The effect of high-frequency conditioning stimulation of human skin on reported pain intensity and event-related potentials

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1Department of Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2Department of Anesthesiology, Pain and Palliative Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; and 3Donders Institute for Brain, Cognition and Behavior, Radboud University Nijmegen, Nijmegen, The Netherlands

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van den Broeke EN, van Heck CH, Ceelen LA, van Rijn CM, van Goor H, Wilder-Smith OH. The effect of high-frequency conditioning stimulation of human skin on reported pain intensity and event-related potentials. J Neurophysiol 108: 2276–2281, 2012. First published August 1, 2012; doi:10.1152/jn.00391.2012.—High-frequency conditioning electrical stimulation (HFS) of human skin induces an increased pain sensitivity to mechanical stimuli in the surrounding nonconditioned skin. The aim of this study was to investigate the effect of HFS on reported pain sensitivity to single electrical stimuli applied within the area of conditioning stimulation. We also investigated the central nervous system responsiveness to these electrical stimuli by measuring event-related potentials (ERPs). Single electrical test stimuli were applied in the conditioned area before and 30 min after HFS. During electrical test stimulation, the reported pain intensity (numerical rating scale) and EEG (ERPs) were measured. Thirty minutes after conditioning stimulation, we observed a decrease of reported pain intensity at both the conditioned and control (opposite arm) skin site in response to the single electrical test stimuli. In contrast, we observed enhanced ERP amplitudes after HFS at the conditioned skin site, compared with control site, in response to the single electrical test stimuli. Recently, it has been proposed that ERPs, at least partly, reflect a saliency detection system. Therefore, the enhanced ERPs might reflect enhanced saliency to potentially threatening stimuli.

MATERIALS AND METHODS

Participants

Twenty-one healthy volunteers (4 men and 17 women ranging in age from 18 to 25 yr; mean age 20 yr) participated in the experiment. Subjects were excluded from the study if they:

1) suffered from a psychiatric or neurological disorder;
2) used centrally acting medication (like antidepressants, antipsychotics, anticonvulsants, or benzodiazepines) or pain medication;
3) experienced pain; or
4) used drugs for recreational use.

All participants signed an informed written consent form. Approval for the experiment was obtained from the local ethical committee.

Design

Experimental conditioning: HFS. Subjects received trains of 100 Hz (pulse width 2 ms) for 1 s repeated 5 times at 10-s intervals with a stimulation intensity of 20× individual electrical detection threshold on the forearm 5 cm distal to the cubital fossa (Klein et al. 2004). The stimulation trains were delivered via a constant current stimulator (Digitimer DS7A) and a specifically designed electrode able to activate peptidergic nociceptive afferents in the skin. The electrode consists of 16 blunt stainless steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins, to achieve spatial summation within the receptive field of spinal cord neurons, 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 concentr-
cally located and has an inner diameter of 22 mm and an outer diameter of 40 mm.

**Test stimuli.** To quantify effects as a result of experimental conditioning stimulation, a block of 40 single painful electrical pulses (pulse width 2 ms at 10× individual electrical detection threshold) was applied before and 30 min after experimental conditioning to the conditioned site and to the control site (opposite arm). We chose 30 min as interval between the conditioning stimulation and the postmeasurement because it has been shown that the increased perceived pain intensity induced after HFS reaches a plateau at 30 min as interval between the conditioning stimulation and the test stimulation (Pfau et al. 2011). The 40 stimuli were delivered randomly to both sites with each site receiving the same number of stimuli (n = 20).

The stimuli were delivered with a random interstimulus interval ranging from 7 to 10 s. Using 2 constant-current stimulators (Digitimer DS7A; 1 for each site), stimuli were delivered to both arms using the same conditioning electrode previously described. To quantify the amount of pain as a result of the test stimulation, subjects were asked to rate, at random times within a train of 5 single pulses, the pain intensity of the last received stimulus on a numerical rating scale (NRS). The NRS ranged from 0 = “no pain” to 10 = “unbearable pain.” After the baseline test stimulation (pre-HFS), subjects were asked to indicate, on a 3-item verbal descriptor list, whether the test stimuli were perceived as pricking, pressing, or dull (Beissner et al. 2010). For this 3-item verbal descriptor list, it has been shown that it can reliably indicate whether the pain sensation evoked by the physical stimulus is the result of predominantly Aδ- or C-fiber activity (Beissner et al. 2010).

**EEG**

To measure brain responses evoked by the single electrical test stimuli, a multichannel (32 channels) EEG (BrainVision with active electrodes; Brain Products) was recorded (band pass 0.1–100 Hz, sample frequency 500 Hz) during the test stimulation. The electrodes were mounted in an elastic electrode cap and arranged according to the international 10–20 system. Left mastoid was used as reference. Eye movements were detected by horizontal and vertical electrooculogram (EOG) recordings. Horizontal EOG was measured from the outer canthus of the left eye, and vertical EOG supraorbitally to the left eye. Impedance was kept under the 20 kΩ for all leads.

**Procedure**

At the beginning of the experiment, individual electrical detection thresholds were determined. This was achieved by delivering an ascending sequence of increased current intensities (single electrical pulses, rectangular shape, pulse width 2 ms), starting from 0 mA and with steps of 0.05 mA, through the conditioning stimulation electrode. This procedure stopped when the current was detected, as verbally reported by the subjects. This procedure was repeated three times. The arm on which the detection threshold was determined (conditioned or control) was balanced across subjects. After this threshold determination, subjects received a block of test stimuli (premeasurement).

During stimulation, subjects were comfortably seated in a chair and instructed to perceive passively the stimuli with eyes closed without making any movements. The NRS delivered during stimulation was preceded by a tone (65 dB), which was presented 1.5 s after the test stimulus. Participants were instructed to open their eyes after the tone and verbally rate the amount of pain of the last received stimulus, after which they closed their eyes again. This was implemented to prevent subjects from becoming drowsy as result of keeping their eyes closed for the period of test stimulation. The stimulated arm (HFS) was balanced (dominant vs. not dominant) across subjects. After this baseline (test) measurement, the experimental conditioning (HFS) followed. After receiving conditioning stimulation, there was a break of 30 min where after the postmeasurement (identical to premeasurement) followed.

**Signal Analysis**

For analyzing ERPs from the EEG, the EEG was analyzed offline using the software BrainVision Analyzer v. 2.0. We used this program because it is compatible with MATLAB 2011a, a program in which scripts can be written for specific functions that are needed for data analysis. As a first step, the continuous EEG was referenced to right mastoid. Next, the EEG signal (500 Hz) was high-pass filtered at 1 Hz and low-pass filtered at 30 Hz. Based on the onset of the stimulus, the EEG was segmented into epochs from −100 ms prestimulus to 2,000 ms poststimulus with a total period of 2,100 ms. Bad segments containing ocular artifacts were corrected using the Gratton-Coles method (Gratton et al. 1983). Segments were also inspected for other artifacts like muscle or jaw and line noise activity and were removed if necessary. As a last step, baseline correction (−100 to 0 ms) was applied to all segments.

All segments, for each subject separately, were averaged to obtain an averaged subject-specific ERP waveform. ERP components were defined in terms of their latency and topographic distribution. To this end, the grand average global field power (GFP) of all subjects was calculated as well as the topographic voltage distribution corresponding to the ERP latencies identified in the GFP plot (Boyle et al. 2008; Skrandies 1990). Then, we identified the electrode in the topographic plot that shows the maximal activity and used this electrode for subsequent analysis. To ensure accurate identification of point of maximal activity, we also inspected the grand average ERPs (of all electrodes) for all subjects.

Individual ERP latencies were determined in the individual GFP plot corresponding to the windows of the grand average GFP latencies (Boyle et al. 2008). The mean amplitude of each ERP component was calculated at the individual GFP latency ±5 ms at the electrode of maximal activity (Boyle et al. 2008) by using a self-written script in the software MATLAB 2011a.

**Statistical Analysis**

For statistical analysis, the software SPSS v. 16.0 was used. A general linear model (GLM) repeated-measures ANOVA was used to test whether there are statistically significant differences regarding behavioral and electrophysiological measurements with respect to the TIME of measurement (pre vs. post) and SITE (control vs. conditioned). In all tests, the significance level was set at P < 0.05.

**RESULTS**

The mean (and SD) individual detection thresholds determined at the beginning of the experiment and used for both the conditioning (20× detection threshold) and test stimulation (10× detection threshold) were 0.39 ± 0.12 mA.

**Behavioral Measurements**

The test stimulus was clearly rated as painful by the subjects as can be seen in Fig. 1. After the baseline test stimulation (pre-HFS), subjects indicated on the three-item verbal descriptor list how they perceived this test stimulus, i.e., pricking, pressing, and dull. All subjects, except one, rated the test stimulus as pricking, one subject as pressing, and none as dull.

To investigate whether there is a change in reported pain intensity (NRS score) in response to the electrical test stimulation after HFS, a GLM repeated-measures analysis was performed. The GLM repeated-measures ANOVA revealed a significant main effect of TIME [F(1,20) = 18.361, P < 0.000, η² = 0.479]. Thirty minutes after experimental conditioning stimulation, the NRS, averaged for both arms, is lower (mean = 3.0) compared with baseline (mean = 3.7; Fig. 1).
Electrophysiological Measurements: ERPs

One subject was left out of the ERP analysis because the EEG contained too many artifacts and was therefore not usable. Based on the grand average GFP and corresponding topographic representations of all subjects ($n = 20$) shown in Fig. 2, we defined three distinctive ERP components:

1) a negative voltage between 100 and 150 ms, maximal at electrode Cz, which we label as N120;
2) a positive voltage between 170 and 210 ms, maximal at Cz, which we label as P188; and
3) a positive voltage between 220 and 340 ms, maximal at Cz, which we label as P260.

The grand average evoked potential waveforms for each measurement (pre and post) and site (conditioned vs. control) are shown in Fig. 3.

N120 amplitude and latency. To investigate whether the N120 amplitude, and corresponding latency, evoked by the test stimulation are changed after HFS, a GLM repeated-measures analysis was performed. This analysis revealed a significant TIME × SITE interaction effect for the N120 amplitude $[F(1,19) = 5.629, P = 0.028, \eta^2 = 0.229]$. Thirty minutes after experimental conditioning stimulation, the N120 amplitude of the stimulation site (mean = $-10.5$) is larger compared with control site (mean = $-7.6$; Fig. 4).

The GLM repeated-measures ANOVA revealed a significant TIME × SITE interaction effect for the N120 latency $[F(1,19) = 7.985, P = 0.011, \eta^2 = 0.296]$. Thirty minutes after experimental conditioning stimulation, the N120 latency of the stimulation site (mean = 118.6) is shorter compared with control site (mean = 124.0; Fig. 4).

P188 amplitude and latency. A GLM repeated-measures analysis was also used to investigate whether the P188 amplitude, and corresponding latency, evoked by the test stimulation, were changed after HFS. The analysis revealed a significant TIME × SITE interaction effect $[F(1,19) = 5.032, P = 0.037, \eta^2 = 0.209]$. Thirty minutes after experimental conditioning stimulation, the P180 amplitude of the stimulation site (mean = 12.7) is larger compared with control site (mean = 10.3; Fig. 4). No significant differences were observed for P180 latency.

P260 amplitude and latency. For the P260 amplitude, and corresponding latency, no statistically significant differences were observed.

DISCUSSION

This is the first study that shows that 30 min after HFS of human skin, the reported pain intensity to single electrical test

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**Fig. 1.** Mean (and SD) numerical rating scale (NRS) scores ($n = 21$) as response to electrical painful test stimulation. ***Main effect of time; pain intensity was significant lower after high-frequency electrical stimulation (HFS) at both arms compared with baseline ($P < 0.001$).

**Fig. 2.** A: global field power (GFP). B: topographic maps. The GFP ($n = 20$) and topographic maps revealed 3 dominant event-related potential (ERP) components in the EEG: a negative charged peak at 120 ms maximum at Cz, a positive charged peak at 188 ms maximum at Cz, and a positive charged peak at 260 ms maximum at Cz. To illustrate best the maximal activity in each representation, we adjusted the scale to its maximal absolute values [for increases (red) and decreases (blue) in voltages]. As a result, the scale differs between the different representations and is therefore left out.
stimuli applied within the conditioned skin site decreases, but ERP amplitudes evoked by the same test stimuli are enhanced. Quality and Perceived Intensity of the Test Stimulus

It is generally accepted that under normal conditions experimentally evoked pain is the result of activation of nociceptive afferents (Aδ- and/or C-fibers). The test stimulus used in the present study was 10× individual electrical detection threshold and always perceived as painful as indicated by the NRS scores, i.e., none of the subjects rated the stimulus as zero pain. This observation indicates that the test stimulus must have activated nociceptive afferents. The quality of the test stimulus

Fig. 3. Grand average evoked potential waveforms. Plotted are the grand averaged evoked potentials waveforms (n = 21) for each measurement (pre, A; post, B) compared between the 2 sites [control (blue) vs. conditioned (red)]. Dotted line on x-axis represents stimulus onset. Upward is positive, and downward is negative charge.

Fig. 4. Mean (and SD) ERP amplitudes (A) and latencies (B) for each site (control vs. conditioned) at every measurement (pre and post). *Statistically significant difference between conditioned vs. control arm (P < 0.05).
was evaluated at the baseline measurement using the three-item
verbal descriptor list (Beissner et al. 2010). Every subject
except one reported that the evoked pain was pricking without
mentioning other descriptors. This observation is in agreement
with the study of Beissner et al. (2010), which also showed that a
painful electrical stimulus evokes predominantly a pricking sen-
sation, although they used a different pulse shape and number.
Similarly, Mouraux et al. (2010) also used an electrical stim-
ulus and observed a predominantly pricking sensation, which
was associated with reaction times corresponding with Aβ-
fibers. However, the authors used a train of three pulses and a
low stimulation intensity (Mouraux et al. 2010).

Decreased Reported Pain Intensity After HFS

Thirty minutes after conditioning stimulation, we observed a
decrease in reported pain intensity in response to the single
electrical test stimuli, which was similar for both skin sites
(conditioned and control). There are at least three hypotheses
that could be put forward to explain this decrease. The first
hypothesis is to ascribe the effect to habituation, a decrease in
response to a stimulus when that stimulus is presented repeat-
edly (Rankin et al. 2008).

The second hypothesis is that the decrease in reported pain
intensity at the conditioned site is also influenced by hypoes-
thesia, which can be induced after HFS with intertrain intervals
of 2 s (De Col and Maihofner 2008).

Assuming that the quality of the perceived pain perception
evoked by the electrical test stimulus is influenced by Aβ-fiber
activity, one could put forward a third hypothesis, i.e., that the
observed decrease in reported pain intensity at the conditioned
site after HFS might be the consequence of long-term depres-
sion (LTD). This is because animal studies show that HFS to
Aβ-fibers induces LTD rather than long-term potentiation
(LTP; Cheng and Randic 2003; Sandkühler et al. 1997).

Our observation that the perceived pain intensity at 30 min
after HFS is not different between the two skin sites seems to
be in contrast with the results demonstrated by Klein et al.
(2008). In their study, Klein et al. (2008) observed the pain
intensity (i.e., NRS scores) every 60 s to single electrical test
stimuli (10× individual detection threshold) before and after
HFS. They observed a habituation response of the pain intensi-
ity at baseline (before HFS) at both sites (conditioned and
control). After HFS, the pain intensity on the control site
further decreased, whereas the pain intensity on the condi-
tioned site increased for at least 45 min. However, compared
with the heterotopic effects observed after HFS (Klein et al.
2008), this homotopic effect is rather small (i.e., between 3 and
7 on a 0–100 NRS). There are several differences between the
present study and the study of Klein et al. (2008), which could
explain the opposite findings. First, the present study had only
two measurements on which the pain intensity was evaluated,
whereas in the study of Klein et al. (2008) there were evalua-
tions every 60 s. Second and more importantly, the method
used for reporting pain is different. Klein et al. (2008) used a
NRS ranging from 0 to 100 with the possibility of using
integers as well as fractions ad libitum, whereas in the present
study the subjects could only rate from 0 to 10 without the
possibility of using integers or fractions. Possibly, this NRS
step is too large to detect the difference in pain perception
produced by HFS in our study. Third, in the study of Klein
et al. (2008), the upper limit of the NRS was labeled as “most
intense pain imaginable,” whereas in the present study we used
the more common label “most unbearable pain” as the upper
limit of the NRS.

Criticisms of the approach of Klein et al. (2008) include,
first, that it is questionable whether volunteers are reliably and
reproducibly capable of scaling pain to such a degree of
accuracy and, second, that the observed effect is too small to be
clinically relevant or detectable. Third, besides the reported
pain intensity to single electrical stimuli at the conditioned site,
Klein et al. (2008) also measured the reported pain intensity to
mechanical stimulation in the surrounding skin area. These two
measurements could have interacted, with as consequence an
increase of the reported pain intensity to the single electrical
test stimuli.

Enhanced ERP Amplitude After HFS

Thirty minutes after conditioning stimulation, we observed
enhanced midlatency ERP amplitudes at the stimulation site in
response to the electrical test stimuli. To our knowledge, this is
the first study to investigate the effect of HFS on ERPs evoked
by single electrical stimuli applied to the homotopic zone. We
were able to demonstrate that HFS conditioning results in
enhanced cortical sensory processing. However, despite the
fact that the test stimuli were perceived as painful, the latency
and waveform of the simultaneously recorded ERPs was simi-
lar to the latency and waveform of ERPs evoked by nonpainful
electrical stimulation (Downman 1994; van den Broeke et al.
2010), suggesting predominant activation of the brain via
fast-conducting Aβ-fibers.

The enhanced ERPs appeared to be dissociated from the
reported reduced pain intensity. Such observations have been
observed previously (Legrain et al. 2010). To explain this
dissociation, Legrain et al. (2010) recently proposed that the
cortical network activated after painful stimulation represents,
at least in part, a saliency detection system that is involved in
detecting and orienting attention toward and reacting to the
occurrence of salient sensory events. The function of this
cortical network is to facilitate the processing of behaviorally
significant (e.g., potentially threatening) sensory input and to
help select an appropriate response (Legrain et al. 2010). In this
context, the enhanced ERPs observed after HFS might reflect
increased saliency as a consequence of conditioning HFS.

Summary

This study is the first to show that after HFS of human skin,
single painful electrical stimuli applied in the conditioned zone
were perceived as less painful, whereas ERPs evoked by the
same stimuli were enhanced. The enhanced ERPs might be a
reflection of increased saliency induced after conditioning
HFS.

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DISCLOSURES

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


REFERENCES


