Neurophysiological Correlates of Nociceptive Heterosynaptic Long-Term Potentiation in Humans

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van den Broeke EN, van Rijn CM, Biurrun Manresa JA, Andersen OK, Arendt-Nielsen L, Wilder-Smith OHG. Neurophysiological correlates of nociceptive heterosynaptic long-term potentiation in humans. J Neurophysiol 103: 2107–2113, 2010. First published February 17, 2010; doi:10.1152/jn.00979.2009. Long-term potentiation (LTP) is a cellular model of synaptic plasticity and reflecting 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 nonpainful 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 nonpainful electrical test stimuli to quantify and confirm the effects of HFS on the behavioral level. ERPs to paired nonpainful electrical stimulation were measured simultaneously. Conditioning HFS resulted in significant heterotopic effects after 30 min, including increased perceived intensity in response to (pinprick) mechanical and paired nonpainful electrical stimulation compared with control. The paired nonpainful electrical stimuli were accompanied by significantly enhanced responses regarding the ERP N1-P2 peak-to-peak and P300 amplitude compared with control. These findings suggest that HFS is capable of producing heterosynaptic spinal LTP that can be measured not only behaviorally but also using ERPs.

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, such as 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 neuronal population (Coenen 1995). During stimulus repetition a typical phenomenon can be observed—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 nonpainful stimulation as a possible electrophysiological cortical correlate of sensitization of the somatosensory system.

METHODS

Participants

Twenty-two healthy men (median age, 26.5 yr; range, 20–57 yr) participated in the experiment. Subjects were excluded from the study if they had a psychiatric or neurological history, used medication, or suffered from preexisting 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 two groups: painful electrical high-frequency stimulation (HFS) or painful electrical single-pulse stimulation (Control). For high-frequency stimulation, sub-
Subjects received trains of 100 Hz (pulse width, 2 ms) of 1 s repeated five times at a 10-s interval, with an intensity of 20-fold the detection threshold on the forearm 5 cm distal to the fossa cubita using a ring electrode (Fig. 1). The ring electrode consisted of 16 blunt stainless steel pins with a diameter of 0.2 mm, protruding 1 mm from the base. The 16 pins, placed in a circle with a diameter of 10 mm, served as a cathode. A stainless steel reference electrode, which served as an anode was concentrically located, had an inner diameter of 22 mm and an outer diameter of 40 mm. This electrode was 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 s at an intensity of 20-fold the detection threshold, repeated five times with a 9-s interval between each single pulse. To avoid interference of lateral dominance, the stimulated arm was balanced across subjects.

**Variables measured**

**BEHAVIORAL MEASURES.** 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 nonpainful electrical paired-pulse stimulation (see following text). A second behavioral test was used to test for heterotopic effects regarding mechanical (pinprick) stimulation (Katsarava 2006). To achieve an optimal standardization across subjects, both electrodes were 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 interstimulus interval and a random interpair interval. Fifty pairs of nonpainful electrical test stimuli (S1–S2), with a fixed interstimulus interval of 500 ms and a random interpair interval ranging from 7 to 10 s, 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 nonpainful 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° 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 five 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.

**FIG. 1.** Top left: positioning of the 2 silver electrodes used for the paired-pulse stimulation on the arm of the subject. Dotted line represents proximal–distal axis. Top right: standardized placement of the 2 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° 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. Bottom: timetable of the experiment for both the high-frequency electric stimulation (HFS) and control group.
TABLE 1. Medians (and interquartile ranges) of the behavioral VAS scores for the static heterotopic mechanical test stimuli and the paired nonpainful heterotopic electrical test stimuli

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Area</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>Proximal</td>
<td>1.4 (0.8–2.1)</td>
<td>2.5 (1.5–4.6)</td>
<td>1.9 (0.8–2.7)</td>
<td>1.9 (1.4–2.5)</td>
</tr>
<tr>
<td>Distal</td>
<td>1.4 (0.6–3.0)</td>
<td>3.4 (1.0–4.3)</td>
<td>1.9 (0.9–2.9)</td>
<td>2.0 (1.3–3.0)</td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>1.6 (0.5–2.1)</td>
<td>2.1 (1.2–4.2)</td>
<td>1.8 (0.8–3.2)</td>
<td>2.0 (1.6–2.2)</td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>1.6 (0.7–2.5)</td>
<td>3.0 (1.1–4.3)</td>
<td>2.3 (0.8–3.0)</td>
<td>2.2 (2.0–2.8)</td>
<td></td>
</tr>
<tr>
<td>Electrical</td>
<td></td>
<td>2.3 (0.9–5.5)</td>
<td>3.1 (1.4–4.8)</td>
<td>2.3 (0.9–4.3)</td>
<td>0.9 (0.4–2.2)</td>
</tr>
</tbody>
</table>

VAS, Visual Analogue Scale; HFS, high-frequency electrical stimulation.

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 2 min (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

At the beginning of the experiment (Fig. 1) 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, Sarasota, FL). After obtaining the detection threshold for the ring electrode, the baseline measurement for the mechanical pinprick test adjacent to the stimulated area:

**Table 2.** Grand median ERP N1/P2 peak-to-peak amplitude: medians (and interquartile ranges) of the amplitudes (in millivolts) of the ERP N1/P2 peak-to-peak amplitude for both the first and second stimuli

<table>
<thead>
<tr>
<th>N1/P2</th>
<th>HFS Pre</th>
<th>Post</th>
<th>Control Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>16.7 (12.7–30.6)</td>
<td>20.6 (17.0–27.7)</td>
<td>16.9 (15.5–20.1)</td>
<td>11.4 (6.9–18.7)</td>
</tr>
<tr>
<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>
<td>6.4 (4.9–13.1)</td>
</tr>
</tbody>
</table>

ERP, event-related potential.

**Table 3.** Grand median ERP P300 amplitude: medians (and interquartile ranges) of the amplitudes (in millivolts) of the ERP P300 amplitude for both the first and second stimuli

<table>
<thead>
<tr>
<th>P300</th>
<th>HFS Pre</th>
<th>Post</th>
<th>Control Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>16.5 (13.4–19.5)</td>
<td>16.1 (14.1–18.3)</td>
<td>14.0 (9.9–18.6)</td>
<td>10.2 (6.5–13.0)</td>
</tr>
<tr>
<td>S2</td>
<td>7.6 (6.1–10.3)</td>
<td>7.7 (4.2–8.3)</td>
<td>6.9 (4.1–8.3)</td>
<td>4.3 (2.8–5.8)</td>
</tr>
</tbody>
</table>

ERP, event-related potential.

**Statistics**

SPSS software (v. 16.0) was used for statistical analysis. Because some variables were not normally distributed, outliers were present and n < 30 nonparametric 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 < 0.05. 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 interquartile 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: 1) distal (U = 25.0, P < 0.01, r = −0.50); 2) proximal (U = 27.5, P < 0.05, r = −0.46); 3) lateral (U = 33.5, P < 0.05, r = −0.38); and 4) medial (U = 33.5, P < 0.05, r = −0.39) (Fig. 2). Within-group (pre vs. post) effects showed significant increases in VAS scores.
SIGNIFICANCE (median pre 2.312; median post 3.077) and the effect VAS score suggested an increase after HFS, it did not reach \( Z \) (Fig. 3). Within-group (pre vs. post) analysis showed a significant decrease of the first stimulus in the control group (\( Z = -1.956, P < 0.05, r = -0.42 \) (Fig. 5). No significant decrease was found after HFS regarding the first or second stimulus of the N1-P2 peak-to-peak amplitude.

**DISCUSSION**

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

![FIG. 2. Static heterotopic mechanical test stimuli applied adjacent to the stimulated skin area. The figure shows the median and interquartile range Visual Analogue Scale (VAS) scores as response to the static mechanical test stimuli.](image)

**FIG. 2.** Static heterotopic mechanical test stimuli applied adjacent to the stimulated skin area. The figure shows the median and interquartile range Visual Analogue Scale (VAS) scores as response to the static mechanical test stimuli. A von Frey monofilament was pressed on 4 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 (●, \( P < 0.05 \), ■, \( P < 0.01 \)). Wilcoxon signed-rank test statistics between pre and post (●●, \( P < 0.05 \), ■■, \( P < 0.01 \), ▲, marginally significant (\( P = 0.053 \)). Red squares represent the post and pre comparison of the HFS group.

**LONG-TERM (INTERSESSION) 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 (\( U = 29.0, P < 0.05, r = -0.44 \)) (Fig. 5). 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 (\( Z = -1.956, P < 0.05, r = -0.42 \) (Fig. 5). No significant decrease was found after HFS regarding the first or second stimulus of the N1-P2 peak-to-peak amplitude.

![FIG. 3. Paired-pulse stimulation.](image)

**FIG. 3.** Paired-pulse stimulation. The figure shows the median and interquartile range VAS scores as response to the paired nonpainful 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 (●, \( P < 0.05 \)). Wilcoxon signed-rank test statistics between pre and post (●●, \( P < 0.01 \)). Blue squares represent the post and pre comparison of the control group.
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 those reported by Klein et al. (2004). They concluded that this increased perceived intensity toward mechanical (pinprick) stimuli in the area surrounding the stimulated area is a perceptual correlate of heterosynaptic spinal LTP (Klein et al. 2004).

FIG. 4. Grand median event-related potentials. Top: plotted are the grand median ERPs for the pre (black line) and post (gray line) condition of the control group. Dotted lines represent stimulus onset. Upward is positive and downward is negative charge. The blue part in the figure represents the difference between pre and post. Bottom: plotted are the grand median ERPs for the pre (black line) and post (gray line) condition of the HFS group. Dotted lines represent stimulus onset. Upward is positive and downward is negative charge. Colored parts represent 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.

FIG. 5. Event-related potential (ERP) N1-P2 peak-to-peak amplitude. Median and interquartile range of the N1-P2 peak-to-peak amplitude on the first and second stimuli (S1 and S2, respectively) in both the control and HFS group. ●, Mann–Whitney U test statistics of the difference scores (post – pre) between HFS and control group (●, P < 0.05); ●●, Wilcoxon signed-rank test statistics between pre and post (●, P < 0.05; ●●, P < 0.01; ●●●, P < 0.001).

FIG. 6. ERP P300 amplitude. Median and interquartile range of the P300 amplitude on the first and second stimuli (S1 and S2, respectively) in both the control and HFS group. ●, Mann–Whitney U test statistics of the difference scores (post – pre) between HFS and control group (●, P < 0.05); ●, Wilcoxon signed-rank test statistics between pre and post (●●●, P < 0.001).
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 (intersession) effects observed in the ERPs to paired nonpainful 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 (intersession) 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.

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

Short-term (intrasession) effects observed in the ERPs to paired nonpainful stimuli

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 nonpainful 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 compared with S1. This effect was present for early (50 ms) and late (160–360 ms) ERP activity (Montoya et al. 2006).

The similarity between the present study and the study reported by Montoya et al. (2006) is the use of nonpainful 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 by Montoya and colleagues. A first possible explanation is the difference regarding the stimulus used. Montoya’s group 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 thus 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 by 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, although according to Rankin et al. (2009) both short-term and long-term habituations 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 affects only long-term habituation (in contrast to short-term habituation) of nonpainful electrical stimuli.

An interesting question is whether these observed changes in ERP amplitudes are solely the result of spinal changes or also supraspinal or (sub)cortical changes. Although 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 (positron emission tomography) study showing changes in the acute and subacute metabolic response of supraspinal 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 stimulation protocol different from that used by Klein et al. (2004) regarding our control condition. As a control, Klein and colleagues placed the conditioning electrode on a control site, about 5 cm from the wrist, without stimulating through this electrode. One could raise the question whether 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-fold the 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 in the perceived intensity and ERP amplitude in our control group could be a perceptual correlate of LTD.

Animal research has shown that ≥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 to observe a significant decline in perceived intensity and ERP amplitude compared with controls. In contrast, the present control condition used only five pulses.

PUNCTATE STIMULATION. To detect increased perceived intensity to punctate pinprick stimuli after HFS, a methodology similar to that described by Klein et al. (2004) has been used. Because we did not have access to similar calibrated stainless steel 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 Aδ-fiber nociceptors (for an overview of this topic, see Meyer and Treede 2004; Ziegler et al. 1999). To be able to use the same VAS scale for measuring changes in the intensity of nonpainful (innocuous) electric and painful (noxious) mechanical pinprick stimulation, we used a modified VAS that allowed scoring of both nonpainful and painful stimuli. Application of this modified VAS scale, used without suggesting the painfulness or nonpainfulness 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 nonpainful stimuli in the present study. This outcome regarding the pinprick stimulation does not affect the validity of the results because, first, 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 that used in other studies (Kawamata et al. 2002; Klein et al. 2004; Meyer and Treede 2004); and, second, 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 min after HFS. These heterotopic effects included increased perceived intensity in response to mechanical (pinprick) and paired nonpainful electrical stimulation compared with controls. The paired nonpainful electrical stimuli were accompanied by significantly enhanced ERP amplitudes compared with controls. Within the context of this experiment we interpreted these results as 1) a lack of long-term habituation and 2) a potential neurophysiological correlate of heterosynaptic LTP induced after HFS in humans.

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