Title:

High-frequency electrical stimulation of cutaneous nociceptors differentially affects pain perception elicited by homotopic and heterotopic electrical stimuli.

Authors:

van den Broeke EN[1*], Urdí M[2], Mouraux A[1], Biurrun Manresa J[3], Torta DM[2]

Affiliations:

[1]Institute of Neuroscience, IoNS, Faculty of Medicine, Université catholique de Louvain, Avenue Emmanuel Mounier 53, B-1200, Brussels, Belgium.

[2]Health Psychology Research Group, Faculty of Psychology and Educational Sciences, KU Leuven, Tiensestraat 102, B-3000 Leuven, Belgium.

[3]Institute for Research and Development in Bioengineering and Bioinformatics (IBB-CONICET-UNER), National University of Entre Rios, Oro Verde, Argentina.

Running title:

High-frequency stimulation and hyperalgesia

*Corresponding author:

Emanuel N van den Broeke
Institute of Neuroscience (IoNS)
Faculty of Medicine
Université catholique de Louvain
B-1200, Brussels
Belgium
E-mail: Emanuel.vandenbroeke@uclouvain.be

ABSTRACT

Animal studies have shown that high-frequency electrical stimulation (HFS) of peripheral C-fiber nociceptors induces both homo- and heterosynaptic long-term potentiation (LTP) within spinal nociceptive pathways. In humans, when HFS is applied onto the skin to activate nociceptors, single electrical stimuli are perceived more intense at the HFS site compared to a control site, a finding that was interpreted as a perceptual correlate of homosynaptic LTP. The present study aimed to investigate if after HFS the pain elicited by electrical stimuli delivered at the skin next to the HFS site is perceived as more intense compared to the pain at a control site (contralateral arm). To test this, HFS was applied to one of the two ventral forearms of twenty-four healthy participants. Before and after HFS, single electrical stimuli were delivered through the HFS electrode, through an identical electrode next to the HFS electrode and through an identical electrode at the contralateral arm. After HFS, the pain elicited by the single electrical stimuli was reduced at all three sites, with the largest reduction at the HFS site. Nevertheless, electrical stimuli delivered to the skin next to the HFS site were perceived as more intense than control stimuli. This result indicates that higher pain ratings to electrical stimuli after HFS at the HFS site cannot solely be interpreted as a perceptual correlate of homosynaptic changes. Furthermore, we show for the first time, in humans, that HFS can *reduce* pain elicited by single electrical stimuli delivered through the same electrode.

NEW & NOTEWORTHY

High-frequency electrical stimulation (HFS) of cutaneous nociceptors can reduce pain perception to single electrical stimuli delivered through the same electrode. Moreover, single electrical stimuli delivered to the skin next to the site at which HFS was applied are perceived as more intense compared to contralateral control site, indicating the presence of heterosynaptic effects for electrical stimuli.

KEYWORDS

High-frequency stimulation; long-term potentiation, nociception; hyperalgesia

1. INTRODUCTION

Animal studies have shown that peripheral noxious stimulation increases synaptic efficacy within spinal nociceptive pathways. For instance, Kronschläger et al. [1] showed that high-frequency electrical conditioning stimulation (HFS) of peripheral peptidergic C-fiber nociceptors induces long-term potentiation (LTP) at both conditioned spinal synapses (homosynaptic LTP), and at remote unconditioned spinal synapses (heterosynaptic LTP). In humans, Klein et al. [2] showed for the first time that after applying HFS onto the skin, electrically evoked pain was perceived as more intense at the HFS site compared to control site (homotopic effect). Furthermore, they showed that the pain elicited by mechanical pinprick stimuli was perceived as more intense at the skin next to the HFS site compared to a control site (heterotopic effect). Based on these findings it was hypothesized that homosynaptic and heterosynaptic LTP plays a role in primary hyperalgesia (increased pain sensitivity in the area of injury) and secondary hyperalgesia (increased pain sensitivity of the surrounding uninjured skin), respectively [1,3,4].

We and others have replicated the HFS-induced heterotopic effect several times. However, the homotopic effect was either not [5,6] or only partially replicated [7]. For this reason, we recently conducted a replication study to assess if HFS increases pain elicited by single electrical stimuli delivered through the same electrode [8]. We found that after HFS electrical stimuli delivered through the same electrode were perceived more intense compared to control stimuli, however, this was mainly due to a decrease of the perceived pain intensity at the control site rather than an increase in perceived pain intensity at the HFS site compared to baseline [8].

Klein et al. suggested that the higher perceived pain intensity elicited by single electrical stimuli at the HFS site (or at least part of it) reflects a perceptual correlate of homosynaptic LTP at C-fiber synapses [2]. This idea could be further substantiated by a later study of the same group in which they found that the higher perceived pain intensity elicited by single electrical stimuli after HFS at the HFS site

was described as hot and burning, descriptors that according to the authors are compatible with the activation of C-fiber nociceptors [9]. Recently, we hypothesized that the higher perceived pain intensity elicited by single electrical stimuli at the HFS site could also reflect (at least partly) a perceptual correlate of heterosynaptic LTP [8]. First, because HFS also triggers LTP at unconditioned C-fiber synapses (heterosynaptic LTP, [1]). Indeed, in humans we have shown that after HFS heat stimuli selectively activating cutaneous C-fibres are perceived more intense when these stimuli were delivered next to the HFS skin compared to the control site [10]. Second, studies using quantitative sensory testing to assess changes in the perception to thermal and mechanical stimuli have shown that within the area at which HFS was applied, HFS predominantly increased pain to mechanical pinprick stimuli [11]. Moreover, a strong correlation was found between the increase in mechanical pinprick pain at the HFS site and the increase in mechanical pinprick pain at the surrounding skin, suggesting that heterosynaptic facilitation dominates at the HFS site [12].

The aim of the present study was to investigate if after HFS the perceived pain intensity elicited by single electrical stimuli delivered at the skin next to the HFS site was higher compared to the perceived pain intensity elicited at the contralateral control site (heterotopic effect). If this is the case, this would indicate that the higher perceived pain intensity elicited by single electrical stimuli after HFS at the HFS site compared to a control site (homotopic effect), as found in previous studies, cannot be solely interpreted as a perceptual correlate of homosynaptic changes.

2. MATERIALS AND METHODS

2.1 Participants

After obtaining approval of the ethical commission (SMEC, KU Leuven: G-202003 1999), twenty-four participants were recruited (14 females, 10 males) with a mean (±SD, min-max) age of 22.9 years (3.31, 20-34). This number of participants was chosen based on our aim of replicating the homotopic effect of our previous replication study and to be able to counterbalance the three conditions (homotopic, heterotopic and control, see below) across participants. Exclusion criteria were: 1) being

younger than 18 or older than 40, 2) having already participated in a study using electrical stimulation of the skin, 3) having used painkillers or anti-inflammatory drugs within 12 hours before the start of the experiment, 4) having heart, vascular, respiratory and/or neurological diseases, 5) having pain, acute or chronic, 6) having a pacemaker or other electronic implant, 7) having hearing and/or vision problems, 8) having a psychiatric history, 9) using drugs for recreational use, 10) using medication regularly (except oral contraceptives), 11) being pregnant, 12) having sleeping problems such as sleep deprivation. The procedures of the present study were explained to each participant and written informed consent was obtained. Participants received either course credits or monetary compensation for their participation in the study.

2.2 Study design

The design of the present study is summarized in Figure 1. In this repeated measures within-subject experiment, HFS was applied to the ventral forearm of the dominant or non-dominant arm (approx. 5 cm from the cubital fossa) using a multi-pin electrode designed to preferentially activate cutaneous nociceptors. Single electrical stimuli were delivered through the multi-pin electrode ('homotopic stimulus'), through an identical multi-pin electrode placed next to the HFS electrode ('heterotopic stimulus') and through another identical multi-pin electrode placed at the contralateral arm that served as control ('control stimulus'). The electrical stimuli were delivered every 5 min, starting 30 min before and ending 60 min after HFS conditioning. Single electrical stimuli were delivered to each site (homotopic, heterotopic and control) in a counterbalanced order across participants and remained the same throughout the experiment for each subject. Of the two electrodes attached on the HFS arm, the most proximal one was always the electrode through which HFS was delivered. To confirm that HFS induced an increase in mechanical pinprick sensitivity of the skin next to the site of HFS, mechanical pinprick stimuli were applied before and after HFS at the skin next to the site of HFS and the contralateral control site. The perceived pain intensity elicited by the single electrical stimuli and mechanical pinprick stimuli was measured using a numeric rating scale (NRS).

-FIGURE 1 HERE-

2.3 High-frequency electrical stimulation (HFS)

HFS consisted of five trains of 100 Hz electrical stimuli (square-wave pulses with a pulse width of 2 ms) that lasted 1 s each and were delivered with a 9 s inter-train interval [8]. The trains were controlled by MATLAB (MathWorks, Nathick, US), generated using a constant current stimulator (DS5, Digitimer Ltd, Welwyn Garden City, UK) and delivered to the forearm using a multi-pin electrode designed to preferentially activate nociceptors. The multi-pin electrode consisted of 10 blunt stainless steel pins (250 μm diameter each) that served as cathode [8]. Three large surface electrodes (PALS platinum 5 x 9, Axelgaard Electrical Stimulation Electrodes, Digitimer, Hertfordshire, UK) served as anode. Two were attached onto the skin of the arm (biceps) at which HFS was applied and one on the same location of the contralateral arm. The intensity at which the HFS was delivered was set at twenty times the individual detection threshold to a single pulse. This intensity was chosen based on the results of our previous study in which we observed a higher perceived heat intensity elicited by CO_2 laser stimuli selectively activating C-fiber nociceptors after HFS at the skin next to the HFS site compared to contralateral control site [10]. To avoid any confounding effect of handedness, the arm onto which HFS was applied (dominant vs. non dominant) was counterbalanced across participants.

2.4 Test stimuli

2.4.1 Single electrical test stimuli

One single electrical pulse (square-wave pulse with a pulse width of 2 ms) was delivered at each time point through each electrode separately with an interval of 20 seconds. After each stimulus, participants were asked to provide a rating of the perceived pain intensity elicited by that stimulus using a numeric rating scale (NRS) ranging from 0 (non-painful) to 100 (most intense pain imaginable) [8]. Participants were instructed to distinguish painful from non-painful sensations by the

166 presence of a sharp or slightly pricking or burning sensation [2,8]. Participants were also told to pay
167 attention to any subtle change in the sensation and were free to use integers as well as fractions [8].
168 The single electrical stimuli were delivered at an intensity of ten times the electrical detection
169 threshold.

170

171 2.4.2 Mechanical pinprick stimuli

172 To confirm that HFS induced an increase in mechanical pinprick sensitivity of the skin surrounding
173 the site at which HFS was delivered, we applied before and after HFS mechanical pinprick stimuli to
174 the skin next to the HFS site and control site using a calibrated mechanical pinprick stimulator (The
175 Pin Prick, MRC Systems GmBH, Heidelberg, Germany) exerting a force of 128 mN [8]. A total of
176 three pinprick stimuli, lasting approximately 1 s each, were delivered for each measurement. During
177 stimulation, the hand-held stimulator tube was kept perpendicular to the volar forearm. After each
178 stimulus, participants were asked to rate the perceived pain intensity of the stimulus on the same scale
179 as the one used for the single electrical stimuli. To avoid sensitization of the skin due to repeated
180 stimulation, the same skin area was never stimulated twice.

181

182 2.5 Experimental procedure

183 The experiment took place in a light- and temperature-controlled room. During the experiment,
184 participants were comfortably seated in a chair with their arms resting on a table in front of them, with
185 palms up. Each participant was first familiarized with the experimental procedures by receiving a
186 description of the general set-up and the stimuli that they would receive. After that, baseline
187 measurements of the mechanical pinprick sensitivity were performed, followed by the assessment of
188 the electrical detection thresholds at each electrode. The same procedure was used as the one used in
189 our previous replication study [8]: a staircase procedure with three ascending and descending
190 staircases of single stimuli (2 ms pulse width). The final electrical detection threshold was the
191 geometric mean of the three series. The order with which the electrical detection thresholds were

192 determined for each electrode was counterbalanced across participants. After the assessment of the
193 electrical detection thresholds, single electrical stimuli were delivered at each electrode every 5
194 minutes starting 30 minutes before the application of HFS. Then, HFS was applied to one of the two
195 arms and followed again by single electrical stimuli delivered at each electrode for 55 min. At the end,
196 the mechanical pinprick testing was repeated.

197

198 2.6 Statistical analysis

199 All statistical analyses were performed in the statistical software package SPSS (version 19). A
200 repeated measures analysis of covariance (RM ANCOVA) was performed to assess the effects of site
201 and time on the ratings elicited by the single electrical stimuli after HFS. Thus, site (levels: homotopic,
202 heterotopic and control) and time (levels: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 min) were
203 considered as fixed factors and the average baseline pain rating was used as covariate for each site and
204 each subject. We performed a second RM ANCOVA to test the effect of site on the ratings elicited by
205 the mechanical pinprick stimuli after HFS. In this case, site (levels: heterotopic and control) was
206 considered as fixed factor and the baseline pain rating was used as covariate for each site and each
207 subject. Finally, a one-way ANOVA was performed to test for differences in the mean detection
208 thresholds between sites. Post-hoc pairwise tests were carried out using the Sidak correction.

209

210 3. RESULTS

211 3.1 Electrical detection thresholds

212 The electrical detection thresholds obtained at each site were 0.16 ± 0.05 (mean ± SD, homotopic),
213 0.17 ± 0.06 (heterotopic) and 0.17 ± 0.07 (control). We did not observe differences between the mean
214 detection thresholds of the three electrodes ($F(2,46) = 0.403$, $P = .671$).

215 3.2 Single electrical test stimuli

Figure 2A shows the mean (and SEM) perceived pain intensity elicited by the single electrical stimuli before and after HFS at the three sites (homotopic, heterotopic and control). The RM ANCOVA revealed significant main effects of site and time but no interaction (Table 1). This means that, when pain ratings were averaged across time points, the perceived pain intensity elicited by the single electrical stimuli was significantly different across the three sites. Post hoc tests showed a significant difference in mean perceived pain intensity between all sites (Table 1). Moreover, the perceived pain intensity was different between the first pain rating after HFS and subsequent pain ratings across sites.

-TABLE 1 HERE-

Figure 2B shows the estimated marginal means (and 95% CI) of the perceived pain intensity elicited by the single electrical stimuli after HFS at the three sites and corrected for pre-existing baseline differences.

-FIGURE 2 HERE-

3.3 Mechanical pinprick stimuli

Figure 3A shows the mean (and SEM) perceived pain intensity elicited by the mechanical pinprick stimuli before and after HFS at both sites (heterotopic and control). The ANCOVA showed a significant main effect of SITE (Table 1). This means that the pain ratings were significantly different between the two sites. Figure 3B shows the estimated marginal means (and 95% CI) of the perceived pain intensity elicited by the mechanical pinprick stimuli after HFS at both sites and corrected for pre-existing baseline differences.

-FIGURE 3 HERE-

3.4 Correlation between mechanical pinprick pain and electrically elicited pain

Since we found a higher perceived pain intensity elicited by both single electrical stimuli and mechanical pinprick stimuli next to the HFS skin as compared to the control site, we wanted to test post-hoc whether this heterotopic effect on the perception elicited by electrical stimuli and mechanical pinprick stimuli were correlated. We did not observe a correlation between the two variables (Pearson r =.178, P =.406).

4. DISCUSSION

The aim of this study was to investigate if after HFS the perceived pain intensity elicited by single electrical stimuli delivered to the skin next to the HFS site ('heterotopic stimulus') was significantly higher compared to the perceived pain intensity at the contralateral control site. We found this to be the case, although the effect size was small. Nevertheless, our result indicates that higher pain ratings to electrical stimuli delivered after HFS at the HFS site as compared to control site, as found in previous studies, cannot solely be interpreted as a perceptual correlate of homosynaptic LTP. Moreover, and contrary to the results of previous studies, we found that after HFS the perceived pain intensity elicited by the single electrical stimuli delivered through the same electrode was lower compared to the control site.

4.1 Homotopic effects

In our previous study [8] we aimed to replicate the higher perceived pain intensity to single electrical stimuli after HFS at the HFS site compared to control site. We observed a reduction of the perceived pain intensity after HFS at both the HFS and control sites. Nevertheless, pain ratings at the HFS site were significantly *higher* compared to the control site. In the present study, the pain ratings elicited by the single electrical stimuli decreased after HFS at all sites and the pain ratings at the HFS site were significantly *lower* compared to the control site. A difference between our previous replication study

265 [8] and the present study is the intensity at which HFS was delivered. In our previous study [8], HFS
266 was delivered at an intensity corresponding to ten times the electrical detection threshold to a single
267 electrical stimulus, while in the present study we delivered HFS at twenty times the electrical detection
268 threshold. Therefore, it could be that the homotopic effects of HFS are dependent on HFS intensity.
269 However, Klein et al. [2] compared the pain ratings elicited by single electrical stimuli at the HFS site
270 delivered at 10 and 20 times the detection threshold and found no significant differences in pain
271 ratings. Notably, the number of participants in that study was smaller (N=7) as compared to the
272 present study (N=24). Also, the electrical detection thresholds and, thus, HFS stimulation intensities
273 tended to be lower in the study by Klein et al. Also Xia et al. [6] investigated the effect of HFS on the
274 perceived intensity elicited by single electrical stimuli delivered through the same electrode. In that
275 study the authors observed a significant higher perceived intensity elicited by the single electrical
276 stimuli at 30, 40, 50 and 60 min after HFS compared to 10 min after applying HFS, but this increase
277 was not different from the control condition, which was not the contralateral arm as in the present
278 study, but a separate condition in which the multi-pin electrode was attached to the skin but no HFS
279 was delivered.

280 The reduction in perceived pain intensity directly after HFS (± 5 min) at the site next to HFS and
281 contralateral control site might reflect a pain-inhibits-pain phenomenon or Diffuse Noxious Inhibitory
282 Controls (DNIC) described by Le Bars [13]. However, the larger pain reduction at the HFS site may
283 possibly reflect another mechanism as it has been suggested that DNIC would serve to enhance
284 contrast between a prominent nociceptive stimulus and background input by inhibiting the activity of
285 neurons relaying heterotopic activity relative to the painful locus. Animal studies have shown that,
286 depending on the membrane potential of spinal dorsal horn neurons, HFS can induce either long-term
287 potentiation (LTP) or long-term depression (LTD) [14]. It could thus be that HFS induced
288 homosynaptic LTD with the larger pain reduction at the HFS site as its perceptual correlate.

289

290 4.2 Heterotopic effects

Both the higher perceived pain intensity elicited by the electrical and mechanical pinprick stimuli at the skin next to the HFS site compared to the control site must involve heterosynaptic facilitation, as the pathways activated by these stimuli were not subjected directly to the high-frequency conditioning stimulation. It is thought that the increase in mechanical pinprick sensitivity is mediated by mechano-sensitive but heat-insensitive A-fiber nociceptors [15]. One possibility could be that the higher perceived pain intensity to single electrical stimuli at the skin next to HFS is mediated by the same afferents that also mediate the increase in mechanical pain sensitivity. However, we did not observe a correlation between the higher perceived pain intensity by the heterotopic electrical stimuli and the heterotopic increase in mechanical pinprick sensitivity, suggesting that the ratings evoked by the two modalities of stimulation are not linearly associated.

Another possibility may be that the higher perceived pain intensity elicited by heterotopic electrical stimuli compared to control stimuli was mediated by C fibers. We have previously shown that the perceived heat intensity elicited by CO2 laser stimuli selectively activating cutaneous C-fiber nociceptors was greater at the heterotopic site compared to a control site [10], suggesting heterosynaptic LTP [1]. Of note, Kronschläger et al. [1] showed in rats that HFS can induce heterosynaptic LTP in the absence of homosynaptic LTP, suggesting that homosynaptic and heterosynaptic LTP are independent phenomena.

4.3 Conclusion

The present study shows that HFS, delivered at twenty times the detection threshold, reduces pain elicited by single electrical stimuli at all sites, with the largest reduction at the HFS site. Nevertheless, electrical stimuli delivered to the skin next to the HFS site were perceived as more intense than control stimuli. This finding indicates that higher pain ratings to electrical stimuli after HFS at the HFS site cannot solely be interpreted as a perceptual correlate of homosynaptic LTP. Furthermore, we show for the first time, in humans, that HFS can reduce pain to single electrical stimuli delivered through the same electrode.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

ENvdB and DMT conceived and designed research; MU performed experiments; ENvdB, MU, JBM analysed data; ENvdB, AM, JBM, DMT interpreted results of experiments; ENvdB prepared figures; ENvdB, AM, JBM, DMT drafted manuscript; ENvdB, MU, AM, JBM, DMT approved final version of manuscript.

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FIGURE LEGENDS

Figure 1. Study design. **A.** HFS was applied to one of the two volar forearms using a multi-pin electrode designed to preferentially activate cutaneous nociceptors. Before and after HFS, single electrical test stimuli were delivered through the multi-pin electrode ("homotopic stimulus"), an identical multi-pin electrode next to the HFS site ("heterotopic stimulus") and another identical multi-pin electrode at the contralateral arm that served as control site ("control stimulus"). **B.** Characteristics

of the multi-pin electrode. **C.** Time-line of the experiment. The single electrical test stimuli were delivered every 5 min for a duration of 25 min before HFS (-30 to -5 min) and for a duration of 55 min after HFS, starting at 5 min after the end of the HFS (5-60 min). Before and after HFS and before and after the application of the single electrical test stimuli, calibrated mechanical pinprick stimuli (128 mN) were applied to the skin next to the HFS site and at the contralateral control site.

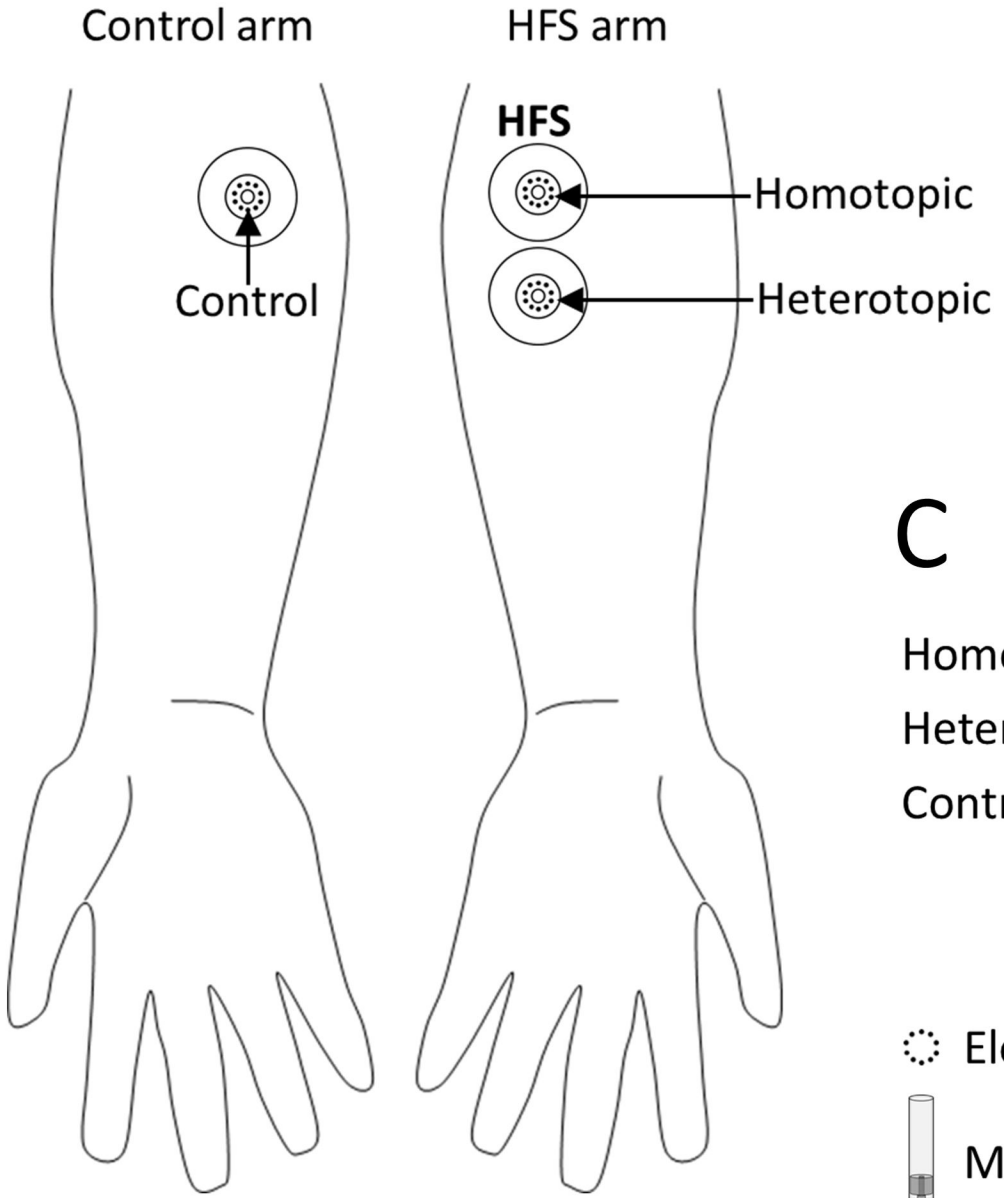
Figure 2. A. Mean (and SEM) perceived pain intensity elicited by the single electrical stimuli before and after HFS at the site at which HFS was delivered (homotopic), at the site next to HFS (heterotopic) and at the contralateral arm (control). Dotted line at zero represents the time at which HFS was delivered. **B.** Estimated marginal means (and 95% CI) of the perceived pain intensity elicited by the single electrical stimuli after HFS at the three sites as calculated by the RM ANCOVA. At the right side of the figure the estimated marginal means (and 95% CI) across all time points are shown. The dotted line represents the average baseline rating across subjects and sites.

Figure 3. A. Mean (and SEM) perceived pain intensity elicited by the mechanical pinprick stimuli applied before and after HFS at the skin next to the site of HFS (heterotopic) and at the contralateral arm (control). **B.** Estimated marginal means (and 95% CI) of the perceived pain intensity elicited by the mechanical pinprick stimuli after HFS (corrected for baseline) at the two sites. Dotted line represents the average baseline rating across subjects and sites.

TABLE LEGENDS

Table 1. Main results of the RM ANCOVA for the pain ratings elicited by single electrical and mechanical pinprick stimuli. * Sidak corrected.

A



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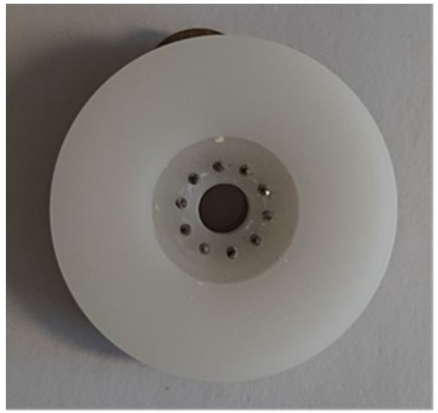

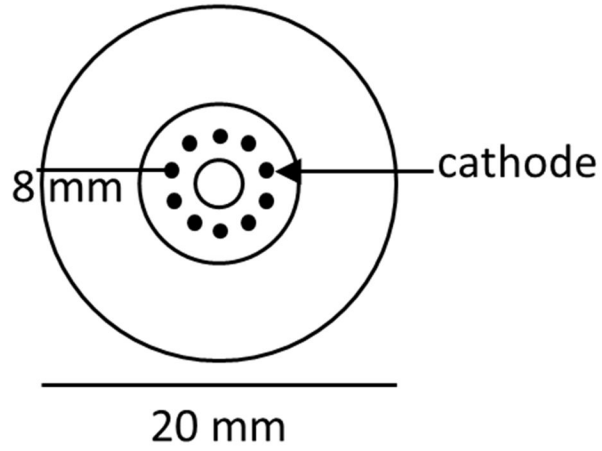

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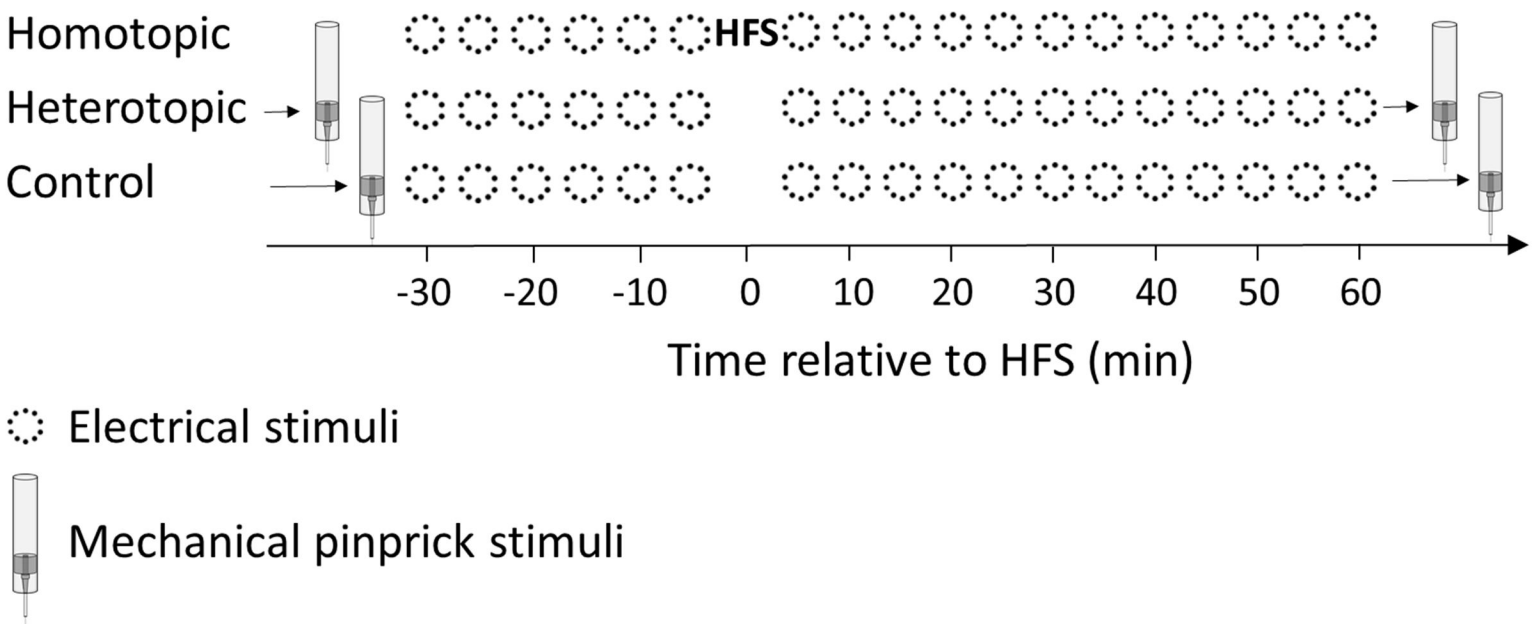

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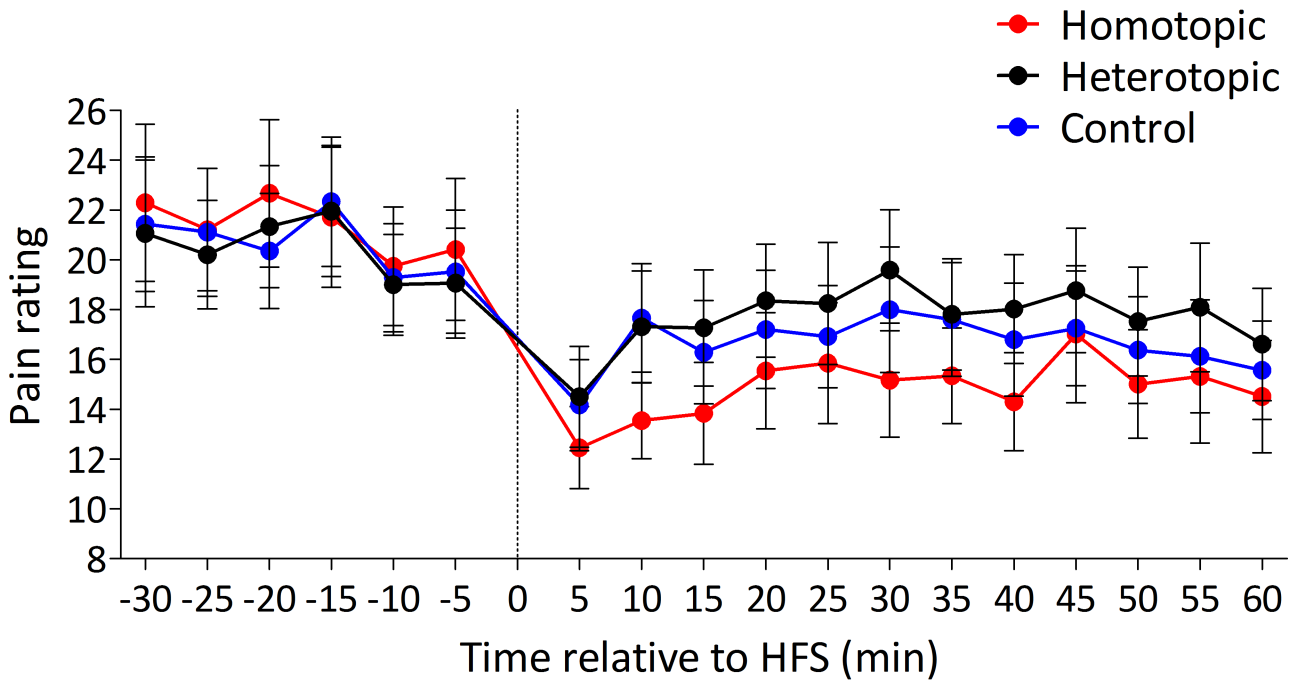

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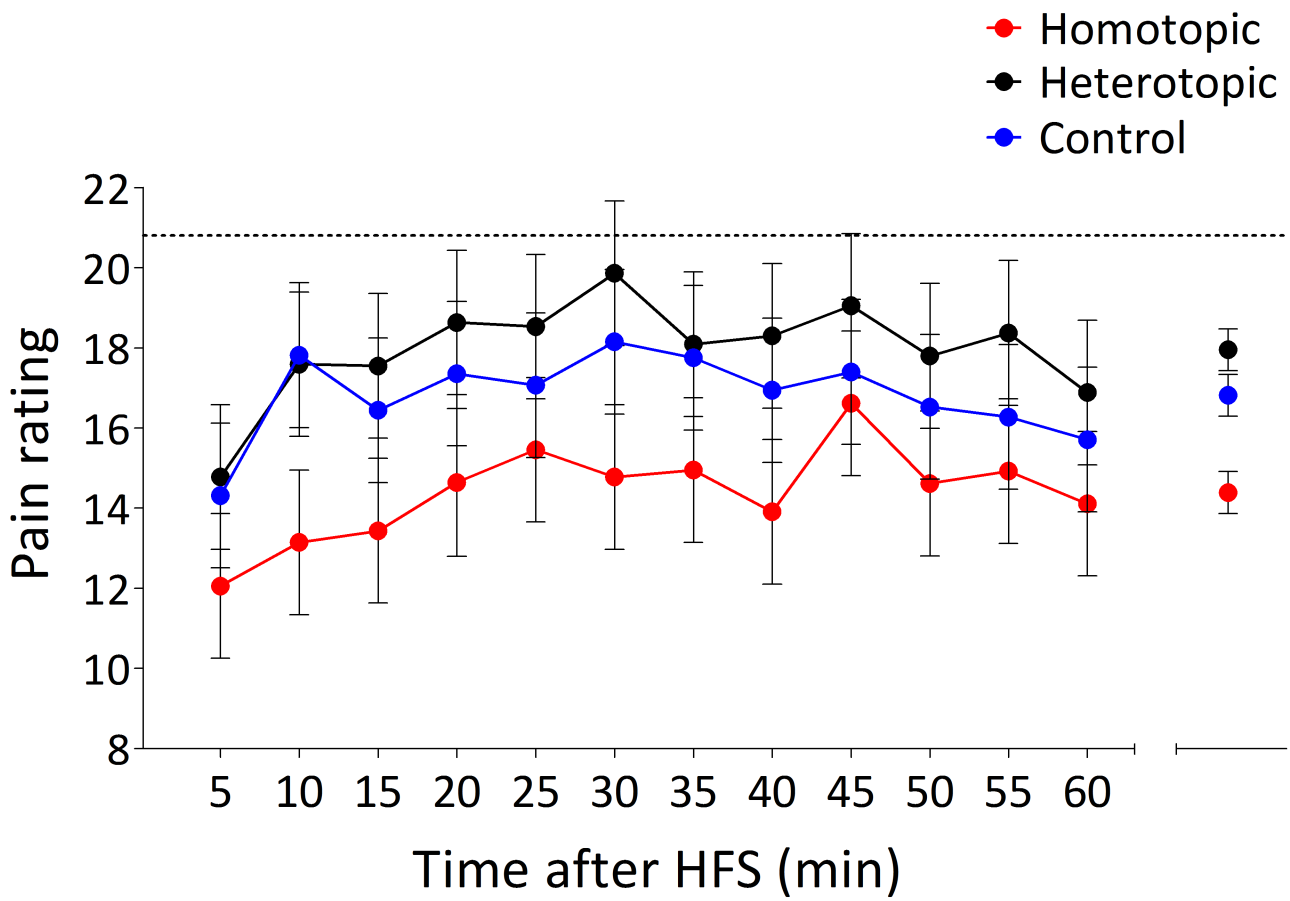
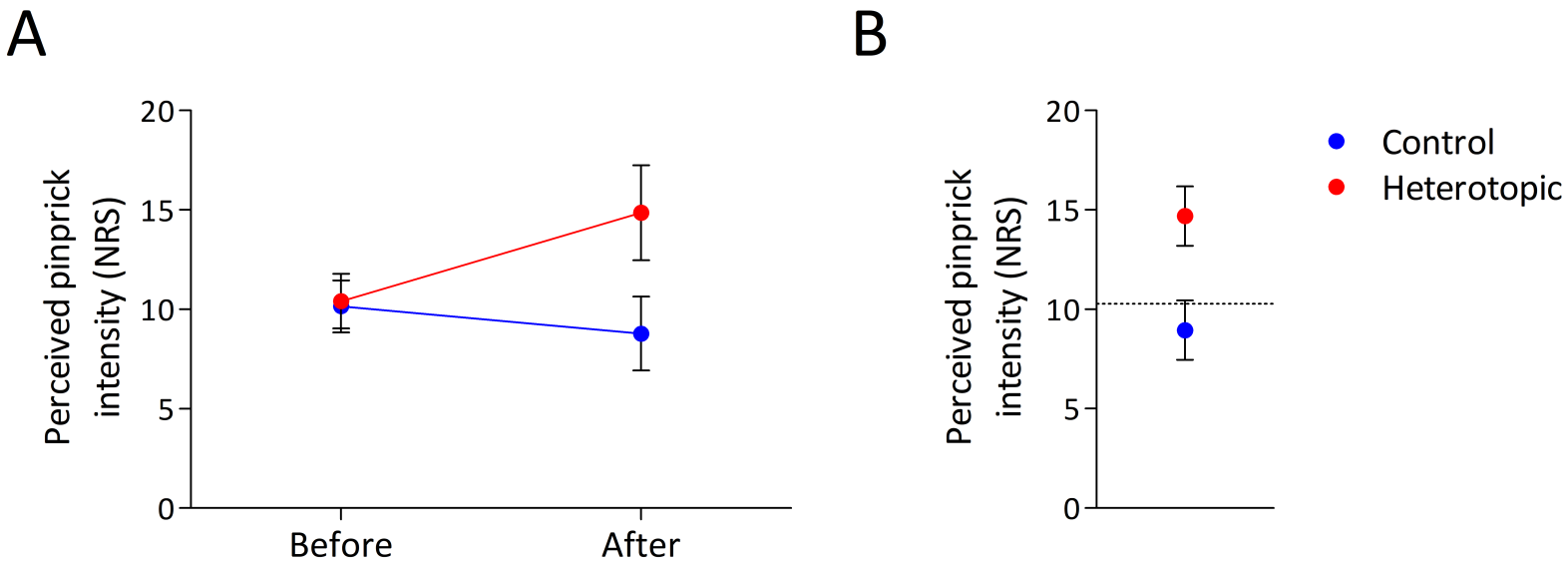
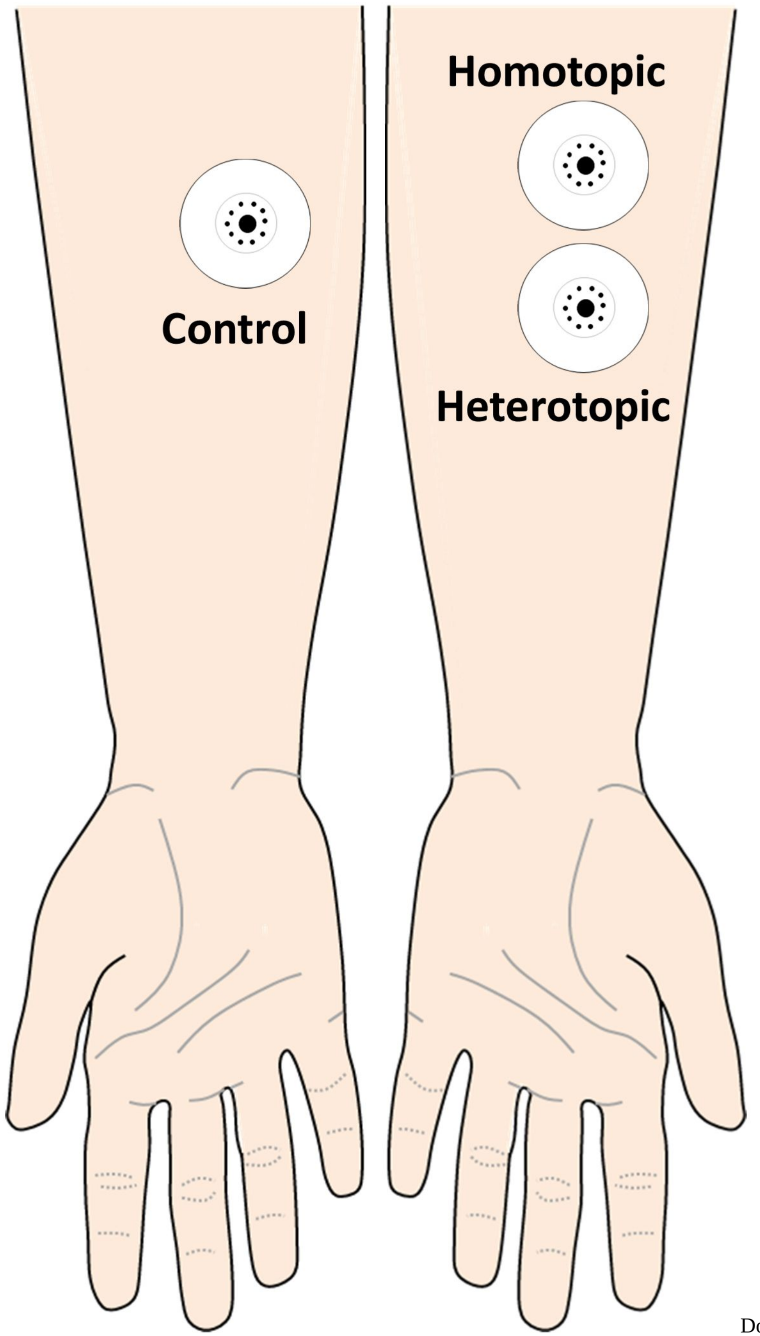


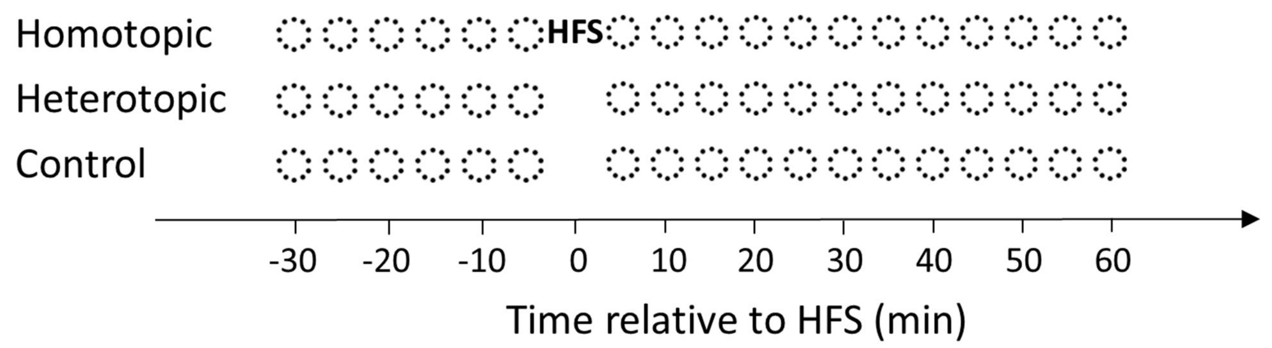


Single electrical stimuli		
main effect of SITE		F (2,803)= 46.385, *P*<.001, partial η^2 =.104
	Post-hoc comparisons*	Heterotopic vs. control: *P*<.01 [1.140 (0.241-2.038)]
	[mean difference + 95% CI]	Homotopic vs. control: *P*<.001 [-2.427 (-3.329-1.524)]
		Homotopic versus heterotopic: P<.001 [-3.566 (-4.470-2.662)]
main effect of TIME		F (11,803)= 3.963, *P*<.001, partial η^2 =.051
	Post-hoc comparisons*	5 min versus 20 min
	(only significant comparisons,	5 min versus 25 min
	P<.05)	5 min versus 30 min
		5 min versus 35 min
		5 min versus 40 min
		5 min versus 45 min
		5 min versus 50 min
		5 min versus 55 min
interaction SITE x TIME		F (22,803)= 0.439, *P*=.989, partial η^2 =.012
main effect of BASELINE		F (1,803)= 262.867, *P*<.001, partial η^2 =.247
Mechanical pinprick stimuli		
main effect of SITE		F (1,22)= 31.696, *P*<.001, partial η^2 =.590
	Mean difference + 95% CI	5.742 (3.627-7.857)
main effect of BASELINE		F (1,22)= 15.304, *P*<.01, partial η^2 =.410

Table 1. Main results of the RM ANCOVA for the pain ratings elicited by single electrical and mechanical pinprick stimuli. * Sidak corrected.



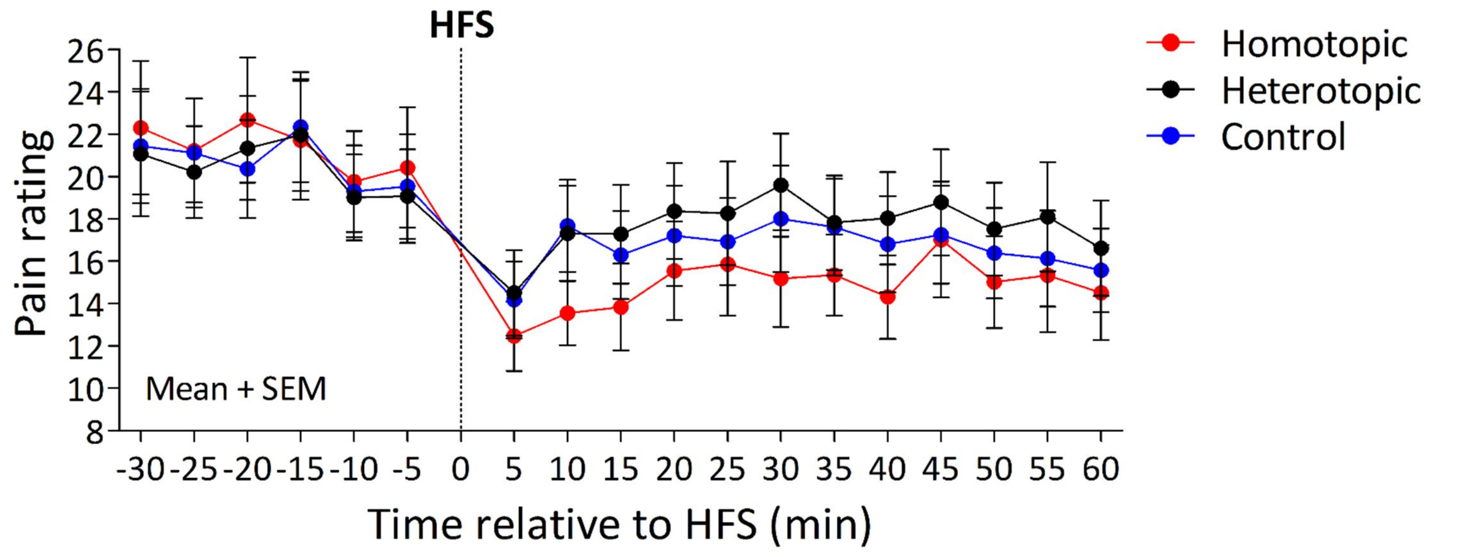
DESIGN



◌ = single electrical stimulus

HFS = high-frequency electrical conditioning stimulation

RESULTS



- ❑ After HFS, the average pain (across time points) elicited by single electrical stimuli **delivered through the same electrode** was **lower** compared to control site.
- ❑ After HFS, the average pain (across time-points) elicited by single electrical stimuli **delivered next to the HFS site** was **higher** compared to control site.