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RESEARCH ARTICLE

Sensory Processing

Repeated exposure to cutaneous high-frequency electrical stimulation prolongs the time course of secondary hyperalgesia

Louisien Lebrun,[1] Alexandre Stouffs,[1] Emmanuel Hermans,[1] Emanuel N. van den Broeke,[1,2] Caterina Leone,[3] Ombretta Caspani,[4] Andrea Truini,[3] Luis Garcia-Larrea,[5] Rolf-Detlef Treede,[4,6] and André Mouraux[1]

[1]*Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium;* [2]*Health Psychology, Faculty of Psychology and Educational Sciences, KU Leuven, Leuven, Belgium;* [3]*Department of Human Neurosciences, Sapienza University, Rome, Italy;* [4]*Mannheim Center for Translational Neurosciences, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany;* [5]*Institut National de la Santé et de la Recherche Médicale, Centre de Recherche en Neurosciences de Lyon, NeuroPain, Université Claude Bernard Lyon 1, Université Jean Monnet, Bron, France; and* [6]*Department of Psychiatry and Psychotherapy, Central Institute for Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany*

Abstract

Exposure to noxious stimuli induces secondary hyperalgesia (SH), likely due to long-term potentiation (LTP) in the dorsal horn or "central sensitization." Previous studies have suggested that repeated exposure to a sensitizing stimulus may promote a persistent form of potentiation. We tested this hypothesis by characterizing the time course and spatial spread of SH induced by repeated exposures to high-frequency electrical stimulation (HFS) in healthy volunteers. Separate groups of 16 participants received either a single session of 5 or 10 HFS trains ("HFS 1x5" and "HFS 1x10" groups) or 2 sessions of 5 HFS trains separated by 1 hour and applied to the same forearm ("HFS 2x5" group) or the contralateral forearm ("HFS 2x5 bilateral" group). HFS trains consisted of 1 second of 100-Hz stimulation (×20 detection threshold). The magnitude and spread of SH were assessed using pinprick stimulation at nine time points up to 24 hours post-HFS. Temporal decay of HFS-induced increase in pinprick sensitivity was significantly slower in the HFS 2x5 versus the HFS 1x5 and HFS 1x10 groups (half-life: 438 vs. 255 vs. 247 min). In contrast, the time course of hyperalgesia area was similar in the HFS 2x5 and HFS 1x5 groups (451 and 470 min) but decayed more rapidly in the HFS 1x10 group (92 min). In the HFS 2x5 bilateral group, preexposure to HFS on one forearm did not affect contralateral hyperalgesia magnitude or half-life but slightly increased its spatial extent. These findings suggest that repeated exposure to noxious stimulation favors the induction of a longer-lasting form of central sensitization.

NEW & NOTEWORTHY This study shows in healthy human volunteers that two sessions of high-frequency electrical stimulation of the skin (HFS) separated by 1 hour induce longer-lasting secondary hyperalgesia as compared to a single session matched in terms of the number of HFS pulses. This finding suggests that repeated exposures to noxious stimulation can favor the induction of a longer-lasting form of central sensitization.

central sensitization; high-frequency stimulation; long term potentiation; secondary hyperalgesia

INTRODUCTION

A key feature of the nociceptive system is its propensity to sensitize when exposed to repeated nociceptive stimulation or tissue injury (1–3). Sensitization of the nociceptive system may involve both changes at the level of peripheral nociceptors leading to an increased sensitivity of free nerve endings and changes at the level of the central nervous system leading to increased synaptic transmission of nociceptive input and transformation of nonnociceptive into nociceptive input. Increased synaptic transmission of nociceptive input has been described extensively at the level of the dorsal horn in animal studies ("spinal central sensitization") (4). Notably, studies in animals have shown that high-frequency electrical

Correspondence: L. Lebrun (louisien.lebrun@student.uclouvain.be); A. Mouraux (andre.mouraux@uclouvain.be).
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stimulation (HFS) of C-fiber nociceptors can induce long-term potentiation (LTP) both at the spinal synapses exposed directly to that nociceptive input (homosynaptic LTP) and at neighboring unconditioned dorsal horn synapses (heterosynaptic LTP) (5, 6).

In humans, HFS delivered transcutaneously using a multipin electrode designed to preferentially activate skin nociceptors, typically consisting of five consecutive 1-s trains of electrical pulses delivered at 100 Hz, has been shown to induce a pronounced and prolonged increase in sensitivity to mechanical pinprick stimulation of the neighboring skin (2, 7–9). This phenomenon of HFS-induced secondary mechanical hyperalgesia has been linked to the spinal central sensitization reported in animal studies (6).

Pfau et al. (10) characterized the time course of HFS-induced pinprick hyperalgesia over a 24-hOUR period. On average, the hyperalgesia half-life was approximately 5 hours. However, in some individuals (10 out of 55), hyperalgesia persisted beyond the study window, suggesting that HFS may trigger a more persistent form of LTP in susceptible individuals. Interestingly, preclinical studies on learning-related LTP in the hippocampus (11, 12) have shown that repeated exposure to HFS (for example, 3 trains of HFS delivered at 10-min intervals) can favor the induction of a more persistent form of LTP, referred to as "late LTP," lasting several days and possibly involving to a greater extent transcriptional processes and protein synthesis (13, 14). Within the nociceptive system, it was shown in rodents that a first injection of NGF into a low back muscle failed to sensitize dorsal horn neurons, whereas a second injection repeated 5 days later induced spinal central sensitization (15). Similarly, repeated surgery in animals (16, 17) and repeated Caesarian surgery in women (18) have been associated with increased postoperative peri-incisional sensitivity to pinprick stimuli and postoperative pain, compatible with the notion that prior induction of central sensitization may potentiate the susceptibility to develop a longer lasting form of central sensitization. This "latent" form of central sensitization suggests that when the increased peripheral sensitivity induced by a first sensitizing event has resolved, the nervous system may have not yet returned to its previous nonsensitized state, as exposure to another sensitizing event can trigger larger or longer-lasting effects.

Prior exposure to a sensitizing event can expand central sensitization not only in the time domain (transition from early to late LTP) but also in the spatial domain (secondary hyperalgesia spreading to more remote body sites). Preclinical studies on nerve injury have demonstrated the activation of a facilitatory brainstem loop that contributes to spinal central sensitization and this spatial domain spread (19–24). This brainstem loop could also enhance responses to stimuli delivered to the contralateral side (22, 25).

The aim of the present exploratory study was to test for the first time in humans whether prior exposure to a conditioning stimulus inducing central sensitization may expand temporally and/or spatially the central sensitization induced by exposure to a second conditioning stimulus.

To address this question, we assessed in healthy human volunteers the time course of the magnitude and spatial spread of secondary hyperalgesia induced by two successive conditioning stimuli, each consisting of five trains of HFS, given 1 hour apart to either the same volar forearm ("HFS 2x5" group) or to the contralateral volar forearm ("HFS 2x5 bilateral" group). As controls, we characterized the effects of a single conditioning stimulus consisting of 5 trains of HFS ("HFS 1x5" group) or 10 trains of HFS ("HFS 1x10" group) to match the total number of stimuli delivered in the HFS 2x5 conditions.

An expansion in the time domain occurring specifically in the HFS 2x5 groups would indicate that HFS creates a memory trace between the two exposures. Furthermore, an expansion in space would suggest increased heterosynaptic LTP and/or involvement of descending modulatory pathways.

METHODS

Participants and Experimental Design

Sixty-four healthy volunteers (34 women and 30 men aged 18–38 yr; mean: 23.7 yr) were included in this between-subjects experiment. The local ethics committee approved the study. All participants provided written informed consent and received a financial compensation at the end of the experiment. The experiment was conducted according to the Declaration of Helsinki.

The inclusion criterion was being aged 18–40 yr. Exclusion criteria were suffering from cardiac or neurological disorders including headaches and practicing sports requiring intense use of the forearms (e.g., volleyball, handball). Moreover, participants with a history of traumatic injury of the upper limb or head, participants with a dermatological condition involving the forearm, and participants having participated in a previous experiment involving HFS were excluded. Finally, participants were asked to sleep at least 6 hours on the night before the experiment (26) and to refrain from recreational drugs and medication including analgesics for a minimum of 3 days preceding the experiment, except for oral contraception.

Participants were randomly assigned to 1 of 4 groups of 16 participants (Fig. 1 and Table 1). As this was an exploratory study, no prior estimates of effect sizes were available. The decision to include 16 participants per group was based on previous studies investigating HFS-induced secondary hyperalgesia, which used similar group sizes to compare different HFS frequencies (8), stimulation patterns (27), or stimulation sites (9).

In all four groups, HFS was delivered as trains lasting 1 s, consisting of 100 pulses delivered at 100 Hz. The first group (HFS 1x5) received a single sequence of five HFS trains delivered using a 10-s intertrain interval (500 pulses in total) on the dominant or nondominant volar forearm [balanced across participants; handedness assessed using the Flinders Handedness survey (28)]. The second group (HFS 1x10) received a single sequence of ten HFS trains on the dominant or nondominant volar forearm, also using a 10-s intertrain interval (1,000 pulses in total). The third group (HFS 2x5) received two consecutive sequences of five HFS trains on the same forearm (dominant or nondominant). The trains within each sequence were separated by a 10-s intertrain interval, and the two HFS blocks were separated by a 60-min pause. Such as in the HFS 1x10 group, a total of 1,000 pulses were delivered. The fourth group (HFS 2x5 bilateral) also received two consecutive blocks of five HFS trains separated by a 60-min pause (total number of

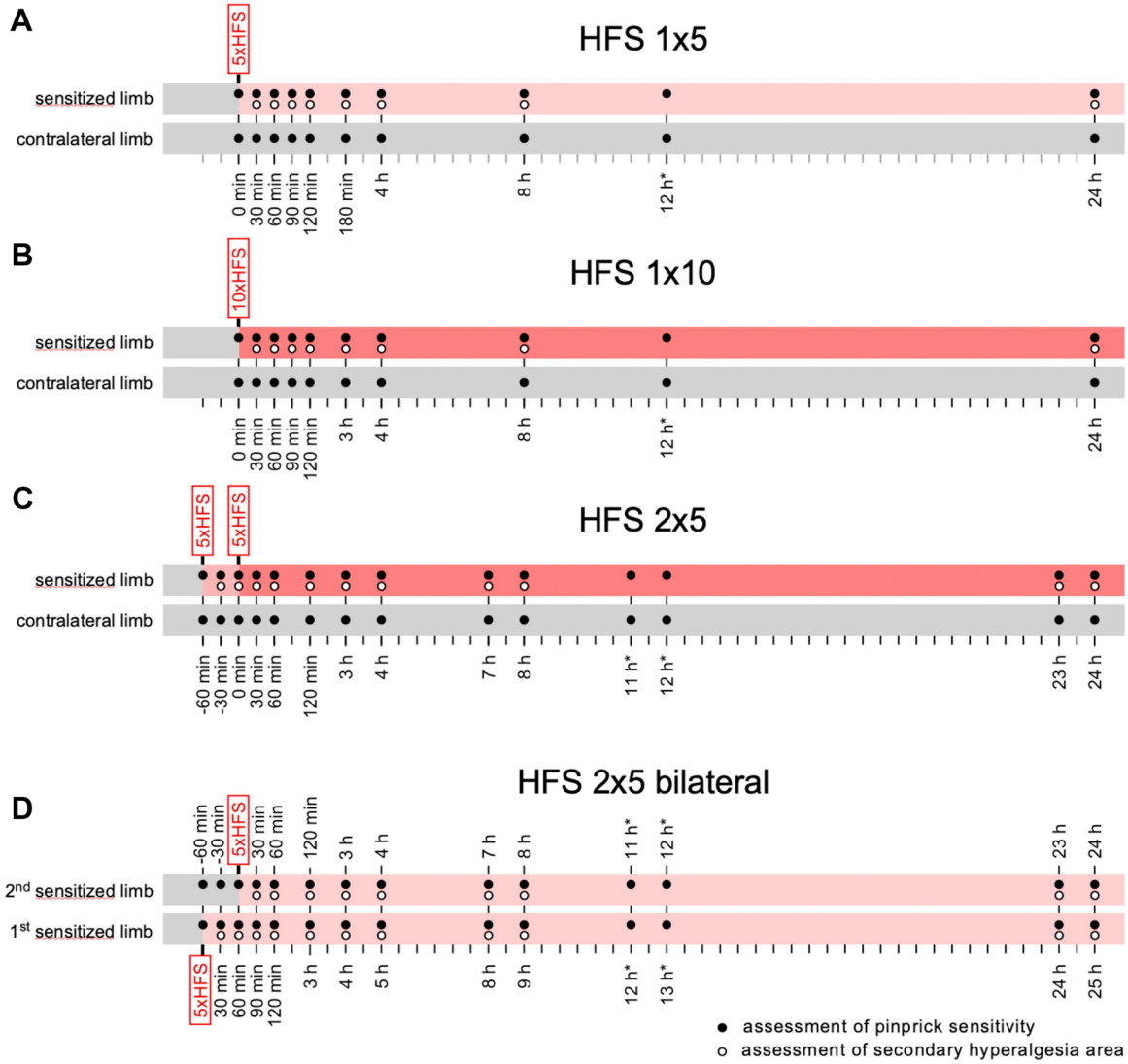

Figure 1. Study design and assessment time points. *A*: participants of the HFS 1x5 group received a single session of high-frequency stimulation (HFS) consisting of five 1-s trains in a row (5xHFS). *B*: participants of the HFS 1x10 group also received a single session of HFS but consisting of ten 1-s trains in a row (10xHFS). *C*: participants of the HFS 2x5 group were exposed to 2 separate sessions of HFS separated by a 1-h break. Such as in the HFS 1x5 group, each session consisted of five 1-s trains of HFS. *D*: participants of the HFS 2x5 bilateral' group received a first session of HFS on one forearm (first sensitized limb) followed by a second session of HFS on the other forearm (second sensitized limb), with a 1-h break in between. Again, the sessions consisted of five 1-s trains of HFS. The time points at which pinprick sensitivity was assessed at both forearms are shown as black dots, while the time points at which secondary hyperalgesia area size was assessed at the sensitized forearm(s) are shown as white dots. Times are expressed relative to the delivery of HFS.

pulses: 1,000). The first HFS sequence was delivered to the dominant or nondominant volar forearm, and the second HFS sequence was delivered to the contralateral forearm. In all four groups, HFS was delivered in the morning, around 8 AM.

All groups consisted of young male and female healthy volunteers (Table 1). There were no significant between-group differences in the electrical detection thresholds (used to determine the intensity of HFS stimulation) and in the pre-HFS ratings of mechanical pinprick stimuli (Table 1).

High-Frequency Electrical Stimulation of the Skin

Participants were seated in a comfortable chair with their arms resting on a desk and volar forearms facing upwards. HFS was delivered 10 cm distal from the cubital fossa (dermatomes C6-C7-T1) (29), using a multipin electrode designed to preferentially activate cutaneous free nerve endings (Center for Sensory-Motor Interaction, Aalborg University, Denmark). The electrode consists of 16 blunt stainless-steel pins (diameter: 0.2 mm) that protrude 1 mm from the base. The pins are placed in a 10-mm-diameter circle that serves as a cathode. The cathode is encircled by a stainless-steel anode electrode with an inner diameter of 22 mm and an outer diameter of 40 mm.

Each electrical pulse consisted of a constant-current square-wave pulse lasting 2 ms, followed by a charge-compensating square-wave pulse of opposite polarity, lasting 4 ms (8). The stimuli were delivered using a constant-current electrical stimulator (DS5; Digitimer, Welwyn Garden City, UK) triggered by a digital-analog interface (NI6343, National Instruments, Austin, TX). The intensity of stimulation was

Table 1. *Demographics (age and sex), detection threshold to a single electrical pulse, and baseline pinprick intensity ratings in each of the four groups of participants*

	HFS 1x5	HFS 1x10	HFS 2x5	HFS 2x5 Bilateral
Age, years	22.7 ± 2.6	23.9 ± 5.0	23.8 ± 4.0	24.3 ± 4.3
Sex (female:male), %	69:31	50:50	50:50	44:56
Electrical detection threshold, mA	0.21 ± 0.07	0.22 ± 0.12	0.24 ± 0.04	0.27 ± 0.09
Pre-HFS pinprick NRS	22.4 ± 12.1	17.9 ± 9.0	17.1 ± 7.9	16.0 ± 7.2

Values are means ± SD. HFS, high-frequency electrical stimulation. HFS 1x5, single session of 5 HFS trains; HFS 1x10, single session of 10 HFS trains; HFS 2x5, 2 sessions of 5 HFS trains separated by a 1-h break and applied to the same forearm; HFS 2x5 bilateral, 2 sessions of 5-HFS trains separated by a 1-h break and applied to the first- and second-sensitized forearms; NRS, numerical rating scale.

set to 20 times the detection threshold for a single pulse, which was estimated in each participant at the beginning of the experiment.

Assessment of Mechanical Pinprick Sensitivity

In all four groups, the sensitivity to mechanical pinprick stimuli was assessed immediately before HFS, and until 24 h after HFS, at both forearms (Fig. 1). In the HFS 1x5 (*group 1*) and HFS 1x10 (*group 2*) groups, the assessment was performed once before HFS ($T = 0$ min), and nine times after HFS ($T = 30$ min, 60 min, 90 min, 120 min, 180 min, 4 h, 8 h, 12 h and 24 h after HFS). In the HFS 2x5 (*group 3*) and HFS 2x5 bilateral (*group 4*) groups, the same time points were assessed relative to each of the two HFS trains, resulting in three time points before the second HFS train ($T = -60$ min, -30 min, and 0 min) and 11 time points after the second HFS train ($T = 30$ min, 60 min, 120 min, 180 min, 4 h, 7 h, 8 h, 11 h, 12 h, 23 h, and 24 h).

The pinprick stimulus consisted of a custom 0.25-mm flat-tip pinprick probe (Center for Advancement of Teaching and Learning, Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium) exerting a 128-mN force (8, 9). At each time point, three pinprick stimuli were applied perpendicular to the skin surrounding the area of HFS stimulation at the sensitized forearm and at the same location on the contralateral forearm. After each stimulus, participants were requested to report the intensity of the elicited sensation using a numerical rating scale ranging from 0 (no perception) to 100 (maximal imaginable pain), with 50 marking the limit between nonpainful and painful sensations (9, 30–33). To avoid sensitization of the skin by repeated mechanical pinprick stimulation, the probe was not applied twice at the same location within each assessment. The order of forearm testing was balanced across participants and remained identical across the different time points. Along the day, participants were trained to perform the pinprick assessment by themselves to perform one self-assessment of pinprick sensitivity scheduled 12 h after HFS. For all time points except this 12-h time point, reported intensities are those obtained during evaluation with the experimenter. Pinprick sensitivity at each time point and at each forearm was expressed as the mean of the three ratings.

Assessment of the Area of HFS-Induced Secondary Hyperalgesia

In each group, the same 128-mN pinprick probe was used to map, from 30 min to 24 h after HFS, the area of increased mechanical pinprick sensitivity surrounding the site of HFS stimulation. In the HFS 1x5 and HFS 1x10 groups, the mapping was performed eight times after HFS ($T = 30$ min, 60 min, 90 min, 120 min, 180 min, 4 h, 8 h, and 24 h). The same time points were assessed relative to each of the two HFS trains in the HFS 2x5 and HFS 2x5 bilateral groups, resulting in a total of two time points before the second HFS train ($T = -30$ min and 0 min) and nine time points after the second HFS train ($T = 30$ min, 60 min, 120 min, 180 min, 4 h, 7 h, 8 h, 23 h, and 24 h).

Participants were asked to close their eyes during the mapping procedure. The stimuli were applied perpendicular to the skin along eight directions separated by a 45° angle starting far from the HFS-treated skin and moving toward the center of the sensitization area. Stimuli were applied every 5 mm. Participants were instructed to report when the sensation increased, and the corresponding stimulation location was marked with a felt-tip pen (9, 31, 33–35). For each of the eight directions, we then measured the distance between the center of the HFS electrode location and the mark. The area of secondary hyperalgesia was computed as the area of a piecewise polynomial fit of a two-dimensional (2-D) closed curve onto the eight marked locations (interpclosed function; Santiago Benito 2023, Matlab Central File Exchange) (9).

Outcome Variables

Magnitude of the post-HFS increase in pinprick sensitivity relative to baseline.

For each time point after HFS ($T_{\text{timepoint}}$), the magnitude of the HFS-induced increase in pinprick sensitivity was computed as the change in pinprick sensitivity at the sensitized forearm relative to the pinprick sensitivity [numerical rating scale (NRS)] before HFS (T_0) at that sensitized forearm [$\Delta\text{NRS}_{\text{HFS}}(T_{\text{timepoint}}) = \text{NRS}_{\text{HFS}}(T_{\text{timepoint}}) - \text{NRS}_{\text{HFS}}(T_0)$]. Furthermore, in the HFS 1x5, 1x10, and 2x5 groups, we also computed the difference between the change in sensitivity at the HFS-sensitized limb and the change in sensitivity at the contralateral nonsensitized limb [$\Delta\Delta\text{NRS}(T_{\text{timepoint}}) = \Delta\text{NRS}_{\text{HFS}}(T_{\text{timepoint}}) - \Delta\text{NRS}_{\text{contralateral}}(T_{\text{timepoint}})$] to account for time-dependent effects on pinprick ratings unrelated to HFS.

Extent of the area of HFS-induced secondary hyperalgesia.

For each time point after HFS and each sensitized forearm, the radius of the area of increased pinprick sensitivity was used as a measure of the spatial extent of HFS-induced secondary hyperalgesia ($r_{\text{timepoint}}$). The radius of the area of increased pinprick sensitivity was computed as the square root of the area per pi.

Duration of the HFS-induced increase in pinprick sensitivity.

In all four groups (HFS 1x5, 1x10, 2x5, and 2x5 bilateral), the increase in pinprick ratings and area radius of secondary hyperalgesia induced by HFS peaked 30–60 min after HFS and then decreased with a time course resembling that of an exponential decay, as also reported in (9, 10). Therefore, the duration of the HFS-induced change in pinprick sensitivity was estimated by fitting an exponential decay function [$Y = Y_0 \times \exp(-K \times (t))$], where t is the time in minutes, Y_0 corresponds to the change in pinprick rating at time $T = 0$ ($\Delta\Delta$NRS or ΔNRS_{HFS}), $T = 0$ corresponds to $T + 30$, and K corresponds to the rate of decay. An initial group-level fitting was conducted in GraphPad Prism (version 8.0.1 for Windows, GraphPad Software, San Diego, CA; www.graphpad.com) using a least squares regression (Levenberg–Marquardt algorithm) (36–38) applied to the group-level means. Both parameters Y_0 and K were constrained to a value greater than 0. The duration of the HFS-induced change in pinprick sensitivity was expressed as the estimated half-life of the effect, corresponding to $\ln(2)/K$ (9). Then, using the group-level parameters as initial estimates, we fitted the same exponential decay function to each participant's individual data. This yielded Y_0 and half-life parameters for each participant in each group.

Duration of the area of HFS-induced secondary hyperalgesia.

The same approach was used to estimate the time course and half-life of the radius of the HFS-induced area of secondary hyperalgesia.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism and JASP (Version 0.14.1.0 for Windows).

Hypothesis 1: the duration of HFS-induced secondary hyperalgesia is prolonged by previous exposure to HFS delivered to the same forearm.

Our first hypothesis was that when a second train of HFS is delivered 1 hour after a first train of HFS delivered to the same forearm (HFS 2x5 group), the previous exposure to HFS may generate a memory trace facilitating the induction of late LTP and thus prolonging the duration of HFS-induced secondary hyperalgesia. To test this hypothesis, we compared the temporal decay parameters (K) of *1*) the poststimulus changes in pinprick sensitivity ($\Delta\Delta$NRS), and *2*) the radius of the area of secondary hyperalgesia (R) in the HFS 1x5, 1x10, and 2x5 groups. These comparisons allowed *1*) evaluating the effect of preexposing the limb to five trains of HFS 1 hour before delivering other trains of HFS, and *2*) determining whether increasing the total number of HFS trains affects the time course (5 trains in the HFS 1x5 group vs. 10 trains in the HFS 1x10 and 2x5 groups). For each comparison, an extra sum-of-squares F test was used to compare the group-level mean fit obtained with a single decay parameter shared for both datasets versus separate decay parameters fitted to each of the two datasets (39–41). Furthermore, the individual estimates of temporal decay (half-life) were compared between the HFS 1x5, 1x10, and 2x5 groups using a one-way ANOVA, followed by post hoc pairwise t tests to explore specific group differences (for this analysis, individual fits with an adjusted $R^2 < 0.5$ were excluded).

Hypothesis 2: the peak magnitude and/or spatial extent of HFS-induced secondary hyperalgesia is increased by previous exposure to HFS delivered to the same forearm.

To test this second hypothesis, we compared the magnitude of the post-HFS increase in pinprick sensitivity ($\Delta\Delta$NRS) and the secondary hyperalgesia area radius (R) in the HFS 1x5, 1x10, and 2x5 groups, at time point $T + 30$. This time point was chosen because previous studies have shown that the HFS-induced increase in pinprick sensitivity peaks ~30 min after exposure to HFS (10). In the HFS 1x5 and HFS 1x10 groups, $T + 30$ corresponded to 30 min after the single HFS train. In the HFS 2x5 group, it corresponded to 30 min after the second HFS train delivered to the same limb. Comparisons between the HFS 1x5 versus 1x10 and 2x5 groups versus 1x10 and 1x10 versus 1x5 groups were conducted using a one-way ANOVA, followed by post hoc pairwise t tests to explore specific group differences.

Hypothesis 3: HFS-induced secondary hyperalgesia delivered to one forearm is facilitated by previous exposure to HFS at the contralateral forearm.

To test this hypothesis, we compared the first-sensitized versus second-sensitized limbs of the HFS 2x5 bilateral group: *1*) the post-HFS increase in pinprick sensitivity (ΔNRS_{HFS}), and *2*) the secondary hyperalgesia area radius (R) 30 min after HFS ($T + 30$) using paired-sample t tests. Furthermore, we compared the temporal decays (K) of ΔNRS_{HFS} and R across the first- and second-sensitized forearms using the extra sum-of-squares F test to compare the group-level mean fit obtained with a single decay parameter versus separate decay parameters fitted to the data of each forearm, followed by paired t tests to compare individual decay (half-life) estimates (individual fits with an adjusted $R^2 < 0.5$ were excluded).

Additional analyses.

To confirm that HFS induced a significant increase in pinprick sensitivity in all four groups, we tested at each sensitized forearm whether the post-HFS increase in pinprick sensitivity ($\Delta\Delta$NRS in the HFS 1x5, 1x10, and 2x5 groups; ΔNRS_{HFS} at each sensitized forearm in the HFS 2x5 bilateral group) was significantly greater than zero using one-sample t tests at time point $T + 30$. To further characterize the differences in HFS-induced secondary hyperalgesia across the four groups, we also performed comparisons of *1*) the post-HFS increase in pinprick sensitivity ($\Delta\Delta$NRS or ΔNRS_{HFS}), and *2*) the secondary hyperalgesia area radius (R) at each post-HFS time point in the HFS 1x5, 1x10, 2x5, and 2x5 bilateral groups using independent-samples or paired-sample t tests (Supplemental Tables S1–S4).

RESULTS

The HFS-Induced Increase in Pinprick Sensitivity Is Prolonged by Previous Exposure to HFS on the Same Forearm

For the group-level fitting, the estimated half-life of the HFS-induced increase in pinprick sensitivity ($\Delta\Delta$NRS) was 285 min in the HFS 1x5 group, 277 min in the HFS

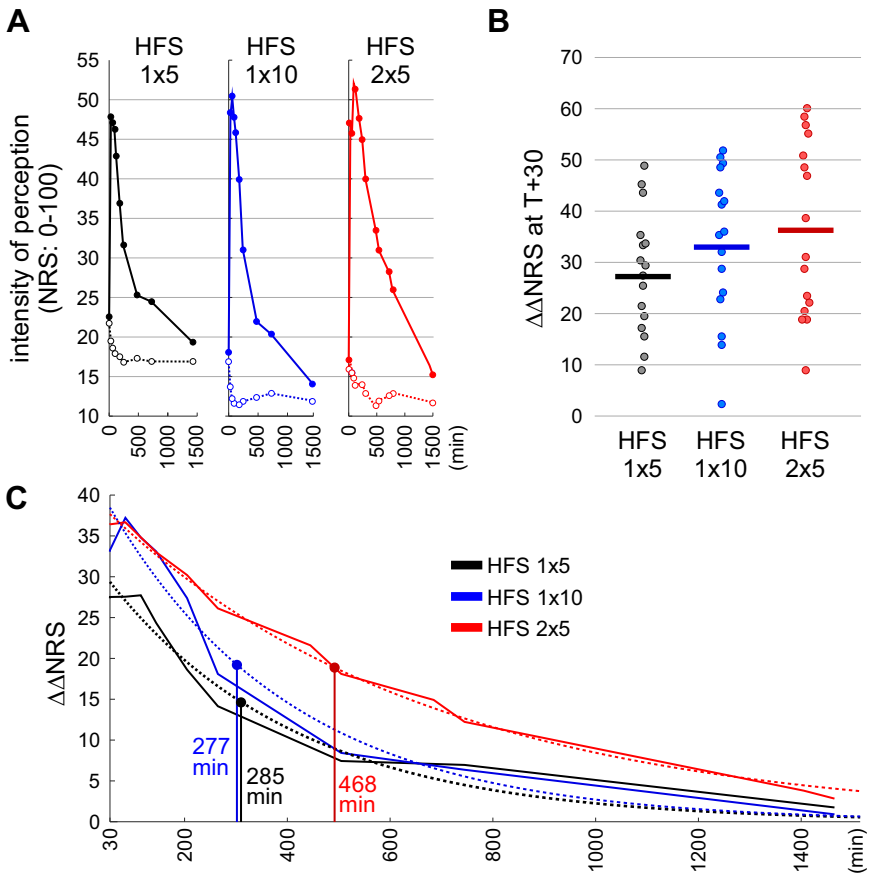

Figure 2. *A*: group-level average time courses of the intensity of pinprick perception on the sensitized forearm (continuous lines) and the nonsensitized contralateral forearm (dashed lines) in the single session of 5 high-frequency electrical stimulation trains (HFS 1x5; black), single session of 10 HFS trains (HFS 1x10; blue), and the 2 sessions of 5 HFS trains separated by a 1-h break (HFS 2x5; red) groups. *B*: change in intensity of perception relative to baseline at the sensitized minus nonsensitized forearms [numerical rating scale (ΔΔNRS)] 30 minutes after the last HFS train ($T + 30$). Scatter plots: data from individual participants. Horizontal line: group-level average. *C*: group-level average time courses of ΔΔNRS (continuous lines) and corresponding fitted exponential decay functions (dashed lines). The vertical bars indicate the estimated half-lives of the group-level fits.

1x10 group, and 468 min in the HFS 2x5 group (Fig. 2). The extra sum-of-squares F test indicated that the temporal decay (K) was slower in the HFS 2x5 group compared to the HFS 1x10 group ($F = 17.71$; < 01). The temporal decay in the HFS 2x5 group was also slower than in the HFS 1x5 group ($F = 19.81$; $P < 0.001$), while the temporal decays in the HFS 1x5 and HFS 1x10 groups were similar ($F = .02$; $P = 0.890$).

For the individual-level fitting, estimates of two participants in the HFS 1x5 group and one participant in the HFS 1x10 group were excluded due to a poor fit (adjusted $R^2 < 0.5$). The average adjusted R^2 for the retained participants were 0.84 ± 0.09, 0.85 ± 0.06, and 0.92 ± 0.14 in the HFS 1x5, HFS 1x10, and HFS 2x5 groups, respectively. The one-way ANOVA comparing the estimated half-lives across the three groups was significant ($F = 3.946$; $P = 0.0269$). Pairwise comparisons (Table 2) showed that preexposure to HFS in the HFS 2x5 group significantly prolonged the HFS-induced increase in pinprick sensitivity as compared to both the HFS 1x5 group ($P = 0.0228$) and the HFS 1x10 group ($P = 0.050$). In contrast, increasing the total number of trains (10 trains in the HFS 1x10 group vs. 5 trains in the HFS 1x5 group) did not affect this duration ($P = 0.650$).

Table 2. *Comparisons of the effects of a single session of HFS to the effects of two separate sessions of HFS delivered to the same forearm, separated by a 1-hour break, each session consisting of five 1-second trains of HFS*

	HFS 1x5	HFS 1x10	HFS 2x5	HFS 1x5 vs. 1x10	HFS 1x5 vs. 2x5	HFS 1x10 vs. 2x5
ΔNRS_{HFS} ($T + 30$)	25.2 ± 11.9	30.1 ± 13.3	35.0 ± 17.5			
ΔNRS_{NS} ($T + 30$)	−2.3 ± 3.2	−3.2 ± 5.8	−1.4 ± 5.3			
ΔΔNRS ($T + 30$)	27.5 ± 12.0	33.3 ± 14.9	36.4 ± 17.2	$P = 0.239$ $d = 0.424$	$P = 0.101$ $d = -0.599$	$P = 0.583$ $d = -0.196$
ΔΔNRS half-life	239 ± 150 min	267 ± 168 min	423 ± 248 min	$P = 0.650$ $d = -0.171$	**$P = 0.023^{*}$** **$d = -0.882$**	**$P = 0.050^{*}$** **$d = -0.735$**
Radius ($T + 30$)	4.8 ± 1.3 cm	5.1 ± 0.9 cm	5.2 ± 0.9 cm	$P = 0.459$ $d = 0.265$	$P = 0.334$ $d = -0.347$	$P = 0.792$ $d = -0.094$
Radius half-life	550 ± 501 min	131 ± 151 min	561 ± 343 min	**$P = 0.003^{**}$** **$d = 1.134$**	$P = 0.940$ $d = 0.027$	**$P < 0.001^{**}$** **$d = 1.626$**

Values are mean ± SD. P values (independent samples t tests) and Cohen's d are shown. HFS, high-frequency electrical stimulation. HFS 1x5, single session of 5 HFS trains; HFS 1x10, single session of 10 HFS trains; HFS 2x5, 2 sessions of 5 HFS trains separated by a 1-h break and applied to the same forearm; HFS 2x5 bilateral, 2 sessions of 5-HFS trains separated by a 1-h break and applied to the first- and second-sensitized forearms; NRS, numerical rating scale; significant results are in bold and $^{*}P < 0.05$, $^{**}P < 0.005$.

The Temporal Decay of the Area of HFS-Induced Secondary Hyperalgesia Is Not Influenced by Previous Exposure to HFS on the Same Forearm but Is Influenced by the Number of HFS Trains Delivered in a Single Session

For the group-level fit, the half-life of the secondary hyperalgesia area radius (R) was 500 min in the HFS 1x5 group, 122 min in the HFS 1x10 group, and 481 min in the HFS 2x5 group (Fig. 3). The F test indicated that the temporal decay (K) was slower in the HFS 2x5 group compared to the HFS 1x10 group ($F = 113.2$; $P < 0.001$). However, the temporal decay in the HFS 1x5 group was also slower than the temporal decay in the HFS 1x10 group ($F = 102.1$, $P < 0.001$), and the temporal decays in the HFS 2x5 and HFS 1x5 groups were similar ($F = .072$, $P = 0.793$).

For the individual-level fits, data from all participants were included in the analysis (all adjusted $R^2 \geq 0.5$). The average adjusted R^2 were 0.91 ± 0.10, 0.96 ± 0.04 and 0.90 ± 0.08 in the HFS 1x5, HFS 1x10 and HFS 2x5 groups. The one-way ANOVA comparing the estimated half-lives across the three groups was significant ($F = 7.395$; $P = 0.0017$). Pair-wise comparisons (Table 2) indicated that preexposure to HFS in the HFS 2x5 group did not affect the temporal decay of the area of secondary hyperalgesia as compared to the HFS 1x5 group ($P = 0.9395$), while exposure to a greater number of trains within a single session in the HFS 1x10 group led to a significantly faster decrease of the area as compared to both the HFS 1x5 ($P = 0.0032$) and the HFS 2x5 ($P < 0.0001$) groups.

The Magnitude and Area of HFS-Induced Secondary Hyperalgesia Is Not Increased by Prior Exposure to HFS on the Same Forearm

At time point $T + 30$, the increase in pinprick sensitivity ($\Delta\Delta$NRS) was 27.5 ± 12.0 in the HFS 1x5 group, 33.3 ± 14.9 in the HFS 1x10 group and 36.4 ± 17.2 in the HFS 2x5 group. The

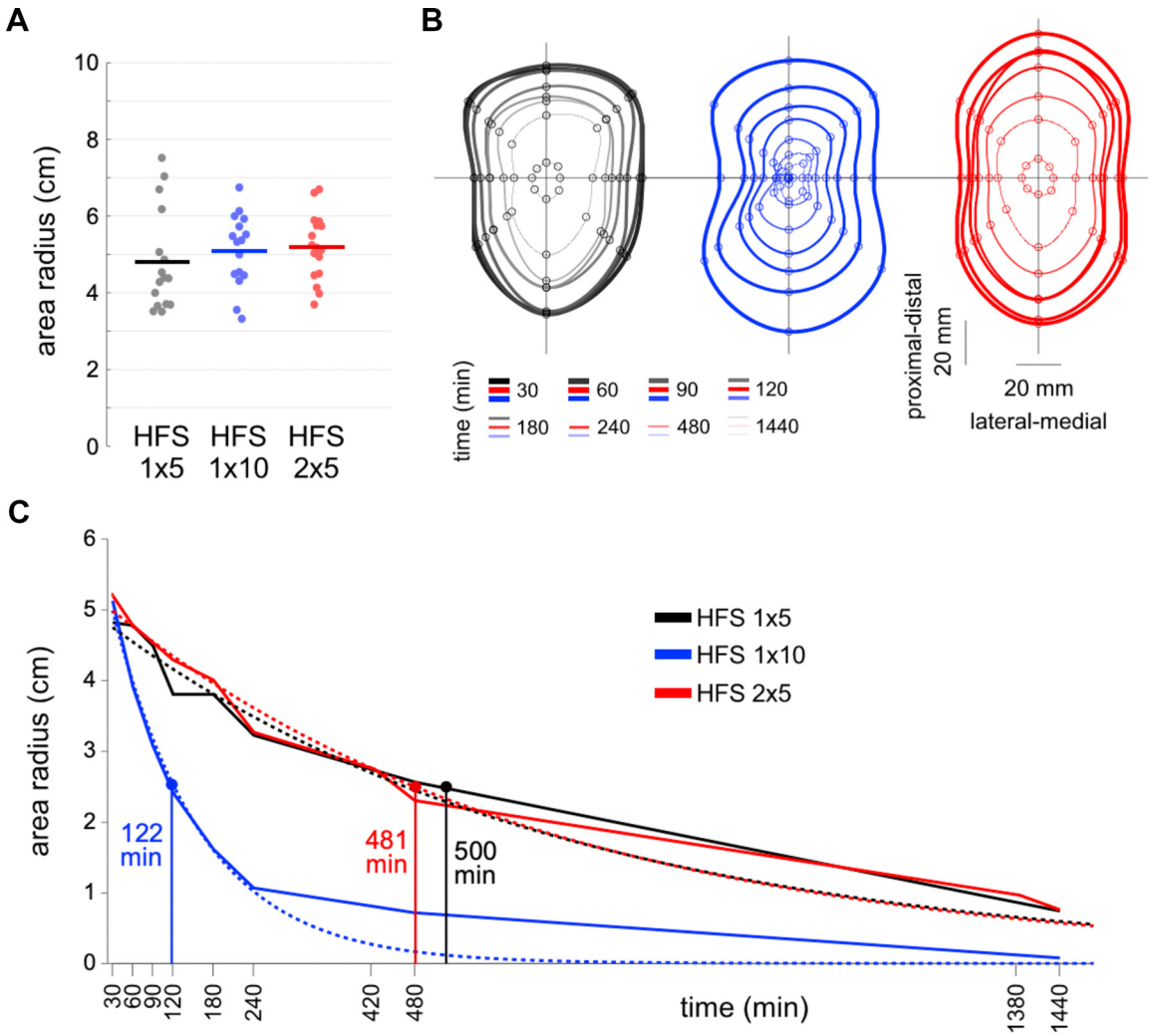

Figure 3. *A*: size (radius) of the area of secondary hyperalgesia in the single session of 5 high-frequency electrical stimulation trains (HFS 1x5; black), single session of 10 HFS trains (HFS 1x10; blue), and 2 separate sessions of HFS separated by a 1-h break (HFS 2x5; red) 30 minutes after the last HFS train ($T + 30$). Scatter plots: data from individual participants. Horizontal lines: group-level average. *B*: group-level average contours of the area of HFS-induced secondary hyperalgesia over time [thickest line: 30 minutes after HFS; thinnest line: 24 hours (1,440 minutes) after HFS]. *C*: group-level average time courses of the area radius (continuous lines) and corresponding fitted exponential decay functions (dashed lines). The vertical bars indicate the estimated half-lives of the group-level fits.

secondary hyperalgesia area radius (R) was 4.8 ± 1.3 cm in the HFS 1x5 group, 5.1 ± 0.9 cm in the HFS 1x10 group, and 5.2 ± 0.9 cm in the HFS 2x5 group. These differences were not significant (ΔΔNRS: $F = 1.473$, $P = 0.240$; R: $F = 0.591$, $P = 0.5579$; Table 2), suggesting that preexposure to HFS in the HFS 2x5 group did not affect the peak magnitude of the HFS-induced increase in pinprick sensitivity or the secondary hyperalgesia area size at $T + 30$.

Effects of Prior Exposure to HFS on the Contralateral Arm

In the HFS 2x5 bilateral group, a similar increase in pinprick sensitivity (ΔNRS_{HFS}) was observed at time point $T + 30$ at the first-sensitized (30.5 ± 15.7) and second-sensitized (31.6 ± 17.5) forearms ($P = 0.582$) (Table 3). There was also no significant difference between the secondary hyperalgesia radius at the first-sensitized (4.4 ± 0.9 cm) and second-sensitized (4.3 ± 0.6 cm) forearms ($P = 0.683$).

The time courses of the increase in pinprick sensitivity and the area of secondary hyperalgesia at the first-sensitized and second-sensitized forearms are shown in Fig. 4. From the group-level fits, the half-life of the HFS-induced increase in pinprick sensitivity (ΔNRS_{HFS}) was 204 min at the first-sensitized forearm and 233 min at the second-sensitized forearm. The half-life of the secondary hyperalgesia radius (R) was 302 min at the first-sensitized forearm and 371 min at the second-sensitized forearm. The F tests indicated no differences in temporal decay for ΔNRS_{HFS} ($F = 1.547$, $P = 0.228$) and for radius ($F = 2.672$, $P = 0.1031$).

For the individual-level fits of the change in pinprick sensitivity (ΔNRS_{HFS}), data from three participants were excluded due to poor fit of the time course at one or both forearms (adjusted $R^2 < 0.5$). The average adjusted R^2 for the retained participants were 0.87 ± 0.06 and 0.86 + 0.07 at the first- and second-sensitized forearms, respectively. The paired-sample t test comparing the estimated half-lives at both forearms showed no significant difference (Table 3).

For the individual-level fits of the area radius (R), data from all participants were included (all adjusted $R^2 \geq 0.5$). The average adjusted R^2 was 0.91 ± 0.07 and 0.93 ± 0.06 at the first- and second-sensitized forearms. The paired-sample t test comparing the estimated half-lives at both forearms showed no significant difference (Table 3).

Additional Analyses

HFS induced a significant increase in pinprick sensitivity in all tested groups. The average change in pinprick sensitivity at time point $T + 30$ was significantly greater than zero in all four tested groups (all $P < 0.001$).

The pairwise comparisons of the post-HFS increase in pinprick sensitivity (ΔΔNRS) at each post-HFS time point in the HFS 1x5, 1x10, and 2x5 groups are provided in Fig. 5 and Table S1. Compared to the HFS 1x5 group, ΔΔNRS was greater in the HFS 2x5 group at time points $T + 180$, $T + 240$, and $T + 480$. Compared to the HFS 1x10 group, ΔΔNRS was greater at $T + 480$. In addition, at time point $T + 60$, ΔΔNRS was greater in the HFS 1x10 group compared to the HFS 1x5 group.

The pairwise comparisons of the area radius (R) in the HFS 1x5, 1x10, and 2x5 groups are shown in Fig. 6 and Table S2. The area radius in the HFS 1x10 group was significantly smaller than in the HFS 1x5 group at time points $T + 120$, $T + 180$, $T + 240$, and $T + 480$ and significantly smaller than in the HFS 2x5 group at time points $T + 60$, $T + 120$, $T + 180$, $T + 240$, and $T + 480$.

Finally, the within-subjects comparisons of the change in pinprick sensitivity (ΔNRS) and area radius (R) at the first-sensitized versus second-sensitized forearms in the HFS 2x5 bilateral group are reported in Fig. 7 and Tables S3 and S4. At the second-sensitized forearm compared to the first-sensitized forearm, the ΔNRS was greater at $T + 120$, while the area radius was greater at $T + 120$ and $T + 180$.

DISCUSSION

In this study, we observed that prior exposure to a conditioning stimulus inducing central sensitization (5 trains of HFS delivered on the volar forearm to activate cutaneous nociceptive terminals) prolongs the duration of secondary hyperalgesia induced by a second and identical conditioning stimulus applied to the same site 1 hour later (HFS 2x5 group), as compared to the secondary hyperalgesia induced by a single session of 5 HFS trains (HFS 1x5 group) or a single session of 10 HFS trains (HFS 1x10 group). This finding suggests that prior induction of central sensitization may create a memory trace or latent sensitization that promotes the susceptibility to develop a more persistent form of central sensitization.

Table 3. *Comparisons of the effects of a HFS at the first- and second-sensitized forearms in the HFS 2x5 bilateral group*

	HFS 2x5 Bilateral: First Forearm	HFS 2x5 Bilateral: Second Forearm	First vs. Second Forearm
$\Delta NRS_{HFS}(T + 30)$	30.5 ± 15.7	31.5 ± 17.5	$P = 0.582$ $d = -0.141$
Half-life (ΔNRS_{HFS})	201 ± 83 min	197 ± 127 min	$P = 0.879$ $d = -0.043$
Radius ($T + 30$)	4.4 ± 0.9 cm	4.3 ± 0.6 cm	$P = 0.683$ $d = -0.104$
Radius half-life	407 ± 360 min	348 ± 245 min	$P = 0.265$ $d = -0.289$

Values are mean ± SD. P values (independent samples t tests) and Cohen's d are shown. HFS, high-frequency electrical stimulation; HFS 2x5 bilateral, 2 sessions of 5 HFS trains separated by a 1-h break and applied to the first- and second-sensitized forearms; NRS, numerical rating scale.

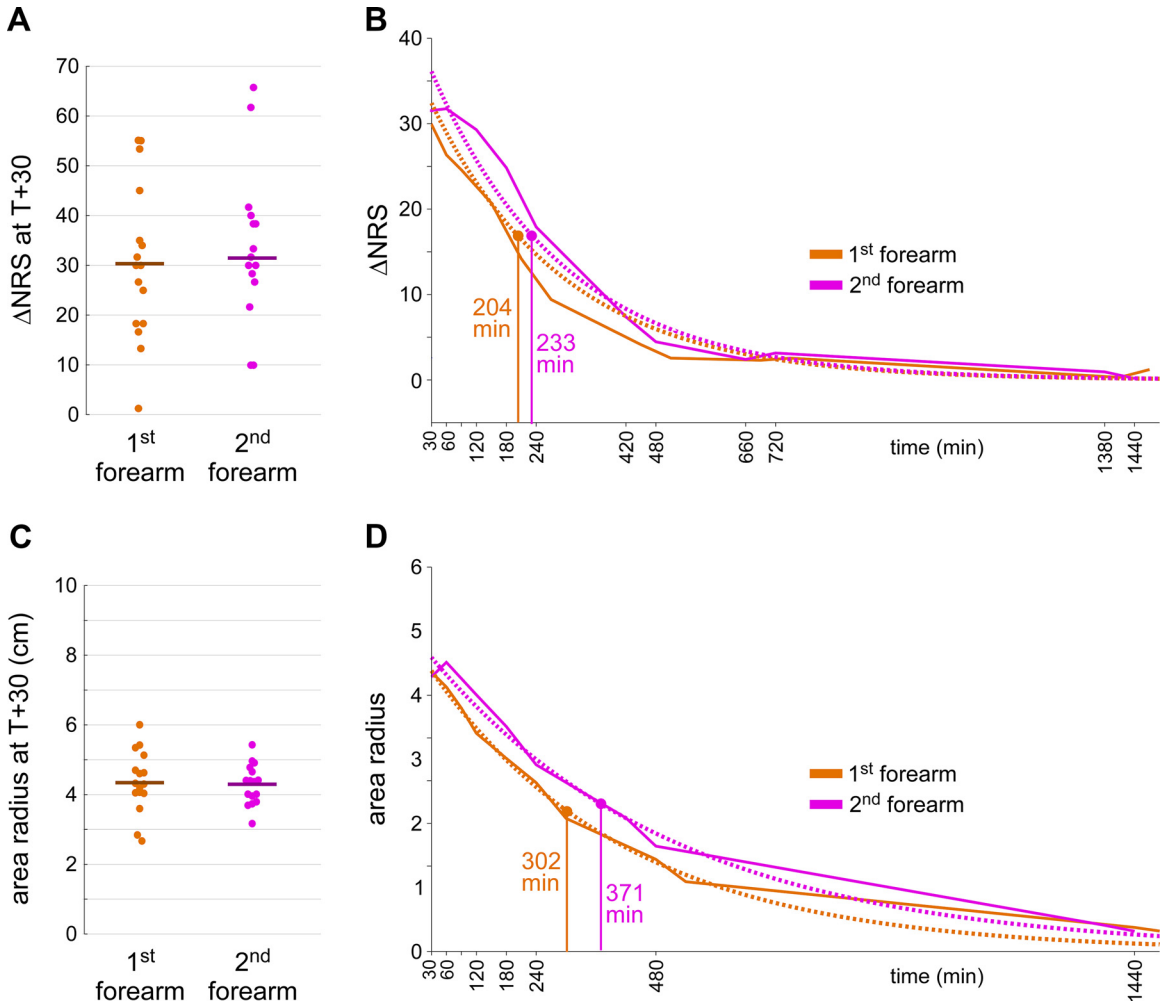

Figure 4. *A*: change in intensity of perception [numerical rating scale (ΔNRS)] 30 minutes ($T + 30$) after high-frequency stimulus (HFS) in 2 separate sessions of HFS separated by a 1-h break applied at the first-sensitized (orange) and second-sensitized (purple) forearms (HFS 2x5 bilateral group). Scatter plots: data from individual participants. Horizontal lines: group-level average. *B*: group-level average time course of ΔNRS (continuous lines) and the corresponding fitted exponential decay functions (dashed lines) at the first- and second-sensitized forearms. The vertical bars and filled dots indicate the estimated half-lives of the group-level fits. *C*: radius of the secondary hyperalgesia area 30 minutes after HFS at the first-sensitized (orange) and second-sensitized (purple) forearms. *D*: group-level average time course of the area radius (continuous lines) and the corresponding fitted exponential decay functions (dashed lines) at the 2 forearms. The vertical bars and filled dots indicate the estimated half-lives of the group-level fits.

Early LTP, Late LTP, and Latent Sensitization

LTP can have different forms varying in duration and underlying mechanisms, with early-phase LTP primarily relying on posttranslational changes such as phosphorylation and late-phase LTP involving transcriptional changes and protein synthesis (10). In the hippocampus, it was shown that repeated stimulation may promote a more persistent form of potentiation (13, 14).

In the present study, preexposure to cutaneous HFS in the HFS 2x5 group (2 sessions of 5 HFS trains delivered to the same forearm 1 hour apart) significantly prolonged the HFS-induced increase in pinprick sensitivity as compared to both groups exposed to a single session of HFS session. This suggests that repeated exposure to a noxious stimulus capable of inducing central sensitization favors the induction of a more persistent form of secondary hyperalgesia (1, 42). Importantly, the increased duration of secondary hyperalgesia observed in the HFS 2x5 group was not seen in the HFS 1x10 group, indicating that this effect was not due to the total number of HFS trains delivered. Instead, it suggests that the time interval between HFS exposures played a critical role, compatible with the notion of latent sensitization. In other words, the development of a more persistent form of HFS-induced secondary hyperalgesia may require not only repeated exposure to HFS but also some time for the processes induced by the first exposure to develop.

Spatial Spread of Secondary Hyperalgesia

Preexposure to HFS in the HFS 2x5 group did not increase the spatial extent of the area of HFS-induced secondary hyperalgesia. Additionally, preexposure to HFS in the HFS 2x5 group did not prolong the temporal persistence of the secondary hyperalgesia area. The finding that preexposure to HFS increased the duration of heightened pinprick sensitivity without affecting the duration of the area size suggests that these two outcome measures of central sensitization are mediated by at least partially different mechanisms. One

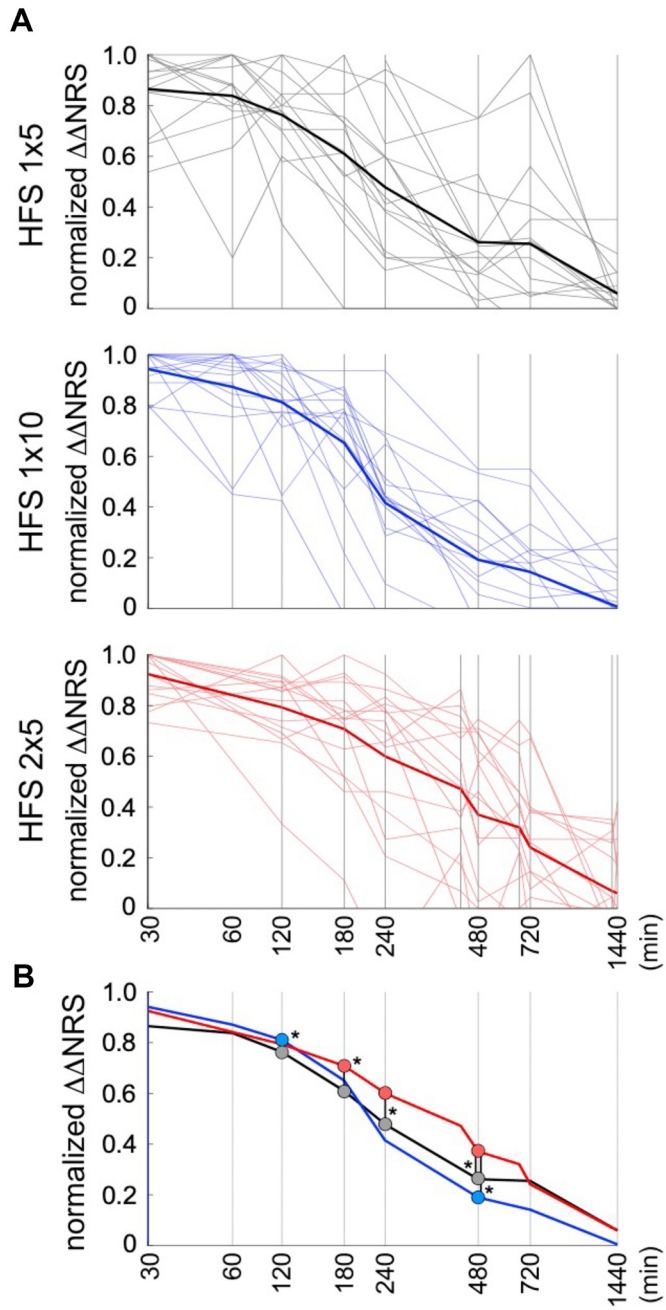

Figure 5. *A*: individual (thin lines) and group-level average (thick lines) time courses of the change in intensity of perception relative to baseline at the high-frequency stimulus (HFS)-sensitized minus the nonsensitized forearms [numerical rating scale (ΔΔNRS)] in the single session of 5 HFS trains (HFS 1x5; black), single session of 10 HFS trains (HFS 1x10; blue), and 2 separate sessions of HFS separated by a 1-h break (HFS 2x5; red) groups. Displayed ratings are normalized to the maximum increase of each participant. *B*: group-level average time courses of the normalized ΔΔNRS. Time points showing a significant difference between groups (independent-sample *t* tests) are indicated by the thick vertical lines and filled dots. $*P < 0.05$, uncorrected for multiple comparisons.

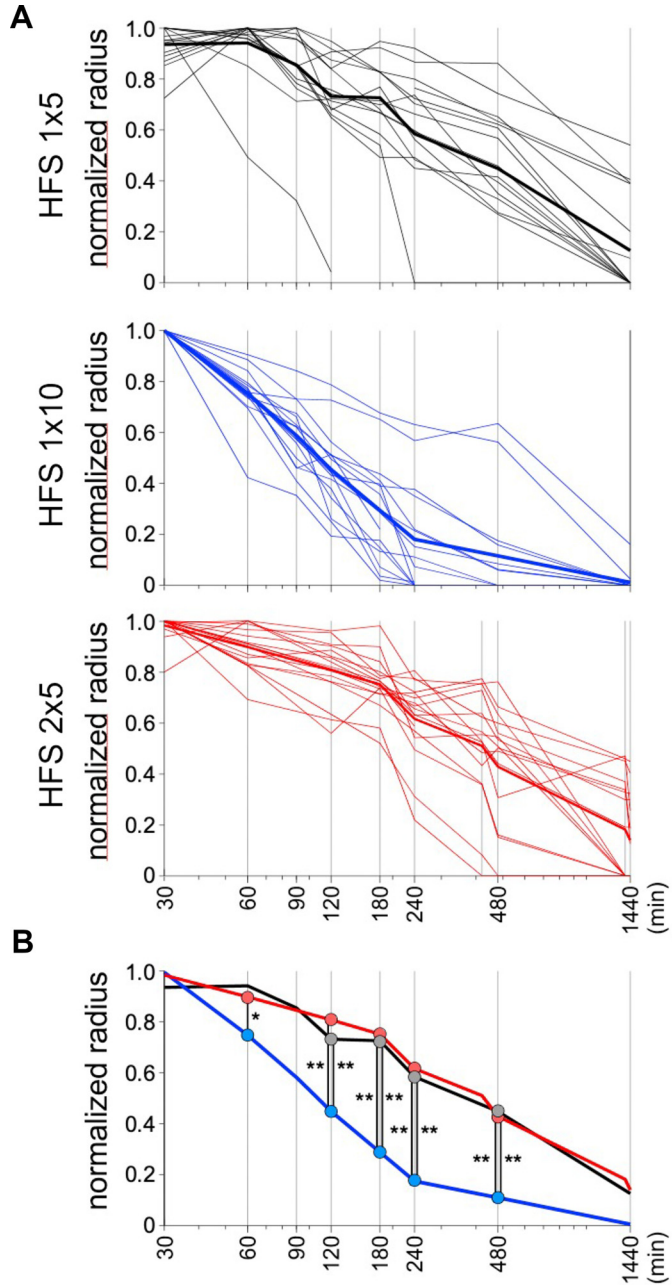

Figure 6. *A*: individual time courses (thin lines) and group-level average (thick lines) time courses of the area radius of high-frequency stimulus (HFS)-induced secondary hyperalgesia in the single session of 5 HFS trains (HFS 1x5; black), single session of 10 HFS trains (HFS 1x10; blue), and 2 separate sessions of HFS separated by a 1-h break (HFS 2x5; red) groups. Values are normalized to the maximum radius of each participant. *B*: group-level average time courses superimposed across the 3 groups. Time points showing a significant difference between groups (independent-sample *t* tests) are indicated by the thick vertical lines and filled dots. $*P < 0.05$, $**P < 0.005$, uncorrected for multiple comparisons.

possible explanation for this dissociation is that the increase in pinprick sensitivity in the skin immediately surrounding the HFS electrode may be primarily driven by homosynaptic LTP (i.e., the processes that increase synaptic efficacy within the synapses exposed to the conditioning stimulus), whereas the spatial spread of secondary hyperalgesia may depend more on the mechanisms driving heterosynaptic LTP (i.e., the processes that increase synaptic efficacy within neighboring synapses not exposed directly to the conditioning stimulus) (1, 43, 44). Indeed, a previous study found that a central plasticity mechanism might involve an "all-or-nothing" modulation of pain, with sharp border of enhanced

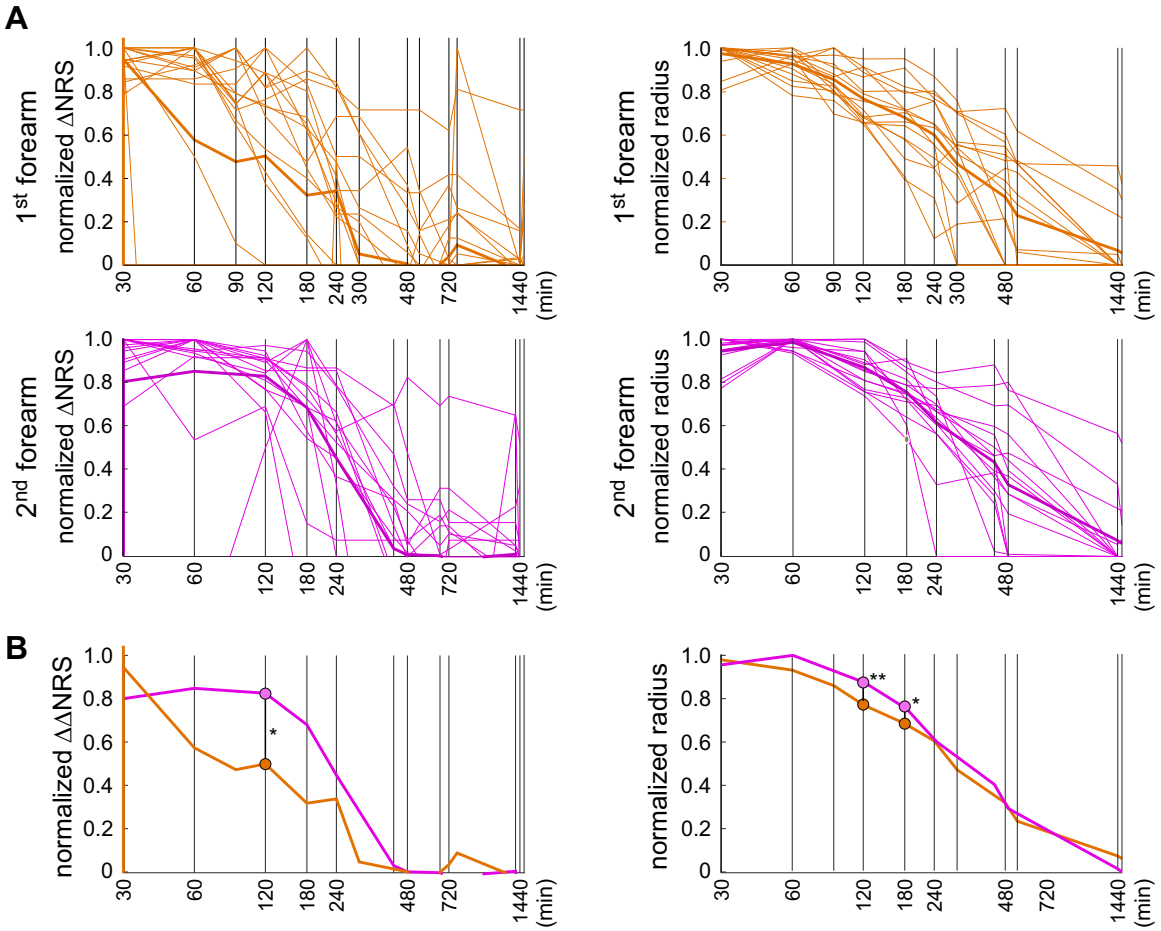

Figure 7. *A*: individual (thin lines) and group-level average (thick lines) time courses of the change in pinprick ratings [numerical rating scale (ΔNRS)] and the secondary hyperalgesia area radius at the first-sensitized (orange) and second-sensitized (purple) forearms in 2 separate sessions of high-frequency stimulus (HFS) separated by a 1-h break (HFX 2x5 bilateral group). *B*: group-level average time courses superimposed. Time points showing a significant difference between the first- and second-sensitized forearms (paired-sample *t* tests) are indicated by the thick vertical lines and filled dots. $^{*}P < 0.05$, $^{**}P < 0.005$, uncorrected for multiple comparisons.

sensitivity and an exponential decrease in pain ratings from this injection site of capsaicin (45). This could witness two underlying different mechanisms. Alternatively, and as discussed below, HFS-triggered activation of descending pathways could have a specific effect on the spatial extent of hyperalgesia.

Notably, the HFS 1x10 group exhibited a much faster temporal decay of the secondary hyperalgesia area, as compared to the HFS 1x5 group and the HFS 2x5 group. This unexpected finding might relate to the notion that HFS of presynaptic afferents can simultaneously induce enhancement (LTP) and depression [long-term depression (LTD)] of synaptic efficacy, with the mechanisms underlying HFS-induced LTP and LTD depending differentially on the number of stimulation trains applied (7, 46). In the human nociceptive system, it was reported that HFS of nociceptive afferents elicits secondary hyperalgesia due to an LTP-like phenomenon whereas sustained low-frequency stimulation (1 Hz) leads to an LTD-like reduction in pain sensitivity (7, 47). Furthermore, Magerl et al. (46) found that very low-frequency stimulation of the skin (0.05 Hz) can prevent and even reverse HFS-induced secondary hyperalgesia. Additionally, the use of burst-like versus continuous stimulation trains can influence the magnitude of HFS-induced secondary hyperalgesia, the first being more likely to induce a stronger secondary mechanical hyperalgesia, the second producing hypoalgesia (27, 48). The present study suggests that the number of HFS trains delivered within a single session may modulate the duration of HFS-induced secondary hyperalgesia.

It is well established that noxious stimulation can activate diffuse noxious inhibitory controls (DNIC) and that activation of this spinal-bulbar-spinal loop reduces the spinal transmission and thus the sensitivity to noxious stimuli applied to remote body locations (49). Since cutaneous HFS is a strong nociceptive stimulus, it is expected to activate DNIC in addition to inducing central sensitization. Interestingly, Vo and Drummond (24) reported that after HFS of the forearm, heterotopic noxious stimulation of the temple produces a stronger reduction in sensitivity to painful stimuli applied to the HFS-exposed forearm and ipsilateral face. This observation suggests that HFS may enhance sensitivity to DNIC within the HFS-exposed hemibody, potentially limiting the spatial spread of secondary hyperalgesia induced by HFS. Such an effect of HFS on descending modulatory pathways could depend on the number of HFS trains delivered, which could

explain the accelerated shrinking of the secondary hyperalgesia area size observed in the HFS 1x10 group.

Contralateral Effects of HFS

In the HFS 2x5 bilateral group, preexposure to HFS on one forearm did not affect the intensity and extent of the secondary hyperalgesia induced by HFS 30 min after HFS delivery ($T + 30$), corresponding to its expected peak, and there was also no effect on the estimated durations.

However, visual inspection of the time courses (Fig. 4) and the exploratory paired comparisons of the change in pinprick sensitivity at each post-HFS time point across the two forearms suggested that 120 min after HFS the increase in pinprick sensitivity was greater at the second-sensitized forearm compared to the first-sensitized forearm (Table S3). Similarly, at $T + 120$ and $T + 180$, the spatial extent of the area of secondary hyperalgesia was greater at the second-sensitized versus first-sensitized forearm (Fig. 4 and Table S4). This observation requiring confirmation may be linked to the notion that HFS-triggered activation of brainstem loops could facilitate responses to painful stimuli on the contralateral side compared to the ipsilateral side (22, 25). It may also relate with the finding that capsaicin injection in healthy volunteers can induce contralateral allodynia (50) or to the observation that patient suffering from neuropathic pain were reporting bilateral pinprick hyperalgesia in spite of unilateral neurological disorder (51).

Clinical Implications

Previous experimental and clinical studies have suggested that prior induction of central sensitization can enhance the central sensitization induced by a second sensitizing event (15–18), and our results indicate that this prior exposure can also favor a more persistent form of central sensitization.

The double HFS-exposure protocol that was used in the present study could serve as a model to investigate in humans the mechanisms underlying the persistence of central sensitization. Additionally, our findings highlight the importance of conducting repeated assessments to capture the temporal dynamics of secondary hyperalgesia, whether it is experimentally induced such as with HFS or evaluated in clinical contexts such as after surgery.

Limitations

The present study was conducted in a small sample of young healthy participants. Moreover, the mechanisms underlying HFS-induced secondary hyperalgesia probably reflect only some of the multiple mechanisms that contribute to hyperalgesia observed in patients. The findings of the present study likely translate reasonably well to individuals undergoing repeated surgery within relatively short time periods, but patients with long-lasting chronic primary pain conditions probably have other mechanisms involved such as reorganization of brain circuits following childhood adversities (52, 53).

Conclusions

In conclusion, we show that two consecutive sessions of five HFS trains, separated by a 1-hour interval, result in a more enduring increase in pinprick sensitivity compared to a single HFS session. This finding suggests that repeated induction of central sensitization may engage mechanisms promoting a more persistent form of secondary hyperalgesia.

DATA AVAILABILITY

Source data are available at https://doi.org/10.6084/m9.figshare.28562921.

SUPPLEMENTAL MATERIAL

Supplemental Tables S1–S4: https://doi.org/10.6084/m9.figshare.28562717.

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DISCLAIMERS

The statements and opinions presented here reflect the author's view and neither Innovative Medicines Initiative nor the European Union, European Federation of Pharmaceutical Industries and Associations, or any Associated Partners are responsible for any use that may be made of the information contained therein.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHORS CONTRIBUTIONS

L.L., A.S., E.H., E.v.d.B., and A.M. conceived and designed research; L.L. and A.S. performed experiments; L.L. and A.M. analyzed data; L.L., R.-D.T., and A.M. interpreted results of experiments; L.L. and A.M. prepared figures; L.L. drafted manuscript; L.L., E.H., E.v.d.B., C.L., A.T., L.G.-L., R.-D.T., and A.M. edited and revised manuscript; L.L., A.S., E.H., E.v.d.B., C.L., O.C., A.T., L.G.-L., R.-D.T., and A.M. approved final version of manuscript.

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