Neurophysiological Correlates of Nociceptive Heterosynaptic Long-Term Potentiation in Humans.

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Abstract

Long-term potentiation (LTP) is a cellular model of synaptic plasticity and reflects an increase of synaptic strength. LTP is also present in the nociceptive system and is believed to be one of the key mechanisms involved in the manifestations of chronic pain. LTP manifested as an increased response in pain perception can be induced in humans using high frequency electrical stimulation (HFS). The aim of this study was to induce spinal heterosynaptic LTP using HFS and investigate its heterotopic effects on event-related potentials (ERPs) to repeated non-painful cutaneous stimuli as a possible electrophysiological cortical correlate of sensitization. Twenty two healthy subjects were randomly assigned to one of the two experimental conditions; HFS and control stimulation. Before and after the stimulation, both conditions received heterotopic mechanical (pinprick) and paired non-painful electrical test stimuli to quantify and confirm the effects of HFS on the behavioral level. ERPs to paired non-painful electrical stimulation were measured simultaneously. Conditioning HFS resulted in significant heterotopic effects after 30 minutes, including increased perceived intensity in response to (pinprick) mechanical and paired non-painful electrical stimulation in comparison with control. The paired non-painful electrical stimuli were accompanied by significantly enhanced responses regarding the ERP N1-P2 peak-to-peak and P300 amplitude in comparison with control. These findings suggest that HFS is capable of producing heterosynaptic spinal LTP which can be measured not only behaviorally but also using ERP’s.
Introduction

Long-term potentiation (LTP) is a cellular model for synaptic plasticity (Cooke and Bliss 2006) and reflects increase of synaptic strength (Bliss and Collingridge 1993). LTP is also present in the nociceptive system (Liu and Sandkühler 1997; Randic et al. 1993; Sandkühler, 2007; Willis 2002; Zhang et al. 2004) and is believed to be one of the key mechanisms involved in the development and maintenance of chronic pain (Ko and Zhuo 2004; Randic et al. 1993; Rygh et al. 2005; Sandkühler 2009; Woolf and Salter 2000). Klein et al. (2004) concluded that nociceptive LTP can be elicited in humans after high frequency electrical stimulation (HFS) of primary nociceptive afferents. Klein et al. (2004) demonstrated the effectiveness of HFS in inducing LTP by observing potential perceptual correlates, e.g. increased subjective pain perception after electrical and mechanical stimuli. In this context, LTP is manifested as a heightened sensitivity in the stimulated area (homotopic effects) as well as the area adjacent to the stimulated area (heterotopic effects) (Klein et al. 2004; Lang et al. 2007). However, to the best of our knowledge no study so far has directly investigated the effect of this cutaneous HFS paradigm for inducing LTP on brain processing. One way to do this is by measuring event-related potentials (ERPs) during stimulus repetition. ERPs are voltage polarity changes in the electroencephalogram (EEG), time-locked to the onset of a stimulus (Fabiani et al. 2000). They represent the synchronized activity of the underlying neural population (Coenen, 1995). During stimulus repetition a typical phenomenon can be observed, namely habituation, defined as a decrease in response to a stimulus when that stimulus is presented repeatedly (Kandel et al. 2000; Rankin et al. 2008). Previous ERP studies with somatosensory stimuli have shown that the ERP response is already habituated after the second stimulus (Kekoni 1999). Sensitization has opposite effects to habituation. It is defined as an enhanced response to a wide variety of stimuli after the presentation of an intense or noxious stimulus (Kandel et al. 2000).

The aim of this study was to induce LTP using HFS and investigate its heterotopic effects on ERPs to repeated non-painful stimulation as a possible electrophysiological cortical correlate of sensitization of the somatosensory system.
**Materials and Methods**

**Participants**

Twenty-two healthy men (median age 26.5 years; range 20-57 years) participated in the experiment. Subjects were excluded from the study if they had a psychiatric or neurological history, used medication, or suffered from pre-existing pain or pain syndrome. All participants signed an informed consent form. Approval for the experiment was obtained from the local Ethical Committee (ECG 03072008).

**Design**

**Experimental conditioning**

Subjects were randomly assigned to one of the two groups: painful electrical high frequency stimulation (HFS) or painful electrical single pulse stimulation (Control). For high frequency stimulation, subjects received trains of 100 Hz (pulse width; 2 ms) of 1 sec. repeated 5 times at 10 sec interval with an intensity of 20 × detection threshold on the forearm 5 cm distal to the fossa cubita using a ring electrode (fig. 1). The ring electrode consists of 16 blunt stainless steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed in a circle with a diameter of 10 mm and serve as cathode. A stainless steel reference electrode which serves as anode is concentrically located and has an inner diameter of 22 mm and an outer diameter of 40 mm. This electrode is designed to activate superficial nociceptive C-fiber afferents with less concomitant recruitment of tactile afferents (Klein et al. 2004). The control condition consisted of one single pulse of 1 sec at an intensity of 20 × detection threshold, repeated five times with a 9 sec interval between each single pulse. In order to avoid interference of lateral dominance, the stimulated arm was balanced across subjects.

**Variables measured**

**Behavioral measures**

In order to quantify the heterotopic effects as a result of HFS on the behavioral level, two tests were used before and after the experimental conditioning. The first behavioral test was obtained using non-painful electrical paired pulse stimulation, see below. A second behavioral test was used to test for heterotopic effects regarding mechanical (pinprick)
stimulation. A calibrated sharp-tipped von Frey monofilament (size: 6.1, target force: 980 mN, Sammons Preston Rolyan, USA) was pressed on four different areas of the skin. These areas were marked at the beginning of the experiment, and were located 1 cm lateral, medial, proximal and distal from the ring electrode. After each stimulus, subjects were asked to rate the sensation on a modified Visual Analogue Scale (VAS) ranging from 0 cm = “I feel nothing” to 10 cm = “unbearable pain” by drawing a vertical line on a horizontal bar. The sequence of the von Frey stimulation in the four different areas was randomized across conditions (pre and post) and subjects.

**Electroencephalogram (EEG)**

A multi-channel EEG (Neuroscan system) was recorded during the experiment (band-pass 0.1-100 Hz, sample frequency 2000 Hz) with nineteen Ag/AgCl electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, O2) mounted in an elastic electrode-cap. The electrodes were arranged according to the international 10-20 system and referenced to linked mastoids. 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 orbital to the left eye. Impedance was kept under the 5 kΩ for all leads.

The ERPs were recorded using the paired pulse paradigm (Montoya et al. 2006; Rentzsch et al. 2008) but with a fixed inter-stimulus interval and a random inter-pair interval. Fifty pairs of non-painful electrical test stimuli (S1-S2) with a fixed inter-stimulus interval of 500 ms and a random inter-pair interval ranging from 7 to 10 sec. were delivered to the arm of the subject. The test stimuli were applied using a constant current stimulator via two large surface silver electrodes (anode and cathode) placed within 1 cm outside the ring electrode (fig. 1), i.e. in the heterotopic skin area. Such non-painful test stimuli are considered to preferentially activate low-threshold tactile afferents (Aβ fibers) in the dermal layer of the skin (Katsarava 2006). To achieve an optimal standardization across subjects, both electrodes were placed at an angle of 45 degrees to the vertical midline. The electrodes were not moved during the experiment. A VAS-score (same VAS as for testing heterotopic effects) was obtained at a random time within a train of 5 paired pulses resulting in a total of 10 VAS-scores obtained during the ERP paradigm. The subject was asked to score the
intensity of the last received stimulus. Stimulation intensity was 50% of the pricking/painful threshold and was kept the same during the whole experiment.

This threshold was determined, by the subjects receiving electrical pulses (pulse width: 2.0 ms) starting from 0 mA and increasing in steps of 0.5 mA. The procedure stopped when the pricking/painful threshold was achieved, as verbally reported by the subjects.

During paired pulse stimulation, subjects were comfortably seated in a sound-attenuating cubicle (inside dimensions: 2.0 × 2.2 × 2.0 m). Subjects were instructed to passively perceive the stimuli with eyes closed, without making any movements. A computer display was placed in front of the subject (0.5 m) together with a computer mouse. The display was used to display the VAS-scale, preceded by a tone (65 dB). Participants were instructed to open their eyes after the tone and use the mouse to mark the VAS-score, after which they closed their eyes again. The subject was asked to score the intensity of the last received stimulus.

The mouse was handled with the hand opposite to the stimulated arm. Prior to paired pulse stimulation, a baseline EEG of two minutes (eyes open and eyes closed) was obtained. During these measurements subjects were instructed to sit as still as possible and make as few eye movements as possible.

Procedure (fig. 1)

At the beginning of the experiment individual thresholds for the paired pulse stimulation (EEG) were determined. After this procedure, the baseline EEG measurement for the paired pulse stimulation followed. Then, the detection threshold for the ring electrode was obtained. To this end, subjects received a single square wave current pulse (duration 2 ms), increasing in 0.1 mA steps, via the electrode until they detected a stimulus. The electrode was connected to a constant current stimulator (World Precision Instruments, USA). After obtaining the detection threshold for the ring electrode, the baseline measurement for the mechanical pinprick test followed. Afterwards, conditioning (HFS or control) took place followed by a 30 minute rest. After the break, both measurements (mechanical pinprick test and paired pulse stimulation) were repeated.
Data analysis

ERPs were extracted from the EEG off-line with Brain Vision Analyzer software version 1.05. First the EEG was down-sampled to 1000 Hertz and re-referenced to linked mastoids. Then data were inspected for ocular artifacts using the Gratton-Coles method (Gratton et al. 1983) and segmented into epochs from -100 ms pre-stimulus to 1000 ms post-stimulus with a total period of 1100 ms (Rentzsch et al. 2008). Bad segments such as muscle or jaw artifacts and line noise activity were removed. After baseline correction (-100 – 0 ms) all epochs were averaged for each subject individually. Based on morphology and latency of the grand median ERP, analyzed from the Cz electrode, three distinct peaks (N100, P200 and P300) were defined. The N100 was defined as the largest negative amplitude value between 100 and 170 ms, the P200 as the largest positive value between 140-290 ms and the P300 as the largest positive value between 230-370 ms. For statistical analysis the maximum value for each amplitude was calculated on every individual grand average ERP.

Statistics

For statistical analysis the software SPSS v. 16.0 was used. Because some variables were not normally distributed, outliers were present and N< 30, non-parametric test statistics were used for data analysis. Difference scores (post minus pre measurement) were first calculated and then compared between the two groups (HFS vs control) with a Mann Whitney U test. For testing within-group effects (pre vs post), the Wilcoxon Signed Rank test was used. In all tests the significance level was set at $p < .05$. Also the effect size ($r$), a measure of the strength of the relationship between two variables, was calculated for the between- and within- group effects (HFS vs control and pre vs post, respectively). The effect size $r$ was calculated as the Z-score divided by the square root of the total number of observations. Medians (and inter-quartile ranges) of the behavioral VAS-scores and ERP peaks for each condition (pre vs post) and group (HFS vs control) are summarized in tables 1 to 3, respectively.
Results

Heterotopic effects: behavioral tests

Significant between-group (HFS vs control) effects were found regarding the mechanical test stimuli applied in the area adjacent to the stimulated area; distal \([U = 25.0, p < .01, r = -.50]\); proximal \([U = 27.5, p < .05, r = -.46]\); lateral \([U = 33.5, p < .05, r = -.38]\) and medial \([U = 33.5, p < .05, r = -.39]\) (fig. 2). Within-group (pre vs post) effects showed significant increases in VAS-scores after HFS in three areas: distal \([Z = -2.938, p < .001, r = -.63]\); proximal \([Z = -2.937, p < .001, r = -.63]\); lateral \([Z = -1.693, p < .05, r = -.36]\) and marginally significant in medial \([Z = -1.646, p = .053, r = -.35]\) (fig. 2).

Besides these effects regarding the mechanical stimuli, between-group (HFS vs control) differences were also found regarding the non-painful electrical stimuli applied during the paired pulse stimulation \([U = 29.0, p < .05, r = -.44]\) (fig. 3). Within group (pre vs post) analysis showed a significant decrease in the control group after conditioning \([Z = -2.401, p < .01, r = -.51]\) (fig. 4). Although the median VAS-score suggested an increase after HFS, it did not reach significance median_{pre} 2.312, median_{post} 3.077 and the effect size is small \((r = -.10)\).

Heterotopic effects: event-related potentials (ERPs)

The grand median ERPs are shown in figure 6.

Short-term (intra session) effects

The pre measurement of both groups showed a statistically significantly attenuated second stimulus (S2) in comparison with the first stimulus of both the N1-P2 peak-to-peak and P300 amplitude (fig. 4 & 5): N1-P2 peak-to-peak amplitude HFS group \([Z = -2.667, p < .01, r = -.57]\), Control group \([Z = -2.934, p < .001, r = -.63]\), and P300 amplitude HFS group \([Z = -2.934, p < .001, r = -.63]\), Control group \([Z = -2.934, p < .001, r = -.63]\). After HFS conditioning, the differences between S1 and S2 in both groups remained comparable with the pre measurement.
The second stimulus of both the N1-P2 peak-to-peak and P300 amplitude continued to be significantly attenuated in comparison with the first one (fig. 4 & 5): N1-P2 peak-to-peak amplitude HFS group \(Z = -2.934, p < .001, r = -.63\), Control group \(Z = -2.667, p < .01, r = -.57\), and P300 amplitude HFS group \(Z = -2.934, p < .001, r = -.63\), Control group \(Z = -2.934, p < .001, r = -.63\). Thus, short-term effects were unaltered in both groups.

**Long-term (inter session) effects**

After conditioning, between-group (HFS vs control) analysis showed a significant difference with respect to the first stimulus (S1) of the N1-P2 peak-to-peak amplitude \(U = 29.0, p < .05, r = -.44\) (fig. 4). There were no significant differences regarding S2. Within-group (pre vs post) analysis revealed a significant decrease of the first stimulus in the control group \(Z = -1.956, p < .05, r = -.42\) (fig. 4). No significant decrease was found after HFS regarding the first or second stimulus of the N1-P2 peak-to-peak amplitude.

Significant between-group (HFS vs control) differences were found with respect to the first stimulus of the P300 amplitude after conditioning \(U = 28.0, p < .05, r = -.46\) (fig. 5). There were no significant differences regarding S2. Within-group (pre vs post) analysis showed a significant decrease of the first stimulus in the control group \(Z = -2.934, p < .001, r = -.63\) (fig. 5). No significant decrease regarding the first or second stimulus of the P300 amplitude was found after HFS conditioning (fig. 5). In summary, a significant inter-session decrement of the first stimulus (S1) was lacking after HFS, but present in the control condition.

We observed no statistically significant differences on the pre measurement between the groups of subjects that were stimulated on the dominant arm vs subjects that were stimulated on the non-dominant arm regarding the VAS-scores, N1-P2 peak-to-peak and P300 amplitude.
Discussion

This study has shown that conditioning HFS resulted in significant heterotopic effects 30 minutes after HFS, including an enhanced perceived intensity to mechanical (pinprick) and paired non-painful electrical stimulation in comparison with controls. The paired non-painful electrical stimuli were accompanied with significantly enhanced responses of the ERP N1-P2 peak-to-peak and P300 amplitudes in comparison with controls.

Potential perceptual correlates of heterosynaptic spinal LTP

The results of the present study regarding the increased perceived intensity to mechanical (pinprick) stimuli are in agreement with Klein et al. (2004). They concluded that this increased perceived intensity towards mechanical (pinprick) stimuli in the area surrounding the stimulated area is a perceptual correlate of heterosynaptic spinal LTP (Klein et al. 2004). Similarly the enhanced ERP amplitudes observed after HFS in the area adjacent to the stimulated area can be interpreted as a neurophysiological correlate of heterosynaptic spinal LTP.

Long-term (inter session) effects observed in the ERPs to paired non-painful stimuli

With regard to behavioral parameters (VAS-scores) the present study showed a significant long-term decrease after conditioning in the control group. This can be interpreted as a behavioral habituation effect and corresponds with the long-term (inter session) effect of the ERP amplitudes. However, in the HFS group a lack of long-term habituation in both the behavioral tests and ERP amplitudes was observed. These results regarding the lack of habituation of the ERPs after HFS are supported by a study performed by Valeriani et al. (2003) who investigated habituation effects in the ERPs in response to CO₂ laser stimuli in patients with migraine. Their design consisted of three repetitive ERP measurements with a 5 min interval. In comparison with healthy controls they observed a reduced habituation effect in the ERP amplitudes in migraine patients.

In order to compare this reduced habituation effect in their patients with the effects after HFS, it would be of interest to investigate if the ERP amplitude and perceived intensity (VAS-scores) start habituating during a later post measurement, e.g. 30 minutes after the first
post measurement, or whether lack of habituation is maintained for a longer period after HFS.

Another interesting finding in the present study is that short term habituation was unaltered after both HFS and control. Thus it seems that HFS does not influence short-term habituation of paired non-painful stimuli. In contrast, Montoya et al. (2006) observed a lack of short-term habituation of paired pulses in patients with chronic pain, i.e. the second stimulus was not significantly attenuated in comparison to S1. This effect was present for early (50 ms) and late (160-360 ms) ERP activity (Montoya et al. 2006).

The similarity between present study and the study of Montoya et al. is the use of non-painful stimuli. However, at least three possible explanations are conceivable regarding the differences in results of short-term habituation between the present study and the study of Montoya et al. A first possible explanation is the difference regarding the stimulus used. Montoya et al. used tactile stimuli instead of the electrical ones used in the present study. We have chosen electrical stimuli because they bypass the processes related to receptor transduction, and therefore allow better synchronization of afferent input. Moreover, recording ERPs in response to electrical stimuli is technically much easier to implement in practice than mechanical stimuli like a tactile stimulus. Furthermore, in the study of Montoya et al. medication use cannot be ruled out as a possible confounder regarding the lack of short-term habituation. A third possible explanation could be the presence of chronic pain, which can be expected to alter somatosensory processing by itself.

It should be noted that in the present study the terms habituation and sensitization are merely used as descriptors of behavioral responses (phenomena) rather than underlying processes. In our opinion the observed behavioral (VAS) and electrophysiological variables are possibly the net outcome of multiple underlying processes. At this moment we can only speculate about these processes.

Why we have observed a lack of long-term habituation but no lack of short-term habituation is still unclear, however according to Rankin et al, (2009) both short-term and long-term habituation are elicited by different underlying cellular mechanisms. If we assume that
heterosynaptic LTP is induced after HFS, the data suggest that this type of LTP only affects long-term habituation (in contrast to short-term) of non-painful electrical stimuli.

An interesting question is whether these observed changes in ERP amplitudes are solely the result of spinal changes or also supra spinal or (sub) cortical changes. While the ERP effects seen must originate in the brain (cortex), it is evident that the present study does not permit definitive distinction as to the origin of the changes observed. To date there is but one animal (PET) study showing changes in the acute and subacute metabolic response of supra spinal areas involved in nociceptive modulation after the induction of LTP via HFS of the peripheral sciatic nerve (Hjornevik et al. 2008).

Methodological considerations

Control condition

It is important to note that we used a different stimulation protocol than Klein et al. (2004) regarding our control condition. As a control, Klein et al. placed the conditioning electrode on a control site, approximately 5 cm from the wrist, without stimulating through this electrode. One could raise the question if this is a valid control because one could expect effects from the perception of the stimulus itself, regardless of the frequency used. To control for these effects one has to give some form of perceived stimulation without producing LTP or the opposite; long-term depression (LTD). This we did by applying stimuli at 20 × detection threshold, and of a number and frequency clearly inadequate to produce effects such as LTP or LTD. It is very unlikely that the observed decrement of the perceived intensity and ERP amplitude in our control group could be a perceptual correlate of LTD. Animal research has shown that at least 900 pulses of low frequency stimulation are necessary to induce LTD (Manahan-Vaughan 2000). Similarly Jung et al. (2009) investigated the optimal protocol for LFS to elicit potential correlates of LTD in humans. The authors needed a minimum of 300 pulses in order to observe a significant decline in perceived intensity and ERP amplitude in comparison with controls. In contrast, the present control condition only used five pulses.
In order to detect increased perceived intensity to punctate pinprick stimuli after HFS, a similar methodology as described by Klein et al. (2004) has been used. As we do not have access to similar calibrated stainless steels wire probes, we used an equivalent calibrated sharp-tipped von Frey monofilament. The use of von Frey monofilaments is a recognized method of detecting and quantifying increased perceived intensity to punctate stimuli (Meyer and Treede 2004). It has been shown that the pricking pain to punctate stimuli is mediated by A-delta fiber nociceptors. (Meyer and Treede 2004 (for an overview of this topic); Ziegler et al. 1999). In order to be able to use the same VAS scale for measuring changes in the intensity of non-painful (innocuous) electric and painful (noxious) mechanical pinprick stimulation, we used a modified VAS that allowed scoring of both non-painful and painful stimuli. Application of this modified VAS scale, used without suggesting the painfulness or non-painfulness of the stimuli applied, resulted in subjects rating their pinprick stimulation below the painful range. Thus, both electric and mechanical pinprick stimulation were rated as non-painful stimuli in the present study. This outcome regarding the pinprick stimulation does not affect the validity of the results because, firstly, despite the different naming by the subjects, the technique used to demonstrate the presence of increased perceived intensity to punctate stimuli is the same as used in other studies (Klein et al. 2004; Meyer and Treede 2004; Kawamata et al. 2002); and secondly, because even if the stimuli were not painful the VAS score used is nevertheless capable of detecting and scaling changes in perceived stimulus intensity.

In summary, conditioning HFS resulted in significant heterotopic effects 30 minutes after HFS. These heterotopic effects included increased perceived intensity in response to mechanical (pinprick) and paired non-painful electrical stimulation in comparison with controls. The paired non-painful electrical stimuli were accompanied by significantly enhanced ERP amplitudes in comparison with controls. Within the context of this experiment we interpreted these results as a lack of long-term habituation and is a potential neurophysiological correlate of heterosynaptic LTP induced after HFS in humans.
Acknowledgement

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References


**Figure legends**

**Fig. 1.** Above left: Positioning of the two silver electrodes used for the paired pulse stimulation on the arm of the subject. Dotted line represent proximal-distal axis. Above right: Standardized placement of the two large surface silver electrodes together with the ring electrode. The anode and cathode of the large surface electrodes were placed in an angle of 45 degrees each from the proximal-distal axis. The diameter of each silver electrode is 8 mm. Both were placed within a circle of 1 cm distance from the ring electrode. Circle of dots in the middle of the ring electrode represents the pins through which the electrical current is transmitted. Below: Time-table of the experiment for both the HFS and control group.

**Fig. 2.** Static heterotopic mechanical test stimuli applied adjacent to the stimulated skin area. The figure shows the median and inter-quartile range VAS-scores as response to the static mechanical test stimuli. A von Frey monofilament was pressed on four different areas of the skin (proximal, distal, lateral and medial), 2.5 cm outside the area of high frequency stimulation. ■ = Mann-Whitney U test statistics of the difference scores (post-pre) between HFS and control group (• = p < .05, •• = p < .01). • = Wilcoxon Signed Rank test statistics between pre and post (• = p < .05, ••• = p < .001, ◦ = marginally significant (p = .053). Red squares represent the pre and post comparison of the HFS group.

**Fig. 3.** Paired pulse stimulation. The figure shows the median and inter-quartile range VAS-scores as response to the paired non-painful heterotopic electrical stimulation adjacent to the stimulated skin area. ■ = Mann-Whitney U test statistics of the difference scores (post-pre) between HFS and control group (• = p < .05). • = Wilcoxon Signed Rank test statistics between pre and post (•• = p < .01). Blue squares represent the pre and post comparison of the control group.

**Fig. 4.** ERP N1-P2 peak-to-peak amplitude. Median and inter-quartile range of the N1-P2 peak-to-peak amplitude on the first (S1) and second (S2) stimulus in both the control and HFS group. ■ = Mann-Whitney U test statistics of the difference scores (post-pre) between HFS and control group (• = p < .05). • = Wilcoxon Signed Rank test statistics between pre and post (•• = p < .05). ••• = p < .001). ••• = p < .001).

**Fig. 5.** ERP P300 amplitude. Median and inter-quartile range of the P300 amplitude on the first (S1) and second (S2) stimulus in both the control and HFS group. ■ = Mann-Whitney U test statistics of the difference scores (post-pre) between HFS and control group (• = p < .05). • = Wilcoxon Signed Rank test statistics between pre and post (••• = p < .001).

**Fig. 6.** Grand median event-related potentials. Top. Plotted are the grand median ERPs for the pre (black line) and post (grey line) condition of the control group. Dotted lines represents stimulus onset. Upward is positive and downward is negative charge. The blue part in the figure represents the difference between pre and post. Down. Plotted are the
grand median ERPs for the pre (black line) and post (grey line) condition of the HFS group.  
Dotted lines represents stimulus onset. Upward is positive and downward is negative charge. 
Colored parts represents the difference between pre and post. The blue part in the figure represents the amount of decrement whereas the purple part represents the amount of increment between pre and post.

Table 1

Medians (and inter-quartile ranges) of the behavioral VAS-scores for the static heterotopic mechanical test stimuli (*mechanical*) and the paired non-painful heterotopic electrical test stimuli (*electrical*).

Table 2

Grand median ERP N1/P2 peak-to-peak amplitude. Medians (and inter-quartile ranges) of the amplitudes in millivolts of the ERP N1/P2 peak-to-peak amplitude for both the first and second stimulus.

Table 3

Grand median ERP P300 amplitude. Medians (and inter-quartile ranges) of the amplitudes in millivolts of the ERP P300 amplitude for both the first and second stimulus.
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<td>16.7 (12.7 - 30.6)</td>
<td>20.6 (17.0 - 27.7)</td>
<td>16.9 (15.5 - 20.1)</td>
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<td>S2</td>
<td>9.2 (7.3 - 12.0)</td>
<td>10.4 (8.5 - 13.0)</td>
<td>9.1 (6.1 - 16.0)</td>
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<td>14.0 (9.9 – 18.6)</td>
<td>10.2 (6.5 – 13.0)</td>
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<td>7.6 (6.1 – 10.3)</td>
<td>6.9 (4.1 – 8.3)</td>
<td>4.3 (2.8 – 5.8)</td>
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