprick in humans.

TRPV1 receptors are upregulated after various types of nerve injury, particularly in uninjured axons (Hudson *et al.*, 2001; Facer *et al.*, 2007; Kim *et al.*, 2008; Wang *et al.*, 2011). In neuropathic pain, hyperalgesia was only present in patients carrying wild-type TRPV1 alleles, but not in those with a presumed lack-of-function TRPV1 single nucleotide polymorphism (Binder *et al.*, 2011). TRPV1 is upregulated in several human diseases, like vulvodynia (Tympanidis *et al.*, 2004), post-mastectomy pain (Gopinath *et al.*, 2005), post-traumatic skin hyperalgesia (Facer *et al.*, 2007), irritable bowel syndrome (Akbar *et al.*, 2010), rectal hypersensitivity and faecal urgency (Chan *et al.*, 2003), burning mouth syndrome (Yilmaz *et al.*, 2007), or in postoperative pain following skin incisions (Pogatzki-Zahn *et al.*, 2005; Banik and Brennan, 2009; Barabas and Stucky, 2013; Mitchell *et al.*, 2014). These observations suggest that those patients may be more prone to hyperalgesia related to pain amplification of the pain-LTP type.

Conversely, the main clinical effect of TRPV1-antagonists may be to prevent the transition from acute to chronic pain by heterotopic LTP and central sensitization, while acute pain sensitivity is only partly affected (*cf.* Patapoutian *et al.*, 2009). Accordingly, the recent introduction of a high concentration capsaicin patch for the treatment of neuropathic pain, which defunctionalizes nociceptive epidermal nerve fibres (Malmberg *et al.*, 2004; Kennedy *et al.*, 2010; Anand and Bley, 2011) reduced sensory abnormalities in patients with neuropathic pain, but primarily so in patients with short disease duration (<6 months), while patients with more chronic neuropathic pain exhibited much weaker pain reduction (Backonja *et al.*, 2008; Simpson *et al.*, 2008; Derry *et al.*, 2013; Höper *et al.*, 2014; Maihöfner and Heskamp, 2014). Based on the present findings we propose that an early and more sustained treatment schedule may be more effective in both prevention and reversal of chronic neuropathic pain through a more complete functional elimination of those nociceptor populations that induce and maintain pain amplification.

Our findings may also help explain a major translation gap in pain medicine. Most drugs are developed to counteract enhanced evoked pain in animal models of nociceptive or neuropathic pain. All of these models of pain amplification likely contain an element of central sensitization. While ongoing pain in humans is mediated by some combination of all types of primary afferent nociceptors, animal models that include central sensitization of the homotopic pain-LTP type will miss the contribution of A-fibre nociceptors, while models including central sensitization of the heterotopic pain-LTP type will miss the contribution of TRPV1-negative nociceptors.

Acknowledgement

Capsaicin patches (Qutenza®) and drug-free vehicle patches were kindly provided by Astellas.

Funding

This study was funded by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft DFG - Tr236/19-1, Ma1251/9-2) and the DFG-funded Heidelberg-Mannheim Center Research Grant on Pain Research (Subproject B09 of SFB 1158).

Conflicts of interest

Johannes Gutenberg University Mainz holds a patent on pinprick stimulators used in this study (Pinprick patent DE/10.07.03/DEA10331250), for which W.M. and R.D.T. are named inventors and for which they receive royalties for marketing of production licenses.

References

Adriaensen H, Gybels J, Handwerker HO, Van Hees J. Response properties of thin myelinated (A-delta) fibers in human skin nerves. J Neurophysiol 1983; 49: 111–22.

Akbar A, Yiangou Y, Facer P, Brydon WG, Walters JR, Anand P, et al. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut 2010; 59: 767–74.

Anand P, Bley K. Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. Br J Anaesth 2011; 107: 490–502.

Anand U, Otto WR, Casula MA, Day NC, Davis JB, Bountra C, et al. The effect of neurotrophic factors on morphology, TRPV1 expression and capsaicin responses of cultured human DRG sensory neurons. Neurosci Lett 2006; 399: 51–6.

Andrew D, Greenspan JD. Peripheral coding of tonic mechanical cutaneous pain: comparison of nociceptor activity in rat and human psychophysics. J Neurophysiol 1999; 82: 2641–8.

Bachy I, Franck MC, Li L, Abdo H, Pattyn A, Ernfors P. The transcription factor Cux2 marks development of an A-delta sublineage of TrkA sensory neurons. Dev Biol 2011; 360: 77–86.

Backonja M, Wallace MS, Blonsky ER, Cutler BJ, Malan P Jr, Rauck R, et al. NGX-4010, a high-concentration capsaicin patch, for the treatment of postherpetic neuralgia: a randomised, double-blind study. Lancet Neurol 2008; 7: 1106–12.

Banik RK, Brennan TJ. Trpv1 mediates spontaneous firing and heat sensitization of cutaneous primary afferents after plantar incision. Pain 2009; 141: 41–51.

Barabas ME, Stucky CL. TRPV1, but not TRPA1, in primary sensory neurons contributes to cutaneous incision-mediated hypersensitivity. Mol Pain 2013; 9: 9.

Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J Neurophysiol 1991; 66: 212–27.

Binder A, May D, Baron R, Maier C, Tölle TR, Treede RD, et al. Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. PLoS One 2011; 6: e17387.

Brenneis C, Kistner K, Puopolo M, Segal D, Roberson D, Sisignano M, et al. Phenotyping the function of TRPV1-expressing sensory neurons by targeted axonal silencing. J Neurosci 2013; 33: 315–26.

Bromm B, Jahnke MT, Treede RD. Responses of human cutaneous afferents to CO2 laser stimuli causing pain. Exp Brain Res 1984; 55: 158–66.

Bromm B, Neitzel H, Tecklenburg A, Treede RD. Evoked cerebral potential correlates of C-fibre activity in man. Neurosci Lett 1983; 43: 109–14.

Campero M, Bostock H. Unmyelinated afferents in human skin and their responsiveness to low temperature. Neurosci Lett 2010; 470: 188–92.

Campero M, Serra J, Ochoa JL. Peripheral projections of sensory fascicles in the human superficial radial nerve. Brain 2005; 128: 892–5.

Cavanaugh DJ, Chesler AT, Braz JM, Shah NM, Julius D, Basbaum AI. Restriction of transient receptor potential vanilloid-1 to the peptidergic subset of primary afferent neurons follows its developmental downregulation in nonpeptidergic neurons. J Neurosci 2011; 31: 10119–27.

Cavanaugh DJ, Lee H, Lo L, Shields SD, Zylka MJ, Basbaum AI, et al. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. Proc Natl Acad Sci USA 2009; 106: 9075–80.

Chan CL, Facer P, Davis JB, Smith GD, Egerton J, Bountra C, et al. Sensory fibres expressing capsaicin receptor TRPV1 in patients with rectal hypersensitivity and faecal urgency. Lancet 2003; 361: 385–91.

Cooke SF, Bliss TVP. Plasticity in the human central nervous system. Brain 2006; 129: 1–15.

Cruccu G, Pennisi E, Truini A, Iannetti GD, Romaniello A, Le Pera D, et al. Unmyelinated trigeminal pathways as assessed by laser stimuli in humans. Brain 2003; 126: 2246–56.

Derry S, Sven-Rice A, Cole P, Tan T, Moore RA. Topical capsaicin (high concentration) for chronic neuropathic pain in adults. Cochrane Database Syst Rev 2013; 2: CD007393.

Dirajlal S, Pauers LE, Stucky CL. Differential response properties of IB(4)-positive and -negative unmyelinated sensory neurons to protons and capsaicin. J Neurophysiol 2003; 89: 513–24.

Facer P, Casula MA, Smith GD, Benham CD, Chessell IP, Bountra C, et al. Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. BMC Neurol 2007; 7: 11.

Finnerup NB, Jensen TS. Mechanisms of disease: mechanism-based classification of neuropathic pain-a critical analysis. Nat Clin Pract Neurol 2006; 2: 107–15.

Fuchs PN, Pappagallo M, Meyer RA. Topical EMLA pre-treatment fails to decrease the pain induced by 1% topical capsaicin. Pain 1999; 80: 637–42.

Garell PC, McGillis SL, Greenspan JD. Mechanical response properties of nociceptors innervating feline hairy skin. J Neurophysiol 1996; 75: 1177–89.

Gopinath P, Wan E, Holdcroft A, Facer P, Davis JB, Smith GD, et al. Increased capsaicin receptor TRPV1 in skin nerve fibres and related vanilloid receptors TRPV3 and TRPV4 in keratinocytes in human breast pain. BMC Women's Health 2005; 5: 2.

Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, et al. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007; 132 (Suppl 1): S26–45.

Guo A, Vulchanova L, Wang J, Li X, Elde R. Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur J Neurosci 1999; 11: 946–58.

Hansen N, Klein T, Magerl W, Treede RD. Psychophysical evidence for long-term potentiation of C-fiber and Adelta-fiber pathways in humans by analysis of pain descriptors. J Neurophysiol 2007; 97: 2559–63.

Heinke B, Balzer E, Sandkuhler J. Pre- and postsynaptic contributions of voltage-dependent Ca^{2+} channels to nociceptive transmission in rat spinal lamina I neurons. Eur J Neurosci 2004; 19: 103–11.

Heppelmann B, Heuss C, Schmidt RF. Fiber size distribution of myelinated and unmyelinated axons in the medial and posterior articular nerves of the cat's knee joint. Somatosens Res 1988; 5: 273–81.

Hines AE, Birn H, Teglbjaerg PS, Sinkjaer T. Fiber type composition of articular branches of the tibial nerve at the knee joint in man. Anat Rec 1996; 246: 573–8.

Hjerling-Leffler J, Alqatari M, Ernfors P, Koltzenburg M. Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. J Neurosci 2007; 27: 2435–43.

Höper J, Helfert S, Heskamp ML, Maihöfner CG, Baron R. High concentration capsaicin for treatment of peripheral neuropathic

pain: effect on somatosensory symptoms and identification of treatment responders. Curr Med Res Opin 2014; 30: 565–74.
Hsieh YL, Lin CL, Chiang H, Fu YS, Lue JH, Hsieh ST. Role of peptidergic nerve terminals in the skin: reversal of thermal sensation by calcitonin gene-related peptide in TRPV1-depleted neuropathy. PloS One 2012; 7: e50805.
Hudson LJ, Bevan S, Wotherspoon G, Gentry C, Fox A, Winter J. VR1 protein expression increases in undamaged DRG neurons after partial nerve injury. Eur J Neurosci 2001; 13: 2105–14.
Ikeda H, Heinke B, Ruscheweyh R, Sandkühler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science 2003; 299: 1237–40.
Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jager T, et al. Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science 2006; 312: 1659–62.
Inui K, Kakigi R. Pain perception in humans: use of intraepidermal electrical stimulation. J Neurol Neurosurg Psychiatry 2012; 83: 551–6.
Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003; 26: 696–705.
Kennedy WR, Vanhove GF, Lu SP, Tobias J, Bley KR, Walk D, et al. A randomized, controlled, open-label study of the long-term effects of NGX-4010, a high-concentration capsaicin patch, on epidermal nerve fiber density and sensory function in healthy volunteers. J Pain 2010; 11: 579–87.
Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA. Spinal neurons that possess the substance P receptor are required for the development of central sensitization. J Neurosci 2002; 22: 9086–98.
Kim HY, Park CK, Cho IH, Jung SJ, Kim JS, Oh SB. Differential Changes in TRPV1 expression after trigeminal sensory nerve injury. J Pain 2008; 9: 280–8.
Klein T, Magerl W, Hopf HC, Sandkuhler J, Treede RD. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. J Neurosci 2004; 24: 964–71.
Klein T, Magerl W, Rolke R, Treede RD. Human surrogate models of neuropathic pain. Pain 2005; 115: 227–33.
Klein T, Magerl W, Treede RD. Perceptual correlate of nociceptive long-term potentiation (LTP) in humans shares the time course of early-LTP. J Neurophysiol 2006; 96: 3551–5.
Klein T, Stahn S, Magerl W, Treede R-D. The role of heterosynaptic facilitation in long-term potentiation (LTP) of human pain sensation. Pain 2008; 139: 507–19.
Kress M, Koltzenburg M, Reeh PW, Handwerker HO. Responsiveness and functional attributes of electrically localized terminals of cutaneous C-fibers in vivo and in vitro. J Neurophysiol 1992; 68: 581–95.
LaMotte RH, Shain CN, Simone DA, Tsai EF. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 1991; 66: 190–211.
LaMotte RH, Thalhammer JG. Response properties of high-threshold cutaneous cold receptors in the primate. Brain Res 1982; 244: 279–87.
Lang S, Klein T, Magerl W, Treede RD. Modality-specific sensory changes in humans after the induction of long-term potentiation (LTP) in cutaneous nociceptive pathways. Pain 2007; 128: 254–63.
Lawson JJ, McIlwrath SL, Woodbury CJ, Davis BM, Koerber HR. TRPV1 unlike TRPV2 is restricted to a subset of mechanically insensitive cutaneous nociceptors responding to heat. J Pain 2008; 9: 298–308.
Lawson SN, Crepps B, Perl ER. Calcitonin gene-related peptide immunoreactivity and afferent receptive properties of dorsal root ganglion neurones in guinea-pigs. J Physiol 2002; 540: 989–1002.
Lawson SN, Crepps BA, Perl ER. Relationship of substance P to afferent characteristics of dorsal root ganglion neurones in guinea-pig. J Physiol 1997; 505: 177–91.
Lozier AP, Kendig JJ. Long-term potentiation in an isolated peripheral nerve-spinal cord preparation. J Neurophysiol 1995; 74: 1001–9.
Ma QP. Expression of capsaicin receptor (VR1) by myelinated primary afferent neurons in rats. Neurosci Lett 2002; 319: 87–90.
Magerl W, Ali Z, Ellrich J, Meyer RA, Treede RD. C- and A delta-fiber components of heat-evoked cerebral potentials in healthy human subjects. Pain 1999; 82: 127–37.
Magerl W, Fuchs PN, Meyer RA, Treede RD. Roles of capsaicin-insensitive nociceptors in cutaneous pain and secondary hyperalgesia. Brain 2001; 124: 1754–64.
Magerl W, Szolcsányi J, Westerman RA, Handwerker HO. Laser Doppler measurements of skin vasodilation elicited by percutaneous electrical stimulation of nociceptors in humans. Neurosci Lett 1987; 82: 349–54.
Magerl W, Wilk SH, Treede RD. Secondary hyperalgesia and perceptual wind-up following intradermal injection of capsaicin in humans. Pain 1998; 74: 257–68.
Maier C, Baron R, Tölle TR, Binder A, Birbaumer N, Birklein F, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. Pain 2010; 150: 439–50.
Malmberg AB, Mizisin AP, Calcutt NA, von Stein T, Robbins WR, Bley KR. Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch. Pain 2004; 111: 360–7.
Maihöfner CG1, Heskamp ML. Treatment of peripheral neuropathic pain by topical capsaicin: Impact of pre-existing pain in the QUEPP-study. Eur J Pain 2014; 18: 671–9.
Martin VT. Ovarian hormones and pain response: a review of clinical and basic science studies. Gend Med 2009; 6 (Suppl 2): 168–92.
McCoy ES, Taylor-Blake B, Zylka MJ. CGRPalpha-expressing sensory neurons respond to stimuli that evoke sensations of pain and itch. PloS One 2012; 7: e36355.
Messlinger K, Pawlak M, Steinbach H, Trost B, Schmidt RF. A new combination of methods for the localization, identification, and three-dimensional reconstruction of the sensory endings of articular afferents characterized by electrophysiology. Cell Tissue Res 1995; 281: 283–94.
Meyer RA, Davis KD, Cohen RH, Treede RD, Campbell JN. Mechanically insensitive afferents (MIAs) in cutaneous nerves of monkey. Brain Res 1991; 561: 252–61.
Mitchell K, Lebovitz EE, Keller JM, Mannes AJ, Nemenov MI, Iadarola MJ. Nociception and inflammatory hyperalgesia evaluated in rodents using infrared laser stimulation after Trpv1 gene knockout or resiniferatoxin lesion. Pain. 2014; 155: 733–45.
Mouraux A, Iannetti GD, Plaghki L. Low intensity intra-epidermal electrical stimulation can activate Adelta-nociceptors selectively. Pain 2010; 150: 199–207.
Naka A, Gruber-Schoffnegger D, Sandkuhler J. Non-Hebbian plasticity at C-fiber synapses in rat spinal cord lamina I neurons. Pain 2013; 154: 1333–42.
Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Luger NM, et al. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. Science 1999; 286: 1558–61.
Nolano M, Simone DA, Wendelschafer-Crabb G, Johnson T, Hazen E, Kennedy WR. Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. Pain 1999; 81: 135–45.
Ochoa J, Mair WG. The normal sural nerve in man. I. Ultrastructure and numbers of fibres and cells. Acta Neuropathol 1969; 13: 197–216.
Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov 2009; 8: 55–68.
Peng YB, Ringkamp M, Campbell JN, Meyer RA. Electrophysiological assessment of the cutaneous arborization of A delta-fiber nociceptors. J Neurophysiol 1999; 82: 1164–77.

Pertovaara A. A neuronal correlate of secondary hyperalgesia in the rat spinal dorsal horn is submodality selective and facilitated by supraspinal influence. Exp Neurol 1998; 149: 193–202.
Pfau DB, Klein T, Putzer D, Pogatzki-Zahn EM, Treede RD, Magerl W. 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. Pain 2011; 152: 1532–9.
Pogatzki-Zahn EM, Shimizu I, Caterina M, Raja SN. Heat hyperalgesia after incision requires TRPV1 and is distinct from pure inflammatory pain. Pain 2005; 115: 296–307.
Qu L, Fan N, Ma C, Wang T, Han L, Fu K, et al. Enhanced excitability of MRGPRA3- and MRGPRD-positive nociceptors in a model of inflammatory itch and pain. Brain 2014; 137: 1039–50.
Rage M, Van Acker N, Facer P, Shenoy R, Knaapen MW, Timmers M, et al. The time course of CO2 laser-evoked responses and of skin nerve fibre markers after topical capsaicin in human volunteers. Clin Neurophysiol 2010; 121: 1256–66.
Rahn EJ, Guzman-Karlsson MC, David Sweatt J. Cellular, molecular, and epigenetic mechanisms in non-associative conditioning: implications for pain and memory. Neurobiol Learn Mem 2013; 105: 133–50.
Randic M, Jiang MC, Cerne R. Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. J Neurosci 1993; 13: 5228–41.
Raymond SA, Thalhammer JG, Popitz-Bergez F, Strichartz GR. Changes in axonal impulse conduction correlate with sensory modality in primary afferent fibers in the rat. Brain Res 1990; 526: 318–21.
Ren K, Dubner R. Pain facilitation and activity-dependent plasticity in pain modulatory circuitry: role of BDNF-TrkB signaling and NMDA receptors. Mol Neurobiol 2007; 35: 224–35.
Ringkamp M, Peng YB, Wu G, Hartke TV, Campbell JN, Meyer RA. Capsaicin responses in heat-sensitive and heat-insensitive a- fiber nociceptors. J Neurosci 2001; 21: 4460–8.
Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkuhler J. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol Pain. 2011; 7: 20.
Rygh LJ, Suzuki R, Rahman W, Wong Y, Vonsy JL, Sandhu H, et al. Local and descending circuits regulate long-term potentiation and zif268 expression in spinal neurons. Eur J Neurosci 2006; 24: 761–72.
Rygh LJ, Svendsen F, Fiska A, Haugan F, Hole K, Tjølsen A. Long-term potentiation in spinal nociceptive systems - how acute pain may become chronic. Psychoneuroendocrinology 2005; 30: 959–64.
Sandkühler J. Understanding LTP in pain pathways. Mol Pain 2007; 3: 9.
Sandkühler J. Models and mechanisms of hyperalgesia and allodynia. Physiol Rev 2009; 89: 707–58.
Schliessbach J, Siegenthaler A, Streitberger K, Eichenberger U, Nuesch E, Juni P, et al. The prevalence of widespread central hypersensitivity in chronic pain patients. Eur J Pain 2013; 17: 1502–10.
Schmalbruch H. Fiber composition of the rat sciatic nerve. Anat Rec 1986; 215: 71–81.
Schmelz M, Schmid R, Handwerker HO, Torebjörk HE. Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. Brain 2000; 123: 560–71.
Serra J, Bostock H, Sola R, Aleu J, Garcia E, Cokic B, et al. Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats. Pain 2012; 153: 42–55.
Shyu B-C, Vogt B. Short-term synaptic plasticity in the nociceptive thalamic-anterior cingulate pathway. Mol Pain 2009; 5: 51.
Simone DA, Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain 1989; 38: 99–107.
Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, Willis WD. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991; 66, 228–46.
Simpson DM, Brown S, Tobias J; NGX-4010 C107 Study Group. Controlled trial of high-concentration capsaicin patch for treatment of painful HIV neuropathy. Neurology 2008; 70: 2305–13.
Sinclair DC, Stokes BAR. The production and characteristics of 'second pain'. Brain 1964; 87: 609–18.
Sinclair DC, Weddell G, Zander E. The relationship of cutaneous sensibility to neurohistology in the human pinna. J Anat 1952; 86: 402–11.
Slugg RM, Meyer RA, Campbell JN. Response of cutaneous A- and C-fiber nociceptors in the monkey to controlled-force stimuli. J Neurophysiol 2000; 83: 2179–91.
Snider WD, McMahon SB. Tackling pain at the source: new ideas about nociceptors. Neuron 1998; 20: 629–32.
Szolcsanyi J. Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultra-violet irradiation. J Physiol 1987; 388: 9–23.
Tan AM, Waxman SG. Spinal cord injury, dendritic spine remodeling, and spinal memory mechanisms. Exp Neurol 2012; 235: 142–51.
Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998; 21: 531–43.
Torsney C. Inflammatory pain unmasks heterosynaptic facilitation in lamina I neurokinin 1 receptor-expressing neurons in rat spinal cord. J Neurosci 2011; 31: 5158–68.
Treede RD, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. J Neurophysiol 1998; 80: 1082–93.
Truini A, Garcia-Larrea L, Cruccu G. Reappraising neuropathic pain in humans–how symptoms help disclose mechanisms. Nature Rev Neurol 2013; 9: 572–82.
Tympanidis P, Casula MA, Yiangou Y, Terenghi G, Dowd P, Anand P. Increased vanilloid receptor VR1 innervation in vulvodynia. Eur J Pain 2004; 8: 129–33.
Urban MO, Gebhart GF. Supraspinal contributions to hyperalgesia. Proc Natl Acad Sci USA 1999; 96: 7687–92.
Vallbo AB, Olausson H, Wessberg J. Unmyelinated afferents constitute a second system coding tactile stimuli of the human hairy skin. J Neurophysiol 1999; 81: 2753–63.
Valtschanoff JG, Rustioni A, Guo A, Hwang SJ. Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. J Comp Neurol 2001; 436: 225–35.
van den Broeke EN, van Heck CH, van Rijn CM, Wilder-Smith OH. Neural correlates of heterotopic facilitation induced after high frequency electrical stimulation of nociceptive pathways. Mol Pain 2011; 7: 28.
Vierck CJ Jr., Kline RH, Wiley RG. Intrathecal substance p-saporin attenuates operant escape from nociceptive thermal stimuli. Neuroscience 2003; 119: 223–32.
Vikman KS, Kristensson K, Hill RH. Sensitization of dorsal horn neurons in a two-compartment cell culture model: wind-up and long-term potentiation- like responses. J Neurosci 2001; 21: U25–30.
Vrontou S, Wong AM, Rau KK, Koerber HR, Anderson DJ. Genetic identification of C fibres that detect massage-like stroking of hairy skin in vivo. Nature 2013; 493: 669–73.
Wang T, Molliver DC, Jing X, Schwartz ES, Yang FC, Samad OA, et al. Phenotypic switching of nonpeptidergic cutaneous sensory neurons following peripheral nerve injury. PloS One 2011; 6: e28908.
Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE. Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. J Neurosci 1999; 19: 10184–90.
Wilder-Smith OH, Arendt-Nielsen L. Postoperative hyperalgesia: its clinical importance and relevance. Anesthesiology 2006; 104: 601–7.
Woodcock J, Witter J, Dionne RA. Stimulating the development of mechanism-based, individualized pain therapies. Nat Rev Drug Discov 2007; 6: 703–10.

Woolf CJ. What to call the amplification of nociceptive signals in the central nervous system that contribute to widespread pain? Pain 2014; 155: 1911–12.

Woolf CJ, Bennett GJ, Doherty M, Dubner R, Kidd B, Koltzenburg M, et al. Towards a mechanism-based classification of pain? Pain 1998; 77: 227–9.

Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000; 288: 1765–9.

Wooten M, Weng HJ, Hartke TV, Borzan J, Klein AH, Turnquist B, et al. Three functionally distinct classes of C-fibre nociceptors in primates. Nat Commun 2014; 5: 4122.

Yilmaz Z, Renton T, Yiangou Y, Zakrzewska J, Chessell IP, Bountra C, et al. Burning mouth syndrome as a trigeminal small fibre neuropathy: increased heat and capsaicin receptor TRPV1 in nerve fibres correlates with pain score. J Clin Neurosci 2007; 14: 864–71.

Yosipovitch G, Maibach HI, Rowbotham MC. Effect of EMLA pretreatment on capsaicin-induced burning and hyperalgesia. Acta Derm Venereol. 1999; 79: 118–21.

Zeilhofer HU. Synaptic modulation in pain pathways. Rev Physiol Biochem Pharmacol 2005; 154: 73–100.

Zhang J, Cavanaugh DJ, Nemenov MI, Basbaum AI. The modality-specific contribution of peptidergic and non-peptidergic nociceptors is manifest at the level of dorsal horn nociresponsive neurons. J Physiol 2013; 591: 1097–110.

Zhou LJ, Yang T, Wei X, Liu Y, Xin WJ, Chen Y, et al. Brain-derived neurotrophic factor contributes to spinal long-term potentiation and mechanical hypersensitivity by activation of spinal microglia in rat. Brain Behav Immun 2011; 25: 322–34.

Zhou LJ, Zhong Y, Ren WJ, Li YY, Zhang T, Liu XG. BDNF induces late-phase LTP of C-fiber evoked field potentials in rat spinal dorsal horn. Exp Neurol 2008; 212: 507–14.

Ziegler EA, Magerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain 1999; 122: 2245–57.

Zylka MJ, Rice FL, Anderson DJ. Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd. Neuron 2005; 45: 17–25.