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

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Abstract

High frequency conditioning electrical stimulation (HFS) of human skin induces an increased pain sensitivity to mechanical stimuli in the surrounding non-conditioned 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 thirty minutes after HFS. During electrical test stimulation the reported pain intensity (NRS) 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 to 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.
1. Introduction

High frequency electrical stimulation (HFS) of human skin (i.e. five trains of 100 Hz for 1 second, repeated in a 10 second interval, at 20x individual detection threshold) results in an gradual increase of pain perception from train to train (Klein et al. 2004; Van den Broeke et al. 2011). More importantly, HFS seems to change electrical and mechanical pain sensitivity in (homotopic) and surrounding (heterotopic) the conditioned skin area. With respect to the homotopic effect it has been shown that after HFS the reported pain intensity to single electrical test stimuli (10x individual detection threshold) applied within the conditioned skin area was elevated (Klein et al. 2004; Hansen et al. 2007; Lang et al. 2007; Pfau et al. 2011). Interestingly, with the use of sensory descriptors Hansen et al. (2007) demonstrated that after HFS the single electrical test stimuli became more ‘stinging’ and ‘burning’, suggesting a facilitation that took place somewhere in the A-delta and C fiber pathways.

The same high frequency electrical stimulation also elevates the reported pain intensity to mechanical pinprick stimuli applied in the surrounding unconditioned skin (heterotopic effect) (Klein et al. 2004; Klein et al. 2008; Pfau et al. 2011; Van Den Broeke et al. 2010; Van Den Broeke et al. 2011) - a phenomenon very similar to secondary hyperalgesia, i.e. an increased mechanical pain sensitivity in the surrounding non-injured skin tissue. Recently, we for the first time investigated central nervous system responsiveness after HFS to heterotopically applied single electric stimuli using event-related potentials (ERPs). ERPs are voltage polarity changes in the electroencephalogram (EEG), time-locked to the onset of a stimulus (Fabiani et al. 2000). The EEG directly measures neuronal activity and the ERPs represent the synchronized activity of the underlying neural population (Fabiani et al. 2000). With the measurement of ERPs it is possible to study sequential stimulus processing of different brain structures in time. The aim of this study was to now use event-related potentials (ERPs) to investigate stimulus processing after HFS to homotopic applied stimuli, i.e. to single electrical stimuli applied within the area of conditioning stimulation.
2. Materials and Methods

2.1 Participants

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

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

All participants signed an informed consent form. Approval for the experiment was obtained from the local Ethical Committee.

2.2 Design

2.2.1 Experimental conditioning: high frequency electrical stimulation (HFS)

Subjects received trains of 100 Hz (pulse width; 2 ms) for 1 sec. repeated 5 times at 10 sec intervals with an 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, Digitimer UK) 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 which serves as anode is concentrically located and has an inner diameter of 22 mm and an outer diameter of 40 mm.

2.2.2 Test stimuli

In order to quantify effects as a result of experimental conditioning stimulation, a block of forty single painful electrical pulses (pulse width; 2 ms at 10 x individual electrical detection threshold) was applied before, and 30 minutes after experimental conditioning to the conditioned site and to the control site (opposite arm). We chose thirty minutes as interval
between the conditioning stimulation and the post measurement because it has been shown that the increased perceived pain intensity induced after HFS reaches a plateau at approximately 30 minutes (Pfau et al. 2011). The forty 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 inter-stimulus interval ranging from 7 to 10 seconds. Using two constant current stimulators (Digitimer DS7A, Digitimer UK, one for each site), stimuli were delivered to both arms using the same conditioning electrode previously described. In order 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 Numeric 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 three-item verbal descriptor list, if the test stimuli were perceived as pricking, pressing and dull (Beissner et al. 2010). For this three-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 A delta or C fiber activity (Beissner et al. 2010).

2.3 Electro-Encephalography (EEG)

In order to measure brain responses evoked by the single electrical test stimuli, a multi-channel (32 channels) EEG (BrainVision with active electrodes, Brain Products GmbH) 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 supra orbitally to the left eye. Impedance was kept under the 20 kΩ for all leads.

2.4 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 for 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 (pre measurement).

During stimulation, subjects were comfortably seated in a chair and were instructed to passively perceive 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 seconds 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 minutes where after the post measurement (identical to pre measurement) followed.

2.5 Signal analysis

For analyzing ERPs form the EEG, the EEG was analyzed offline using the software BrainVision Analyzer v. 2.0 (Brain Products GmbH). 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 re-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 pre-stimulus to 2000 ms post-stimulus with a total period of 2100 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 – 0 ms) was applied to all segments.

All segments, for each subject separately, were averaged to obtain an averaged subject-specific event-related potential 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 which shows the maximal activity and used this electrode for subsequent analysis. To insure 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.

2.6 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 \( p < .05 \).

3. Results

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

3.1 Behavioral measurements

The test stimulus was clearly rated as painful by the subjects as can be seen in figure 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 if 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 < .000, eta² = .479). Thirty minutes after experimental conditioning stimulation, the NRS, averaged for both arms, is lower (M = 3.0) compared to baseline (M = 3.7) (fig. 1).

3.2 Electrophysiological measurements: Event-related potentials (ERPs)

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

1. A negative voltage between 100-150 milliseconds (ms), maximal at electrode Cz, which we label as N120,
2. A positive voltage between 170-210 ms, maximal at Cz, which we label as P188,
3. A positive voltage between 220-340 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 figure 3.

3.2.1 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 = .028, eta² = .229). Thirty minutes after experimental conditioning stimulation the N120 amplitude of the stimulation site (M = -10.5) is larger compared to control site (M = -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 = .011, eta² = .296). Thirty minutes after experimental conditioning stimulation the N120 latency of the stimulation site (M = 118.6) is shorter compared to control site (M = 124.0) (Fig. 4).
A GLM repeated measures analysis was also used to investigate if the P188 amplitude, and corresponding latency, evoked by the test stimulation, were changed after HFS. The analysis revealed a significant \( \text{TIme x Site} \) interaction effect (\( F (1,19) = 5.032, p = .037, \eta^2 = .209 \)). Thirty minutes after experimental conditioning stimulation the P180 amplitude of the stimulation site (\( M = 12.7 \)) is larger compared to control site (\( M = 10.3 \)) (Fig. 4). No significant differences were observed for P180 latency.

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

4. Conclusion and Discussion

This is the first study that shows that 30 minutes after high frequency electrical conditioning stimulation of human skin, the reported pain intensity to single electrical test stimuli applied within the conditioned skin site decreases but ERP amplitudes evoked by the same test stimuli are enhanced.

4.1 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 (A delta and/or C fibers). The test stimulus used in the present study was 10 x 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 was evaluated at the baseline measurement using the three-item verbal descriptor list (Beissner et al. 2010). Almost 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. (Beissner et al. 2010) which also showed that a painful electrical stimulus evokes predominantly a pricking sensation, although they used a different pulse shape and number. Similarly, Mouraux et al. (2010) also used an electrical stimulus and observed a predominantly pricking sensation which was
associated with reaction times corresponding with A-delta fibers. However the authors used a train of three pulses and a low stimulation intensity (Mouraux et al. 2010).

4.2. 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 repeatedly (Rankin et al. 2008). The second hypothesis is that the decrease in reported pain intensity at the conditioned site is also influenced by hypoesthesia, which can be induced after high frequency electrical stimulation with inter train intervals of 2 seconds (De Col and Maihöfner 2008). Assuming that the quality of the perceived pain perception evoked by the electrical test stimulus is influenced by A-delta 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 depression (LTD). This because animal studies show that HFS to A-delta 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 minutes 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. observed the pain intensity (i.e. NRS scores) every 60 seconds to single electrical test stimuli (10 x individual detection threshold) before and after HFS. They observed a habituation response of the pain intensity at baseline (before HFS) at both sites (conditioned and control). After HFS, the pain intensity on the control site further decreased, while the pain intensity on the conditioned site increased for at least 45 minutes. However, in comparison with the heterotopic effects observed after HFS (Klein et al. 2008), this homotopic effect is rather small (i.e. between three and seven on a zero to hundred NRS). There are several differences between the present study and the study of Klein et al. (Klein et al. 2008), which could explain the opposite findings. Firstly, the present study had only two measurements on which the pain intensity was evaluated, while in the study of Klein et al. there were evaluations every 60 seconds. Secondly, and more importantly the
method used for reporting pain is different. Klein et al. used a NRS ranging from zero to
hundred with the possibility of using integers as well as fractions ad libitum, while in the
present study the subjects could only rate from zero to ten 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. Thirdly, in the study of Klein et al. the upper limit
of the NRS was labeled as "most intense pain imaginable" while in the present study we used
the more commonly used label "most unbearable pain" as the upper limit of the NRS.

Criticisms of Klein’s approach include, firstly, that it is questionable whether volunteers are
reliably and reproducibly capable of scaling pain to such a degree of accuracy, and secondly,
that the observed effect is too small to be clinically relevant or detectable. Thirdly, besides
the reported pain intensity to single electrical stimuli at the conditioned site Klein et al. 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.

4.3 Enhanced ERP amplitude after HFS

Thirty minutes after conditioning stimulation we observed enhanced mid-latency 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 similar to the latency and waveform of ERPs evoked by non-painful electrical
stimulation (Dowman 1994; Van Den Broeke et al. 2010), suggesting predominant activation
of the brain via fast-conducting A beta fibers.

The enhanced ERPs appeared to be dissociated from the reported reduced pain intensity.
Such observations have been observed previously (Legrain et al. 2010). In order to explain
this dissociation Legrain et al. 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 towards 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.

4.4 Summary
This study is the first study that shows that after high frequency electrical conditioning
stimulation of human skin, single painful electrical stimuli applied in the conditioned zone
were perceived as less painful while ERPs evoked by the same stimuli were enhanced. The
enhanced ERPs might be a reflection of increased saliency induced after conditioning HFS.

Competing interests
All authors declare that they have no competing interests

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Figure legends

Figure 1. Mean (and SD) NRS-scores (N=21) as response to electrical painful test stimulation. *** = main effect of time; pain intensity was significant lower after HFS at both arms compared to baseline (p < .001).

Figure 2. A) Global Field Power (GFP) and B) topographic maps. The GFP (N=20) and topographic maps revealed three dominant 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 best illustrate 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 leaving out.

**Figure 3.** Grand average evoked potential waveforms. Plotted are the grand averaged evoked potentials waveforms (N=21) for each measurement (pre (A) and post (B)) compared between the two sites (control (blue) vs. conditioned (red)). Dotted line on X-axis represents stimulus onset. Upward is positive and downward is negative charge.

**Figure 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 < .05).