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High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input

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van den Broeke EN, Mouraux A. High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. *J Neurophysiol* 111: 1564–1573, 2014. First published January 22, 2014; doi:10.1152/jn.00651.2013.—High-frequency electrical stimulation (HFS) of the human skin induces increased pain sensitivity in the surrounding unconditioned skin. The aim of the present study was to characterize the relative contribution of the different types of nociceptive and nonnociceptive afferents to the heterotopical hyperalgesia induced by HFS. In 17 healthy volunteers (9 men and 8 women), we applied HFS to the ventral forearm. The intensity of perception and event-related brain potentials (ERPs) elicited by vibrotactile stimuli exclusively activating nonnociceptive low-threshold mechanoreceptors and thermonociceptive stimuli exclusively activating heat-sensitive nociceptive afferents were recorded before and after HFS. The previously described mechanical hyperalgesia following HFS was confirmed by measuring the changes in the intensity of perception elicited by mechanical punctate stimuli. HFS increased the perceived intensity of both mechanical punctate and thermonociceptive stimuli applied to the surrounding unconditioned skin. The time course of the effect of HFS on the perception of mechanical and thermal nociceptive stimuli was similar. This indicates that HFS does not only induce mechanical hyperalgesia, but also induces heat hyperalgesia in the heterotopical area. Vibrotactile ERPs were also enhanced after HFS, indicating that nonnociceptive somatosensory input could contribute to the enhanced responses to mechanical pinprick stimuli. Finally, the magnitude of thermonociceptive ERPs was unaffected by HFS, indicating that type II A-fiber mechanoheat nociceptors, thought to be the primary contributor to these brain responses, do not significantly contribute to the observed heat hyperalgesia.

high-frequency stimulation; secondary hyperalgesia; event-related potentials; mechanical; heat

THE CENTRAL NERVOUS SYSTEM has the ability to change and adapt in a use-dependent way (Cooke and Bliss 2006). This has also been shown for nociceptive pathways and is thought to play a key role in the development and maintenance of chronic pain, in particular, some forms of hyperalgesia (Latremoliere and Woolf 2009; Sandkühler 2009). Indeed, sustained nociceptive input can induce activity-dependent changes in synaptic strength within nociceptive pathways, possibly leading to an amplification of nociceptive signals. This has been clearly demonstrated by Ikeda et al. (2003), who showed in vitro that high-frequency electrical stimulation (HFS) of peptidergic C-fibers induces long-term potentiation of excitatory synaptic transmission between peripheral C-fibers and secondary lamina I dorsal horn neurons projecting to the parabrachial area in the brain stem.

In humans, HFS applied onto the human skin has been shown to enhance the perception of pain elicited by nociceptive test stimuli delivered to the conditioned skin as well as the skin surrounding the conditioned area (Klein et al. 2004, 2008; van den Broeke et al. 2010, 2011, 2014; Vo and Drummond 2013). Furthermore, HFS-induced hyperalgesia within the surrounding unconditioned skin has been suggested to affect only the perception of mechanical nociceptive stimuli (Klein et al. 2008), thus mimicking the phenomenon of "secondary hyperalgesia" observed following a skin lesion, i.e., increased pain sensitivity to mechanical nociceptive stimuli delivered to the area surrounding the injured skin (Meyer and Treede 2004). At present, the exact mechanism underlying this heterotopical hyperalgesia is unknown but is thought to involve heterosynaptic facilitation and, hence, to constitute a suitable model to study the mechanisms underlying central sensitization of nociceptive pathways (Klein et al. 2008).

One important issue that needs to be clarified is the relative contribution of the different types of afferent fibers to the increased pain perception. It has been suggested that the heterotopic hyperalgesia induced by HFS is primarily mediated through a selective enhancement of the synaptic transmission of mechanical nociceptive input (Klein et al. 2008). This notion is based on the results of Lang et al. (2007), who found that HFS reduces pain thresholds to mechanical stimuli without concomitantly inducing changes in heat pain thresholds. However, they did not actually measure the intensity of the percept elicited by thermonociceptive stimuli. Furthermore, their conclusion is contradicted by the results of other studies showing increased heat pain sensitivity in the area surrounding the injured or conditioned skin when using other models to induce central sensitization (Hardy et al. 1950; Kilo et al. 1994; Pedersen and Kehlet 1998; Serra et al. 1998; Sumikura et al. 2006). Finally, one must take into consideration the fact that mechanical nociceptive stimuli inevitably also activate nonnociceptive low-threshold mechanoreceptors (LTMs) and, hence, that at least part of the HFS-induced changes in the perception of these stimuli could be related to changes in the transmission of nonnociceptive somatosensory input within lemniscal pathways.

The aim of the present study was to characterize better the effect of HFS on the different types of nociceptive and nonnociceptive afferents within the so-called area of heterotopical

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hyperalgesia. For this purpose, we compared the heterotopical effect of HFS on the intensity of the percept and the magnitude of the event-related brain potentials (ERPs) elicited by *1*) nonnociceptive vibrotactile stimuli exclusively activating LTMs, and *2*) thermonociceptive stimuli generated using an infrared CO_2 laser exclusively activating heat-sensitive afferents (Plaghki and Mouraux 2003). The previously described effect of HFS on the perception of nociceptive mechanical stimuli was confirmed by measuring the changes in the intensity of perception elicited by mechanical punctate stimuli.

METHODS

Participants

Seventeen healthy volunteers [9 men and 8 women aged 22–37 yr (mean age: 28 yr)] participated in the experiment. Approval for the experiment was obtained from the local Ethical Committee. All participants signed an informed consent form and received financial compensation for their participation. One participant was excluded from the study because we failed to deliver thermal stimuli that were perceived as painful while remaining within the limits above which we could have induced a burn lesion.

Experimental Design

The design of the experiment is summarized in Fig. 1. During the sensory testing and the HFS conditioning procedure, the participants were comfortably seated in a chair with their arm resting as comfortable as possible on a pillow.

HFS. HFS was delivered to the volar forearm, 10 cm distal to the cubital fossa. The stimulation consisted of 5 trains of 100 Hz (pulse width: 2 ms) lasting 1 s. The time interval between each train was 10 s. The intensity of stimulation was individually adjusted to 20× the detection threshold to a single pulse (0.31 ± 0.09 mA, mean ± SD).

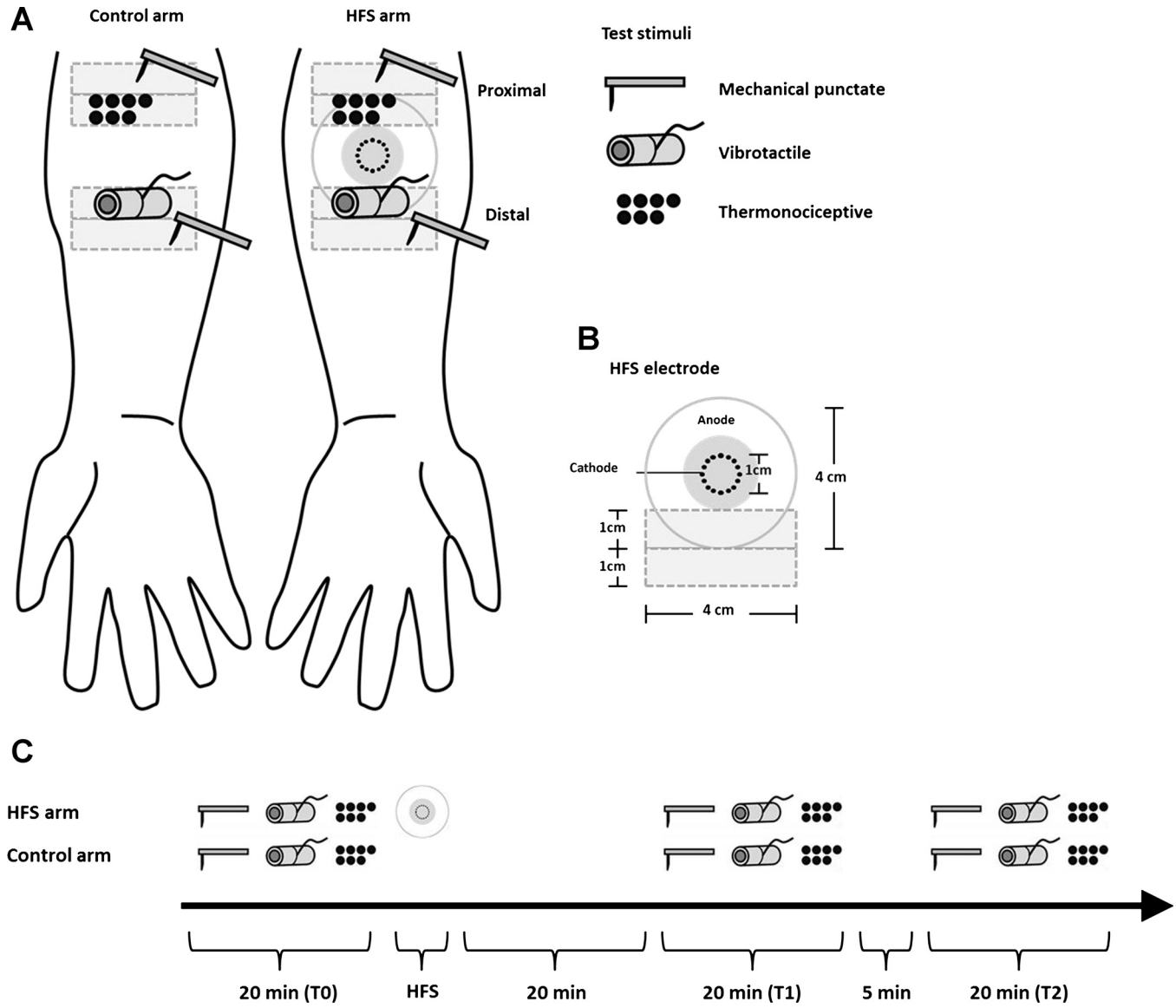

Fig. 1. Experimental setup. *A*: high-frequency stimulation (HFS) was applied to the volar forearm of 1 arm. Test mechanical punctate, vibrotactile, and thermonociceptive stimuli were applied to the skin surrounding the area onto which HFS was applied as well as to the same skin area on the contralateral arm, which served as control. *B*: the electrode used to deliver HFS consisted of 16 blunt, stainless steel pins placed in a 10-mm diameter circle (cathode) surrounded by a concentrically located, stainless steel anode. The heterotopic test area is shown in light gray. *C*: the effect of HFS on the responses elicited by the test stimuli was assessed at 3 different time points: before HFS (T0), 20 min after HFS (T1), and 45 min after HFS (T2).

The stimulation trains were generated by a constant-current electrical stimulator (Digitimer DS7A) and delivered to the skin using a specifically designed electrode previously demonstrated to activate peptidergic nociceptive afferents in the skin (Klein et al. 2004). The electrode, designed and built at the Center for Sensory-Motor Interaction (Aalborg University, Denmark), consists of 16 blunt, stainless steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed in a circle with a diameter of 10 mm and serve as cathode. A stainless steel reference electrode that serves as anode is concentrically located and has an inner diameter of 22 mm and an outer diameter of 40 mm. To avoid interference of handedness, handedness was determined using the Flinders Handedness survey (Nicholls et al. 2013), and the arm onto which HFS was applied was balanced across participants.

Heterotopic sensory stimulation. The heterotopical effect of HFS was characterized using three different types of sensory stimuli: mechanical punctate stimuli, vibrotactile stimuli, and thermonociceptive laser stimuli. The test stimuli were applied to the skin surrounding the area onto which HFS was applied as well as to the same skin area on the contralateral arm, which served as control to take into account a possible time-dependent habituation. The measurements were performed before HFS (T0), 20 min after HFS (T1), and 45 min after HFS (T2). The order of presentation of the three types of test stimuli was randomized across measurements and participants. The arm onto which the stimuli were applied first (HFS vs. control arm) was balanced across measurements and participants.

Mechanical punctate stimuli were delivered by pressing a calibrated, sharp-tipped Semmes-Weinstein monofilament (size: 5.18, 15 g, target force: 147 mN) with a 90° angle to the skin surface until it bends. The stimuli were applied twice within an area of 4 cm^2, at a distance of 2.0 cm distal and proximal relative to the center of the conditioning stimulation.

Vibrotactile stimuli consisted of constant-amplitude sinusoidal mechanical vibration delivered at 300 Hz for 50 ms using a vibrotactile transducer (length: 2.8 cm; width: 1.2 cm; Haptuator; Tactile Labs). The vibrotactile stimuli were repeated 20× using a random interstimulus interval ranging from 5 to 10 s and delivered to an area of 4 cm^2 at a distance of 1.0 cm distal or proximal (balanced across subjects) relative to the center of the conditioning stimulation.

Thermonociceptive laser stimuli consisted of brief (50 ms) pulses of radiant heat generated by a CO_2 laser (wavelength: 10.6 μm) designed and built in the Department of Physics of the Université Catholique de Louvain (Plaghki et al. 1994). Beam diameter at target site was 3.75 mm. To avoid skin overheating and minimize nociceptor sensitization or habituation, the target of the laser beam was slightly displaced after each stimulus using a mirror set on a two-axis, computer-controlled device. The stimulus energy was individually adjusted to elicit a percept qualified as painful. At the beginning of the experiment, the pain threshold was determined using a staircase procedure (11.4 ± 2.8 mJ/mm^2). This threshold was defined as the minimum energy required to elicit a clear pricking percept *1*) qualified as painful, i.e., rated as ≥50 on a numerical rating scale (NRS) extending from 0 (no perception) to 100 (maximum pain) with 50 marking the border between nonpainful and painful domains of sensation (see also *Behavioral Measures*), and *2*) detected with a reaction time <600 ms (350 ± 70 ms), i.e., compatible with the conduction velocity of nociceptive Aδ-fibers. The arm onto which the threshold was determined (conditioned or control arm) was balanced across participants. The stimuli were repeated 20× using a random interstimulus interval ranging from 5 to 10 s and delivered to an area of 4 cm^2 at a distance of 1.0 cm distal or proximal (balanced across subjects) relative to the center of the conditioning stimulation.

Behavioral Measures

The effect of HFS on the intensity of perception elicited by the three types of test stimuli was assessed by asking participants to rate the intensity of the stimuli on a NRS ranging from 0 (no perception) to 100 (maximal pain) with 50 representing the transition from nonpainful to painful domains of sensation. Inclusion of both the nonpainful and painful domains of sensation allowed us to use the same scale for every type of stimulus. For mechanical punctate stimuli, ratings were obtained following the delivery of each stimulation pair. For vibrotactile and thermonociceptive stimuli, ratings were obtained following five stimuli pseudorandomly selected within the stimulation trains. Participants were also asked to rate the intensity of the percept elicited by each of the five trains of HFS.

Electrophysiological Measures

The EEG was recorded using 32 actively shielded Ag-AgCl electrodes mounted in an elastic electrode cap and arranged according to the International 10-20 system (WaveGuard 32-channel EEG cap; Advanced Neuro Technologies). Participants were instructed to keep their gaze fixed on a black cross displayed at a distance of ~1 m at an angle of 30° below eye level and to sit as still as possible without making any movements. The EEG signals were amplified and digitized using a sampling rate of 1,000 Hz using an average reference (HS64; Advanced Neuro Technologies). Eye movements were recorded using two surface electrodes placed at the upper-left and lower-right sides of the left eye. Impedance was kept under 10 kΩ for all leads.

The EEG was analyzed offline using BrainVision Analyzer v. 1.05 (Brain Products). As a first step, the continuous EEG was band-pass filtered between 0.5 and 40 Hz using a zero-phase Butterworth filter (12 dB per octave). The EEG was then segmented into epochs extending from −500 to +1,000 ms relative to stimulus onset. Epochs containing ocular artifacts (i.e., eye movements and eye blinks) were corrected using the Gratton et al. (1983) method. After baseline correction (reference interval: −500 to 0 ms), segments with amplitude values exceeding ±100 μV were rejected as these were likely contaminated by artifacts. Separate average waveforms were computed for each participant, stimulation type (vibrotactile and thermonociceptive), and time point (T0, T1, and T2). ERPs were defined in terms of their amplitude, latency, and topographic distribution as follows. The grand average global field power (GFP) of all participants was calculated (Fig. 2; Skrandies 1990; van den Broeke et al. 2012). Subsequently, we calculated the topographic voltage distribution corresponding to the ERP latencies identified in the GFP plots. Then, we identified the electrode in the topographic plot that showed the maximal activity and used this electrode for subsequent analysis. Two distinct peaks (N1 and P2) were identified in the vibrotactile ERP at electrode Cz. The N1 was defined as the most negative peak within the time interval extending from 100 to 170 ms after stimulus onset. The P2 was defined as the most positive peak within the time interval extending from 170 to 400 ms. Two distinct peaks (N2 and P2) were identified in the thermonociceptive ERP, also at electrode Cz. The N2 was defined as the most negative peak within the time interval extending from 150 to 260 ms, and the P2 was defined as the most positive peak within the time interval extending from 260 to 500 ms. Peak amplitudes were expressed relative to baseline. Peak latencies were expressed relative to stimulus onset.

Statistical Analyses

Statistical analyses were conducted using SPSS 18 (SPSS, Chicago, IL). To check whether the data were normally distributed, we inspected the frequency distribution of the data, skewness, and kurtosis values and applied the Wilk-Shapiro test.

Statistical comparison of the intensity of percept elicited by each of the five trains of HFS was performed using one-way repeated-measures ANOVA.

To characterize the effect of HFS on the behavioral (intensity of the percept elicited by mechanical punctate, vibrotactile, and thermono-

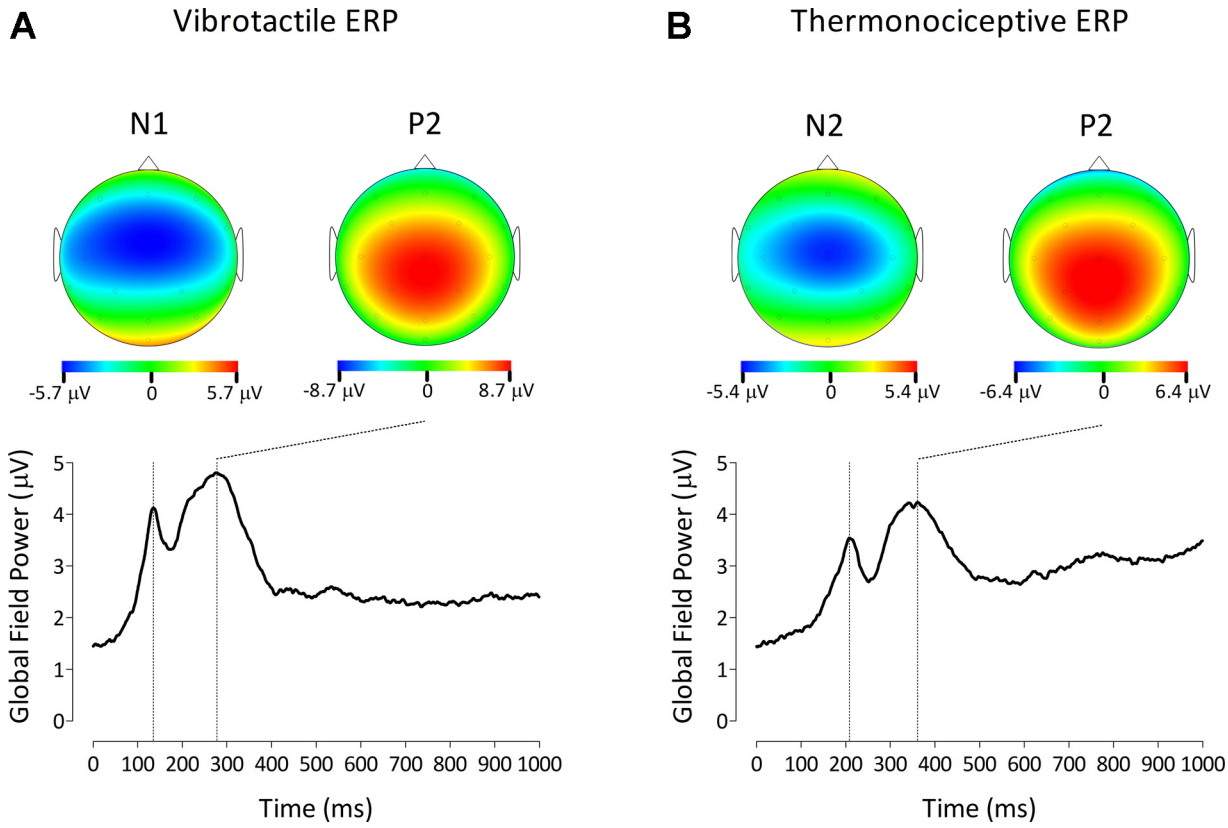

Fig. 2. Grand average global field power (GFP) and corresponding topographic maps of vibrotactile and thermonociceptive event-related brain potentials (ERPs). The GFP is calculated for each modality separately across all conditions and all subjects. *A*: 2 different peaks can be identified in the vibrotactile ERP. First, a peak appearing between 100 and 170 ms, maximal and negative at Cz, labeled N1. Second, a peak appearing between 170 and 400 ms, maximal and positive at Cz, labeled P2. *B*: 2 different peaks can also be identified in the thermonociceptive ERP. First, a peak between 150 and 260 ms, maximal and negative at Cz, labeled N2. Second, a peak appearing between 260 and 500 ms, maximal and positive at Cz, labeled P2.

ciceptive stimuli as measured using the NRS) and electrophysiological measures (N1 and P2 waves elicited by vibrotactile stimuli and N2 and P2 waves elicited by thermonociceptive laser stimuli), a general linear model repeated-measures ANOVA was performed using two within-subject factors: time (T0, T1, and T2; corresponding to before, 20 min after, and 45 min after HFS) and treatment (control vs. conditioned arm). In this model, the specific effect of HFS can be isolated from time-dependent habituation by assessing the interaction between the factors time and treatment. For the statistical evaluation of the intensity of percept obtained during mechanical punctate stimulation, we also included the factor area (distal vs. proximal) in the repeated-measures ANOVA.

The assumption of sphericity was tested using Mauchly test of sphericity. In those cases where the data violated the assumption of sphericity, F values were corrected using the Greenhouse-Geisser procedure. For post hoc tests, P values were Bonferroni-corrected for the number of tests. The level of significance was set at $P < 0.05$ (2-sided).

RESULTS

HFS Conditioning

Each train of HFS elicited a percept rated as clearly painful; mean (and SD) NRS scores were: *train 1*: 84 (11); *train 2*: 88 (9); *train 3*: 89 (8); *train 4*: 91 (8); and *train 5*: 90 (9). A one-way repeated-measures ANOVA revealed a statistically significant effect of time [$F_{\text{Greenhouse-Geisser}}(1.953, 31.255) = 5.678$, $P = 0.008$, $\eta^2 = 0.262$]. The intensity of the percept was significantly increased between the first and second train [$F(1, 16) = 6.513$, $P = 0.021$, $\eta^2 = 0.289$].

Perception of Mechanical Punctate Stimuli

The perception elicited by mechanical punctate stimuli delivered to the control and HFS-conditioned arm before (T0) and after (T1 and T2) conditioning is shown in Fig. 3. To investigate whether there were differences in perceived intensity between the two areas, we also included the factor area (distal vs. proximal) in the repeated-measures ANOVA.

The repeated-measures ANOVA revealed a statistically significant time × treatment interaction [$F_{\text{Greenhouse-Geisser}}(1.344, 21.498) = 25.152$, $P < 0.001$, $\eta^2 = 0.646$]. This interaction shows that the intensity of the percept elicited by mechanical punctate stimuli was significantly different between the two arms at the different measurement times. The univariate within-subject contrasts revealed that the perceived intensity was significantly enhanced at the conditioned arm after HFS at both T1 [$F(1, 16) = 33.468$, $P < 0.001$, $\eta^2 = 0.677$] and T2 [$F(1, 16) = 31.702$, $P < 0.001$, $\eta^2 = 0.665$]. Post hoc tests revealed a statistically significant increase of perception at T1 [paired t-test, $t(16) = -3.705$, $P < 0.05$] and T2 [paired t-test, $t(16) = -3.262$, $P < 0.05$] on the conditioned arm. The area × time × treatment interaction was not significant, indicating that there were no differences in perceived intensity after HFS between the proximal and distal areas. Mechanical hyperalgesia was present in all subjects.

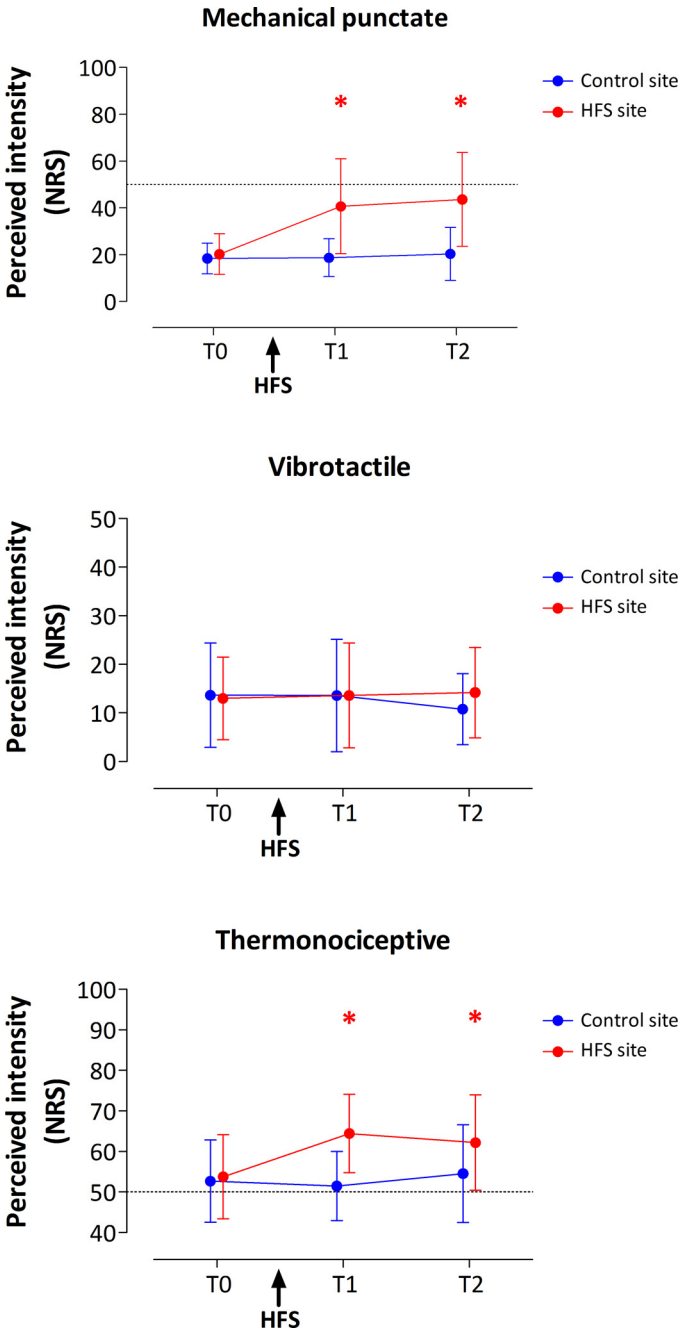

Fig. 3. Effect of HFS on the perception of mechanical punctate stimuli (delivered to the distal and proximal area relative to the site of HFS), vibrotactile stimulation, and thermonociceptive laser stimulation. Group-level mean and SD of the intensity of perception [numerical rating scale (NRS) scores] were obtained at the 3 different time points: T0, T1, and T2. Note that HFS significantly increased the perception of mechanical punctate and thermonociceptive stimuli at both T1 and T2. In contrast, HFS did not appear to modulate the perception elicited by vibrotactile stimuli. Asterisks denote statistical significance of post hoc tests ($P < 0.05$).

Perception of Vibrotactile Stimuli

The perception elicited by nonnociceptive vibrotactile stimuli delivered to the control and HFS-conditioned arm before (T0) and after (T1 and T2) conditioning is shown in Fig. 3. The repeated-measures ANOVA revealed no statistically significant interaction between the two factors.

Perception of Thermonociceptive Stimuli

The perception elicited by thermonociceptive laser stimuli delivered to the control and HFS-conditioned arm before (T0) and after (T1 and T2) conditioning is shown in Fig. 3. The repeated-measures ANOVA revealed a significant time × treatment interaction [$F(2, 32) = 12.506$, $P < 0.001$, $\eta^2 = 0.439$]. The univariate within-subject contrasts revealed that the perceived intensity was significantly enhanced at the conditioned arm after HFS at both T1 [$F(1, 16) = 20.897$, $P < 0.001$, $\eta^2 = 0.566$] and T2 [$F(1, 16) = 7.586$, $P = 0.014$, $\eta^2 = 0.322$]. Post hoc tests revealed a statistically significant increase of perception at T1 [paired t-test, $t(16) = -5.808$, $P < 0.05$] and T2 [paired t-test, $t(16) = -6.441$, $P < 0.05$] on the conditioned arm.

Vibrotactile ERPs

Group-level average waveforms of the ERPs elicited by vibrotactile stimuli delivered to the control and HFS-conditioned arm before (T0) and after (T1 and T2) conditioning are shown in Fig. 4. The mean (and SD) amplitudes of the N1 and P2 waves are shown in Fig. 5. The N1 and P2 latencies are summarized in Table 1.

The repeated-measures ANOVA revealed a significant time × treatment interaction [$F_{\text{Greenhouse-Geisser}}(1.473, 23.572) = 3.935$, $P = 0.045$, $\eta^2 = 0.197$] on the magnitude of the N1 wave. The univariate within-subject contrasts revealed that the N1 amplitude was significantly enhanced at the conditioned arm after HFS at both T1 [$F(1, 16) = 6.953$, $P = 0.018$, $\eta^2 = 0.303$] and T2 [$F(1, 16) = 6.340$, $P = 0.023$, $\eta^2 = 0.284$]. Post hoc tests revealed a statistically significant increase of N1 amplitude at T1 on the conditioned arm [paired t-test, $t(16) = 4.765$, $P < 0.05$] and a statistically significant decrease of N1 amplitude at T2 on the control arm [paired t-test, $t(16) = -3.561$, $P < 0.05$].

The repeated-measures ANOVA revealed no statistically significant differences in P2 amplitude and N1 and P2 latencies.

Thermonociceptive ERPs

Group-level average waveforms of the ERPs elicited by thermonociceptive laser stimuli delivered to the control and HFS-conditioned arm before (T0) and after (T1 and T2) conditioning are shown in Fig. 4. The mean (and SD) amplitudes of the N2 and P2 waves are shown in Fig. 5. The N2 and P2 latencies are summarized in Table 1. The repeated-measures ANOVA revealed no statistically significant differences.

DISCUSSION

The aim of this study was to examine whether, in addition to enhancing the responses to mechanical punctate stimuli, HFS also enhances the responses vibrotactile stimuli selectively activating nonnociceptive LTM and laser stimuli selectively activating heat-sensitive nociceptive afferents. After HFS, both the intensity of perception to mechanical punctate stimuli and the intensity of perception to heat stimuli were significantly increased, thus demonstrating the presence of both mechanical and heat secondary hyperalgesia. The time course of this enhancement was similar, involving both T1 and T2. The magnitude of the brain response elicited by vibrotactile stimuli

(N1 wave) was also significantly enhanced following HFS. This indicates that HFS enhances the responses to nonnociceptive vibrotactile input conveyed within the lemniscal pathway. The time course of this enhancement involved both T1 and T2, indicating that nonnociceptive somatosensory input could contribute to the enhanced responses to mechanical pinprick stimuli. In contrast, HFS did not significantly modulate the magnitude of thermonociceptive ERPs, suggesting that the HFS-induced heat hyperalgesia is mediated by afferents that do not significantly contribute to these heat-evoked brain responses.

Effect of HFS on the Responses to Mechanical Stimuli

In agreement with previous reports, we demonstrate an increased mechanical punctate sensitivity of the skin surrounding the conditioned area after HFS (Klein et al. 2004, 2008; van den Broeke et al. 2010, 2011, 2014; Vo and Drummond 2013). This increased mechanical punctate sensitivity seems very similar to the observed secondary hyperalgesia after skin injury.

In primates, it has been shown that high-intensity mechanical punctate stimuli (e.g., von Frey probes) are capable of activating Aδ- and C-fiber nociceptors (Slugg et al. 2004). However, in healthy humans, von Frey stimulation usually does not cause pain. One possible explanation for this discrepancy could be that von Frey monofilaments also activate LTMs and that this activation interacts with the perception of mechanical nociceptive input (Meyer and Treede 2004). Alternatively, it is well-known that the perception of pain requires some amount of temporal and/or spatial summation. Therefore, von Frey monofilaments could activate a too small number of nociceptors for a too short duration to elicit a sensation consistently qualified as painful.

Several previous studies have attempted to assess the relative contribution of A- and C-fibers to the enhancement of sharp pricking pain in the area of secondary hyperalgesia.

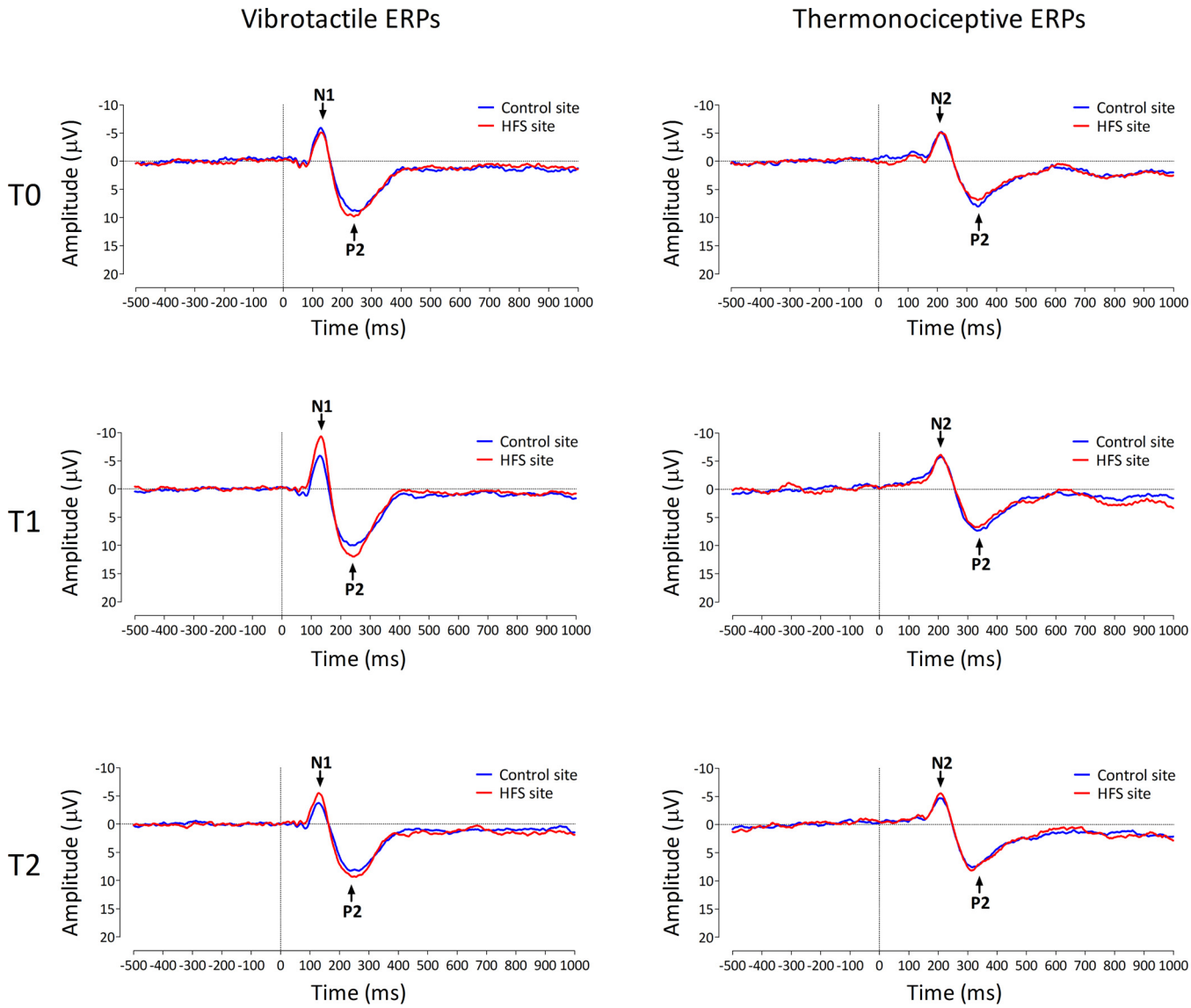

Fig. 4. Effect of HFS on the ERPs elicited by vibrotactile and thermonociceptive laser stimulation. The waveforms show the group-level average ERP waveforms of the signals measured from Cz vs. average reference, T0, T1, and T2 following stimulation of the HFS-treated arm (red) and the control arm (blue). Note the increase of the N1 wave elicited by vibrotactile stimuli delivered to the treated arm at T1 and T2.

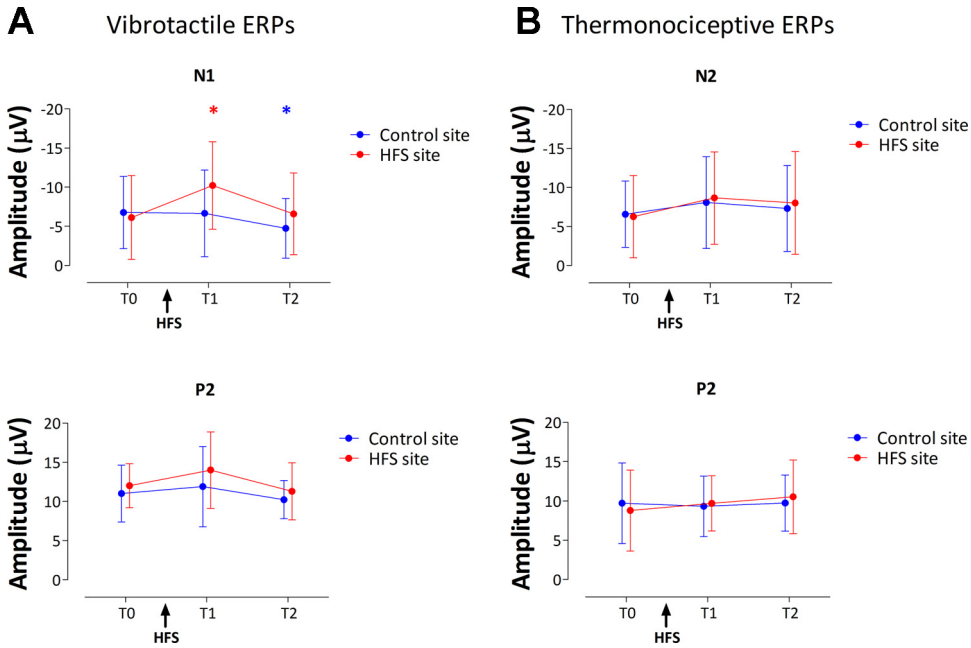

Fig. 5. Mean (and SD) of vibrotactile and thermonociceptive ERP amplitudes T0, T1, and T2 following stimulation of the HFS-treated arm (red) and the control arm (blue). The amplitude of the vibrotactile N1 was significantly increased at T1 and T2. In contrast, the amplitude of the vibrotactile P2 as well as the amplitudes of the thermonociceptive N2 and P2 were unaffected by HFS. Asterisks denote statistical significance of post hoc tests ($P < 0.05$).

Ziegler et al. (1999) applied prolonged pressure to the superficial branch of the radial nerve to block the conduction of myelinated afferents without affecting the conduction of unmyelinated afferents (unmyelinated afferents are more resistant to pressure than myelinated afferents; Nahra and Plaghki 2003; Torebjörk and Hallin 1973; Yarnitsky and Ochoa 1991). During nerve compression, they observed substantially reduced pricking pain to punctate stimuli (75%). Furthermore, they found that intradermal injection of capsaicin significantly enhanced perception of the punctate stimuli only in the absence of nerve compression. Taken together, these observations indicate that myelinated afferents significantly contribute to the perception of punctate stimuli as well as to the enhancement of this perception in the area of secondary hyperalgesia.

In a second study performed by the same group, Magerl et al. (2001) investigated whether secondary hyperalgesia involves capsaicin-sensitive or capsaicin-insensitive A-fibers. For this purpose, they treated a small skin area on the hand dorsum with topical capsaicin to induce a denervation of capsaicin-sensitive epidermal free nerve endings (Nolano et al. 1999). An adjacent area was treated with a vehicle and served as a control. Compared with the control area, they observed a significant but small reduction of pinprick pain within the capsaicin-treated skin (−32%). Then, they applied a superficial radial nerve block to interrupt the conduction of myelinated afferents innervating both the capsaicin and control skin areas. They found that pinprick pain was substantially reduced in the control area (−82%) and entirely abolished in the capsaicin-treated area (−98%). Finally, in a second experiment, they performed an intradermal injection of capsaicin in between the control and capsaicin-treated skin to induce secondary hyperalgesia. They found that this enhanced the perception of pinprick pain in both the vehicle and capsaicin-treated skin. Taken together, these results suggest that the pinprick pain elicited by punctate mechanical stimuli receive only a minor contribution from capsaicin-sensitive afferents and, most importantly, that the enhancement of pinprick pain characterizing secondary hyperalgesia is primarily mediated by capsaicin-insensitive A-fibers, which include type I A-fiber mechano-heat nociceptors (AMH-I) and high-threshold mechanoreceptors (HTM; Magerl et al. 2001).

However, the results of these experiments do not exclude the alternative interpretation that the increase in pinprick sensitivity observed in the area of secondary hyperalgesia is mediated, at least in part, by nonnociceptive Aβ-fiber afferents conveying vibrotactile sensations. Indeed, such as AMH-I and HTM, these afferents are *1*) mechanosensitive and thus expected to respond to punctate mechanical stimulation, *2*) myelinated and thus sensitive to nerve compression, and *3*) capsaicin-insensitive. Contradicting this alternative hypothesis is an observation performed in one single patient hypothesized to suffer from a large-fiber neuropathy affecting the conduction within large-

Table 1. *N1, N2, and P2 latencies*

		T0		T1		T2	
		Control Site	HFS Site	Control Site	HFS Site	Control Site	HFS Site
Vibrotactile ERP N1	Latency, ms	127.8 (9.5)	131.6 (13.5)	128.6 (9.5)	131.5 (10.0)	126.9 (15.1)	129.9 (13.6)
Vibrotactile ERP P2	Latency, ms	239.4 (48.0)	237.2 (31.2)	253.9 (43.6)	242.0 (25.6)	254.7 (36.4)	253.3 (43.7)
Thermonociceptive ERP N2	Latency, ms	218.6 (19.6)	215.4 (20.7)	209.4 (30.8)	204.2 (34.3)	204.1 (32.4)	197.2 (27.9)
Thermonociceptive ERP P2	Latency, ms	363.1 (45.5)	367.7 (44.4)	349.2 (53.6)	340.0 (34.0)	329.8 (51.7)	342.4 (34.4)

Values are means (SD). HFS, high-frequency stimulation; T0, time before HFS; T1, 20 min after HFS; T2, 45 min after HFS; ERP, event-related brain potential; N1, a peak appearing between 100 and 170 ms, maximal and negative at Cz; N2, a peak between 150 and 260 ms, maximal and negative at Cz; P2, a peak appearing between 170 and 400 ms, maximal and positive at Cz.

diameter Aβ-fibers but not small-diameter Aδ-fibers (Treede and Cole 1993). Indeed, they found that this patient developed pinprick hyperalgesia following capsaicin injection, thus suggesting that this phenomenon is not primarily mediated by Aβ-fibers.

Recently, Iannetti et al. (2013) recorded ERPs in response to pinprick stimulation before and after intradermal injection of capsaicin in the adjacent skin. The pinprick stimulation elicited a typical biphasic ERP waveform (N1 and P2 waves) with latencies compatible with the conduction of myelinated Aβ- or Aδ-fiber afferents. After capsaicin injection, they observed an enhancement of both the intensity of perception and the magnitude of the N1 wave. Taking into consideration the observation in a single patient with a lesion of the spinothalamic tract showing a reduction of the ERPs elicited by stimulation of the hypoalgesic area, the authors concluded that pinprick-evoked ERPs reflect activities primarily mediated by Aδ-fibers and, hence, that pinprick hyperalgesia following capsaicin injection is mainly mediated by Aδ-fibers.

At first glance, our results may appear to support this conclusion. Indeed, we found that HFS significantly increased the perceived intensity of the mechanical punctate stimulation, whereas it did not affect the perception elicited by vibrotactile stimulation. However, HFS induced a clear-cut enhancement of the ERPs elicited by vibrotactile stimulation (Fig. 4), demonstrating that nonnociceptive vibrotactile input conveyed through Aβ-fibers and the lemniscal pathway is processed differently after HFS. In a previous study, we assessed the effect of HFS on the ERPs elicited by nonpainful transcutaneous electrical stimuli applied to the surrounding unconditioned skin (van den Broeke et al. 2010). Such as in the present study, we observed an enhancement of the N1 wave 30 min after HFS at the conditioned arm compared with control arm. Furthermore, we also observed an increase in the perception elicited by these stimuli. A possible explanation for the different effect of HFS on the percept elicited by transcutaneous electrical stimulation (van den Broeke et al. 2010) and mechanical vibrotactile stimulation (present study) could be that both types of stimuli do not activate the same types of somatosensory afferents. Indeed, transcutaneous electrical stimulation may be expected to activate indistinctly all large-diameter afferents, whereas mechanical vibrotactile stimulation may be expected to activate predominantly rapidly adapting tactile mechanoreceptors.

In summary, by showing that HFS significantly enhances the ERPs elicited by vibrotactile stimuli selectively activating LTMs, our results demonstrate that the effect of HFS is not restricted to mechanical nociceptive input conveyed by AMH-I. Because the time course of the effect of HFS on the N1 wave of vibrotactile ERPs was not different from the time course of the effect of HFS on the perception of mechanical punctate stimulation (both were enhanced at T1 and T2), our results suggest that nonnociceptive vibrotactile input could contribute to the phenomenon of mechanical hyperalgesia.

Effect of HFS on the Responses to Thermal Stimuli

Such as the perception elicited by mechanical punctate stimuli, the perception elicited by nociceptive radiant heat stimuli applied to the heterotopic area was significantly enhanced after HFS. This shows that HFS induces both mechanical and heat secondary hyperalgesia, challenging the conclusions of Lang et al. (2007) but supporting the results of other studies (Hardy et al. 1950; Kilo et al. 1994; Pedersen and Kehlet 1998; Serra et al. 1998; Sumikura et al. 2006). Importantly, the time course of the increased perception to laser stimuli after HFS was similar to the time course of the effect of HFS on the perception of punctate mechanical stimuli (Fig. 3).

Both Aδ- and C-fiber heat-sensitive afferents contribute to the perception elicited by nociceptive radiant heat stimuli (Mouraux and Plaghki 2007). Based on their responses to noxious heat, these afferents can be categorized as either slowly adapting (AMH-I and slowly adapting C-fibers, which respond gradually following the onset of a thermal stimulus and for which response exhibits little or no adaptation when the thermal stimulus is maintained over time) or rapidly adapting (AMH-II and quickly adapting C-fibers, which respond immediately after the onset of a thermal stimulus but quickly adapt if the thermal stimulus is maintained; Meyer and Campbell 1981; Treede et al. 1995).

In the present study, we used short-lasting laser heat stimuli that probably do not elicit a strong response within slowly adapting nociceptors (Bromm et al. 1984). Hence, the perception elicited by the thermal stimuli was probably mainly related to the activation of AMH-II and quickly adapting C-fibers, and the increased perception following HFS could be explained by an enhancement of the responses elicited by activation of these afferents. Alternatively, HFS could also change the responsiveness of slowly adapting nociceptors. For example, Ringkamp and Meyer (2009) showed that slowly adapting, heat-sensitive afferents can respond in a more phasic manner following tissue injury. Therefore, these afferents could also contribute to the enhanced perception after HFS.

Whether an increase in baseline skin temperature resulting from a flare response within the skin surrounding the area of HFS could have contributed to the observed heat hyperalgesia should also be considered (Ali et al. 1996). However, previous studies have shown that, following skin burn injury, the temperature in the area of flare increases by only 0.3°C, and this increase is clearly insufficient to explain the observed enhancement of perception (Pedersen and Kehlet 1998).

Contrasting strongly with the observed heat hyperalgesia that clearly increased the perceived intensity of laser stimuli delivered to the HFS site at both T1 and T2, the magnitude of the ERPs elicited by the same laser stimuli were not increased following HFS. Because laser-evoked ERPs are thought to be exclusively related to the activation of AMH-II (the relatively short latency of the N2 and P2 waves is incompatible with the slow conduction velocity of unmyelinated C-fibers; Mouraux et al. 2012), the observed dissociation between a marked effect of HFS on the perception elicited by laser stimulation and the lack of effect of HFS on the ERPs elicited by the same stimuli could indicate that HFS-induced thermal hyperalgesia is mediated by quickly adapting, heat-sensitive C-fibers. An involvement of C-fibers in secondary thermal hyperalgesia has also been proposed by Serra et al. (2004). Using microneurographic recordings, the authors showed that a subclass of mechano-insensitive C-fibers are sensitized following adjacent intradermal capsaicin injection. This raises the possibility that at least part of the induced secondary heat hyperalgesia results from

peripheral sensitization of C-fibers in the surrounding skin (Serra et al. 2004).

Conclusion

The present study confirms that HFS applied onto the human skin induces a mechanical hyperalgesia in the surrounding unconditioned skin, similar to the phenomenon of secondary hyperalgesia following skin lesion and likely to be primarily mediated by an enhancement of mechanical nociceptive input conveyed by AMH-I and/or HTMs.

However, our results show that the effect of HFS is not restricted to an enhancement of the responses to mechanical nociceptive input, as it also clearly enhances the brain responses to nonnociceptive vibrotactile stimuli selectively activating nonnociceptive Aβ-fiber LTMs of the lemniscal pathway. This raises the possibility that nonnociceptive vibrotactile input contributes to the phenomenon of mechanical secondary hyperalgesia. In addition, HFS induced a significant thermal hyperalgesia, as demonstrated by the increased perception to thermonociceptive laser stimuli and hypothesized to result from an enhancement of thermonociceptive input conveyed by quickly adapting, heat-sensitive C-fiber afferents.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

E.N.v.d.B. and A.M. conception and design of research; E.N.v.d.B. performed experiments; E.N.v.d.B. analyzed data; E.N.v.d.B. and A.M. interpreted results of experiments; E.N.v.d.B. prepared figures; E.N.v.d.B. and A.M. drafted manuscript; E.N.v.d.B. and A.M. edited and revised manuscript; E.N.v.d.B. and A.M. approved final version of manuscript.

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