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# How different experimental models of secondary hyperalgesia change the nociceptive flexion reflex

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HIGHLIGHTS

- Central sensitization manifesting with secondary hyperalgesia is similarly induced by high-frequency stimulation and topical capsaicin.
- High frequency stimulation and topical capsaicin do not significantly activate the endogenous pain modulatory system.
- RIII reflex variables reflecting central sensitization are similarly modulated by high frequency stimulation and topical capsaicin.

ABSTRACT

*Objective:* In this neurophysiological study in healthy humans, we assessed how central sensitization induced by either high-frequency stimulation (HFS) or topical capsaicin application modulates features of the RIII reflex response. The ability of these stimuli to engage the endogenous pain modulatory system was also tested.
*Methods:* In 26 healthy participants we elicited an RIII reflex using suprathreshold stimulation of the sural nerve. Subsequently HFS or capsaicin were applied to the foot and the RIII reflex repeated after 15 minutes. Contact heating of the volar forearm served as the heterotopic test stimulus to probe activation of the endogenous pain modulatory system.
*Results:* HFS significantly reduced the pain threshold by 29% and the RIII reflex threshold by 20%. Capsaicin significantly reduced the pain threshold by 17% and the RIII reflex threshold by 18%. Both HFS and capsaicin left RIII reflex size unaffected. Numerical Rating Scale (NRS) pain scores elicited by the heterotopic noxious heat stimulus were unaffected by capsaicin and slightly increased by HFS.
*Conclusions:* HFS and capsaicin similarly modulated the pain threshold and RIII reflex threshold, without a concomitant inhibitory effect of the endogenous pain modulatory system.
*Significance:* Our neurophysiological study supports the use of the RIII reflex in investigating central sensitization in humans.


## 1. Introduction

Central sensitization, defined as an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input (https://www.iasp-pain.org/Education/Content.aspx?ItemNumber = 1698#Centralsensitization), is a

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key contributor to chronic pain, in particular neuropathic and nociplastic chronic pain syndromes (Arendt-Nielsen et al., 2018).

After tissue injury, chemical mediators generate an area of primary hyperalgesia due to the peripheral sensitization of nociceptive fibres (Treede et al., 1992). Inputs into the spinal cord induced by a given stimulus consequently increase. As a secondary effect, sustained peripheral nociceptive input may induce second-order neuron sensitization in the central nervous system (Liu and Sandkühler, 1997; Benrath et al., 2005), which manifests with a zone of secondary hyperalgesia, defined as the undamaged area surrounding the injury site with increased sensitivity to mechanical stimulation (Raja et al., 1984; LaMotte et al., 1991; Koltzenburg et al., 1992; Dahl et al., 1993). Hence, central sensitization is a set of mechanisms that occur after each acute injury and are manifest as secondary hyperalgesia to pinprick stimulation.

Different experimental pain models have been devised to investigate the mechanisms underlying central sensitization in humans (Quesada and Kostenko, 2021). Topical application of capsaicin and high-frequency electrical stimulation (HFS) are commonly used in human studies since they intensely activate nociceptive nerve fibres and lead to secondary hyperalgesia via central sensitization.

Although central sensitization is considered one of the leading mechanisms underlying chronic pain, how to reliably quantify central sensitization within the dorsal horn of the human spinal cord remains a controversial issue. Previous human studies used the RIII component of the nociceptive flexion reflex to quantify central sensitization. The RIII reflex of the lower limb is a pure nociceptive reflex mediated by A-delta fibres and usually elicited by electrical stimulation of the sural nerve that corresponds to the subclinical withdrawal of the stimulated limb, whose anatomical substrate is entirely located at the spinal level (Meinck et al., 1981; Shahani and Young, 1971; Jankowska, 1992). Several studies showed that the RIII reflex threshold is reduced during capsaicin-induced central sensitization (Grönroos and Pertovaara, 1993; Andersen et al., 1995), as well as in patients with chronic pain (García-Larrea and François, 1990; Leroux et al., 1995; Desmeules et al., 2003; Banic et al., 2004; Coffin et al., 2004). However, it is still unclear whether reflex size is similarly affected by central sensitization (Andersen et al., 1995; Andersen et al., 1996; Arendt-Nielsen et al., 1994; Manresa et al., 2010). Moreover, noxious conditioning stimuli capable of inducing central sensitization may also trigger endogenous pain modulatory systems. Accordingly, HFS and capsaicin may simultaneously induce an area of secondary hyperalgesia surrounding their application sites and a global inhibition of nociceptive signal processing via the endogenous modulatory system.

More precise information on how central sensitization induced by different experimental pain models modulates RIII reflex variables and whether the effect of the endogenous modulatory system activated by heterotopic noxious conditioning stimulation affects RIII modulation may be useful to improve the use of this nociceptive reflex as an objective biomarker of central sensitization.

In this neurophysiological study in healthy humans, we verified how central sensitization induced by HFS and topical capsaicin application modulates pain threshold, RIII reflex threshold and RIII reflex size. We also tested whether the endogenous modulatory system, as assessed by the modulation of a heterotopic noxious test heat stimulus influences RIII reflex changes due to central sensitization.

## 2. Methods

### 2.1. Participants

We consecutively enrolled 26 healthy subjects (mean age 30.7 years, range 26–38 years; 16 males) without chronic pain disorders, symptoms or signs of peripheral or central nervous system disorders or other medical conditions, drug intake in the past two weeks, dermatological disorders or skin lesions affecting the area of capsaicin application, jet lag, irregular working hours, sleep restrictions in the last week, or past drug abuse. Participants were asked to refrain from caffeine, nicotine, and alcohol for at least 4 hours before their arrival at the laboratory.

### 2.2. Study protocol

Written informed consent was obtained from all participants. This study was approved by the local institutional review board (Rif. 2020/03) and performed according to the Declaration of Helsinki regarding the use of humans in experimental studies.

To investigate how central sensitization modulates nociceptive RIII flexion reflex variables, we compared three main RIII outcome variables, namely the pain threshold, the RIII reflex threshold and RIII reflex size before and after the induction of secondary hyperalgesia (corresponding to about 15 minutes after the HFS and capsaicin application). In experiment 1, secondary hyperalgesia was induced in 14 participants with HFS. In experiment 2, secondary hyperalgesia was induced in 12 participants with topical capsaicin preceded by heating. In both experiments, we verified whether the endogenous modulatory system influenced the effect of HFS and capsaicin on the RIII reflex (Fig. 1); more specifically, we tested whether the two experimental pain models induced potential heterotopic changes in pain sensitivity.

### 2.3. High-frequency electrical stimulation

We induced secondary hyperalgesia in 14 participants using HFS. We delivered HFS with a constant current electrical stimulator (DS5, Digitimer, UK) using a dedicated electrode. The multipin electrode has been designed to preferentially activate cutaneous nociceptors (HFS Electrode "EPS-P10", MRC Systems GmbH, Heidelberg, Germany) composed of a rectangular anode (area: 24x20 mm2) and a cathode (diameter: Ø = 21 mm) equipped with 10 needle pins arranged on a circle with a diameter of 5 mm. The anode was covered by a gel cushion. HFS was applied to the lateral side of the foot below the lateral malleolus, in the sural nerve territory. We determined the electrical detection threshold, defined as the lowest intensity at which subjects detected a single square wave electrical pulse, with the method of limits. The geometric mean of three suprathreshold and three subthreshold values corresponded to the detection threshold. If subjects declared any irradiating sensation along the foot, indicating an electrode placement directly above the nerve branches, the position of the device was promptly changed. Similarly, the blood vessel course was avoided. The HFS protocol consisted of five trains of electrical pulses delivered at 100 Hz (pulse width: 2 ms). Each train lasted 1 s, with a 10-s interval between each train. Stimulation intensity was set to 20x the detection threshold and subjects were asked to rate each single train on a 0–100 numerical rating scale (NRS). We then calculated the average pain magnitude of the five electrical train pulses. The active site was counterbalanced between subjects.

### 2.4. Capsaicin and pre-heating conditioning

We induced secondary hyperalgesia in 12 participants using topical application of capsaicin on the lateral part of the foot, in the sural nerve territory, preceded by heating. Using a thermode (QST.Lab, Strasbourg, France) with a flat surface area of 160 $mm^2$ and a heating ramp of 50 °C/s, we heated the area to be treated with capsaicin (i.e., the lateral part of the foot below the lateral malleolus), with a target temperature of 47–50 °C, according to the subject's tolerance, for 5 min (Dirks et al., 2000). On the same heated area, we then applied a commercially available capsaicin

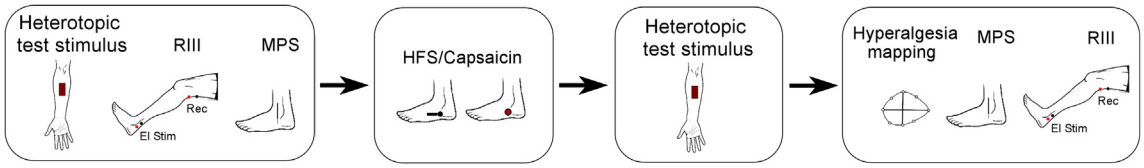


**Fig. 1.** Experimental procedures. The RIII reflex, perception of the heterotopic noxious test heat stimulus, and mechanical pain sensitivity were collected before and after high frequency stimulation and capsaicin topical application. MPS: mechanical pain sensitivity; El Stim: electrical stimulation of the sural nerve; Rec: recording on the biceps femori; HFS: high-frequency stimulation.

cream (2.5% capsicum oleoresin corresponding to 0.1% capsaicin associated with inactive ingredients) for 30 min. The induction side varied randomly among participants.

### 2.5. Mapping and rating hyperalgesia

We mapped the area of increased sensory perception with a calibrated 128-mN pinprick probe (MRC Systems GmbH, Heidelberg, Germany). Participants were asked to close their eyes and report when the pinprick sensation was felt different, i.e., more intense or associated with a burning sensation. Stimuli were applied starting from the neutral areas and proceeding toward the centre of the primary hyperalgesia area along the four perpendicular radii. We measured the four radii in mm, marked the area, and calculated the geometric mean.

To assess the magnitude of pinprick hyperalgesia, we tested mechanical pain sensitivity with three calibrated pinprick probes (16, 64, and 256 mN, flat contact area of 0.2-mm diameter). For each participant, we obtained a stimulus–response function by rating pinprick-evoked pain related to each stimulation with a 0–100 NRS. We assessed dynamic mechanical allodynia with innocuous stimuli: a cotton wisp, a cotton wool tip (Q-Tip) fixed to an elastic strip, and a standardized brush (MRC Systems GmbH, Heidelberg, Germany). Participants rated stimulus intensity with a 0–100 NRS. Light touch stimuli were applied in a balanced test sequence intermingled with pinprick stimuli. Two consecutive stimuli were separated by at least 10 s to avoid a potential windup phenomenon.

### 2.6. Nociceptive flexion reflex recording

We recorded the nociceptive flexion RIII reflex on both sides using standard surface electrode recording from the short head of the biceps femoris muscle and stimulating the ipsilateral sural nerve at the lateral malleolus (Sandrini et al., 2005), in the area of secondary hyperalgesia. Subjects were seated in a comfortable position, with the limb completely relaxed, a knee flexion of 110-130°, and their eyes covered to prevent expectation. Before the start of the RIII measurement, participants were provided with explanations and instructions regarding the procedure. Electrical stimulation consisted of a train of five rectangular pulses with a duration of 0.5 ms each at a frequency rate of 200 Hz. Acquisition parameters were a screen sensitivity of 200 μV/division, an acquisition time base of 300 ms, a high-pass filter of 20 Hz and a low-pass of 2 KHz, and a sampling rate of 3000 Hz. The pain threshold was defined as the lowest stimulation intensity generating a painful sensation. The RIII threshold was defined as the stimulation intensity generating stable reflex responses at a rate of $\geq$ 60% after a series of 20 stimuli. To obtain the thresholds, we used a staircase of ascending and descending stimulus intensities with 0.5 mA steps. The stimulus intensity to record the RIII reflex corresponded to 1.5x the reflex threshold. We consecutively recorded 12 trials with an interstimulus interval that manually ranged between 10–25 s to avoid summation or habituation and asked subjects to rate pain perception in each trial on a 0–100 NRS. The EMG recording was then rectified, averaged, and stored offline. RIII size was calculated as the area under the curve (AUC) of the rectified EMG, in a time window between 70 and 160 ms. After secondary hyperalgesia induction, we re-defined the RIII reflex threshold and the pain threshold to verify any perception changes, but we kept the same stimulation intensity used before the sensitization process.

### 2.7. Assessment of the endogenous modulatory system

We investigated whether the endogenous modulatory system activated by HFS and capsaicin influenced the central sensitization-induced modulation of RIII variables. Using a Sense-Q Model, 2001-TSA, Analyzer Sensory T (Medoc, Israel), consisting of a 30x30 mm Peltier thermode, we have therefore measured the perception of a heterotopic noxious heat stimulus before and after HFS and capsaicin.

We used a sequential paradigm (Kennedy et al., 2016). Noxious test stimuli were delivered before and immediately after the conditioning stimuli, i.e. HFS and capsaicin application. Contact heat applied to the right/left volar forearm (contralateral to secondary hyperalgesia) served as the heterotopic test stimulus. Test stimulus intensity was predetermined individually for each participant based on the psychophysical parameter of "pain-60": using the method of limits with a baseline temperature of 32 °C and an increase rate of 2 °C/s, we asked each subject to press a stop button as soon as they perceived a painful sensation with an intensity of at least 60 on a 0–100 NRS for three consecutive trials (Leone and Truini, 2019). The average of the three trials was considered the target temperature (pain-60) for the test stimulus.

Using the ramp and hold method, we first delivered two consecutive heat test stimuli using pain-60 as a target temperature. We then assessed the pain rating related to the test noxious stimulus after HFS- and capsaicin application. The difference in the average pain scores of the test stimuli before and after the two experimental pain models was used to evaluate the endogenous modulatory effect (negative values indicate an inhibitory effect of the endogenous modulatory system).

### 2.8. Statistical methods

Given that the main outcome variables were not normally distributed, as assessed with the Shapiro-Wilk normality test, we then used Wilcoxon's non-parametric rank sum test to compare variables before and after secondary hyperalgesia induction and the Mann-Whitney test to compare the area of secondary hyperalgesia induced by the two different experimental pain models. To verify whether one experimental model of central sensitization was more effective than the other, we performed a mixed effects analysis with time (pre *vs* post) and model (HFS *vs* capsaicin) as fixed effects for the three different outcomes: pain threshold, RIII reflex threshold and RIII reflex size. Results are presented as mean ± standard deviation (SD) in the text and mean ± SD and median and interquartile ranges in tables. We also calculated the normalized ratios of the RIII reflex variables before and after HFS

and capsaicin between the lower limb receiving HFS/capsaicin and the control lower limb (Supplementary Table 1).

A two-tailed significance level of 5% was considered statistically significant.

## 3. Results

### 3.1. Experiment 1

In all subjects, electrical stimulation of the sural nerve (19.7 ± 1 0.4 mA, 0.5 ms) evoked a reproducible RIII reflex on both sides.

HFS (9.5 ± 3.7 mA) evoked a pain rating of 94.5 ± 7.4, as assessed with the 0–100 NRS. HFS induced an area of secondary hyperalgesia in all participants, with a mean radius of 61 ± 11 mm (beyond the 20 mm radius of the HFS electrode) a 3.5-fold increase in mechanical pain sensitivity and mild dynamic mechanical allodynia (Table 1).

The perception rating related to the heterotopic test heat stimulus delivered on the contralateral volar forearm after HFS was significantly higher than that to the heat stimuli delivered on the volar forearm before HFS (6.3 ± 13.76; p = 0.027), (Supplementary Table 2).

In the lower limb, pain threshold and RIII reflex threshold significantly decreased after HFS (the pain threshold by 29% and the RIII reflex threshold by 20%), (p < 0.05; Table 1). Conversely, RIII reflex size expressed as the AUC did not significantly differ (Fig. 2). In the control lower limb, RIII reflex variables did not change significantly after HFS (Table 1).

The normalized ratio between the RIII reflex variables changes between the lower limb receiving HFS and the control lower limb further showed that HFS reduced the pain threshold and the RIII reflex threshold and left unchanged the RIII reflex size (Supplementary Table 1).

### 3.2. Experiment 2

In all subjects, electrical stimulation of the sural nerve (18.75 ± 8.38 mA, 0.5 ms) evoked a reproducible RIII reflex on both sides.

Capsaicin application evoked a pain rating of 14.3 ± 9.1, as assessed with the 0–100 NRS. The area of capsaicin-induced secondary hyperalgesia had a mean radius of 44 ± 10 mm. Capsaicin induced an increase of 2.9-fold the mechanical pain sensitivity and mild dynamic mechanical allodynia. Pain did not persist after capsaicin removal in any of the subjects.

Pain ratings to heterotopic test heat stimulus delivered on the contralateral volar forearm after capsaicin application were similar to those in response to heat stimuli delivered on the volar forearm before capsaicin application (-1.4 ± 3.7), (Supplementary Table 2).

In the lower limb receiving capsaicin, pain threshold and RIII reflex threshold significantly decreased (the pain threshold by 17% and the RIII reflex threshold by 18%), (p < 0.05). Conversely, RIII reflex size expressed as the AUC did not significantly differ (Fig. 3). In the control lower limb, RIII reflex variables did not change after capsaicin application (Table 2).

The normalized ratio between the RIII reflex variables changes between the lower limb receiving capsaicin and the control lower limb further showed that capsaicin reduced the pain threshold and RIII reflex threshold and slightly increased the RIII reflex size (Supplementary Table 1).

Although HFS was associated with a higher pain rating and a larger area of secondary hyperalgesia than capsaicin (p < 0.01), the two experimental procedures induced similar changes in the different RIII variables (both procedures similarly reduced pain threshold and RIII reflex threshold and left RIII reflex size unaffected), with no significant advantage of one experimental model over the other (Supplementary Table 3).

## 4. Discussion

In this neurophysiological study in healthy humans, we showed that HFS- and capsaicin-induced central sensitization reduced pain threshold and RIII reflex threshold after stimulation of the sural nerve in the area of secondary hyperalgesia. However, both experimental pain models left RIII reflex size unaffected. The endogenous modulatory system did not produce any inhibitory effect on the central sensitization-induced modulation of the RIII reflex variables (while capsaicin did not change the perception to the heterotopic noxious test heat stimulus, HFS tended to slightly increase the perception to the heterotopic noxious test heat stimulus).

In this study, we used HFS and capsaicin to test how central sensitization modulates different RIII reflex variables (Quesada and Kostenko, 2021). While HFS activates all cutaneous nociceptors, consistent with a non-specific activation of cutaneous nerve fibres through electrical stimulation, capsaicin specifically activates C nociceptors through the TRPV receptor (Henrich et al., 2015). Although both experimental pain models are widely used to induce central sensitization in human and animal studies, the methodologies reported in literature vary (Quesada and Kostenko, 2021). In this study, we applied HFS using a recent paradigm consisting of five trains of stimulation with a frequency of 100 Hz and an intensity 20x the detection threshold since this paradigm leads to more robust secondary hyperalgesia (Klein et al., 2004). To increase the efficacy of topical capsaicin at the lower limb, we used a pre-heating of the area (Dirks et al., 2000).

In our experiments, we tested whether the RIII reflex consistently reflected dorsal horn excitability changes during central sensitization. Accordingly, we measured different RIII reflex variables before and after HFS- and capsaicin-induced central sensitization. We found that the pain threshold and the RIII reflex threshold significantly decreased during central sensitization. This finding is in line with previous human studies showing that different experimental pain models producing secondary hyperalgesia (Grönroos and Pertovaara, 1993), as well as clinical conditions presumably associated with central sensitization (García-Larrea and François, 1990; Desmeules et al., 2003; Banic et al., 2004; Coffin et al., 2004), modulate the RIII reflex. However, we showed that RIII reflex size did not change during HFS- and capsaicin-induced central sensitization. Previous studies have reported conflicting evidence on how HFS- and capsaicin-induced central sensitization influence RIII reflex size (Andersen et al., 1995; Andersen et al., 1996; Manresa et al., 2010). While some studies have reported that central sensitization alone does not increase RIII reflex size (Andersen et al., 1995; Andersen et al., 1996;), others have shown that central sensitization induced by continuous low-frequency stimulation (Manresa et al., 2010) or concomitant thermal stimulation (Andersen et al., 1995) increases RIII reflex size, thus indicating that the modulation of this variable likely requires ongoing somatosensory afferent summation to the dorsal horn. Accordingly, we did not find any RIII reflex size modulation probably because HFS and topical capsaicin elicited short-term painful sensations and did not trigger ongoing afferent summation.

Our human data, showing that capsaicin-induced secondary hyperalgesia modulates RIII reflex variables agree with experimental data in monkeys demonstrating spinal central sensitization due to capsaicin (Simone, 1991); in this study we also provide the previously unreported data that HFS induces a similar effect.

Using an additional heterotopic noxious test heat stimulus, we provide previously unreported findings on whether HFS and capsaicin trigger the endogenous modulatory system. Previous studies

**Table 1**
HFS modulation of RIII reflex variables.

| | Active side | | | | Control side | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | Post | p | Effect size* | Baseline | Post | p | Effect size* |
| Reflex Threshold (mA) | 13.8 (7.1)<br>13.5 (6.9;18) | 11 (5.9)<br>10.2 (4.2;16.8) | **0.02** | 0.43 | 12.1 (6.5)<br>12.2 (5.8;17.3) | 11.6 (5.5)<br>12 (5.62;14.5) | 0.2 | - |
| Pain Threshold (mA) | 12.3 (5.3)<br>12 (6.8;18) | 8.8 (5.3)<br>7.8 (4.2;11.5) | **0.001** | 0.66 | 11.9 (5.9)<br>12 (6.8;16.3) | 11.6 (5.9)<br>12 (5.6;15.5) | 0.5 | - |
| NRS Pain** (0–100) | 56.5 (17.9)<br>59.5 (50;69.5) | 50.3 (22.9)<br>51 (38;68.5) | 0.2 | - | 56.1 (18.9)<br>59 (46;69.5) | 54.1 (21.42)<br>57 (35;64.5) | 0.2 | - |
| AUC (AUC units) | 20.6 (14.1)<br>19.6 (10.4;25.3) | 17.9 (12.4)<br>15.43 (6.9;25) | 0.3 | - | 19.2 (12.22)<br>19.5 (9.8;21.93) | 17.2 (13.08)<br>14.8 (6.8;21.6) | 0.2 | - |
| MPS (0–100) | 4.2 (4.9)<br>2.97 (0.34;5.18) | 14.6 (13.9)<br>8.69 (5.1;21.1) | **0.0001** | 0.99 | - | - | - | - |
| DMA (0–100) | 0.1 (0)<br>0.1 (0.1;0.1) | 1.7 (5.46)<br>0.3 (0.1;0.49) | **0.003** | 0.4 | - | - | - | - |

Each value is expressed as Mean (SD) (1st line) and Median (25;75 IRQ) (2nd line)
NRS: Numerical Rating Scale; AUC: area under the curve as measured with arbitrary units; MPS: mechanical pain sensitivity; DMA: dynamic mechanical allodynia.
* Cohen's effect size.
** Pain to electrical stimulation.

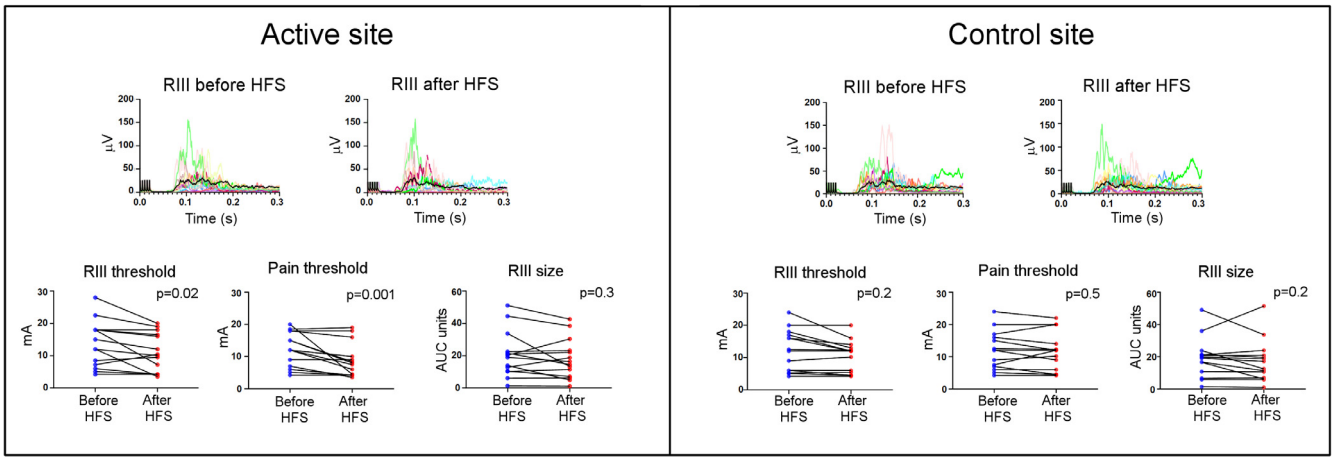


**Fig. 2.** RIII reflex modulation induced by high-frequency stimulation. Upper panel: RIII reflex recording before and after high-frequency stimulation (HFS)-induced secondary hyperalgesia. The different averaged recordings for each subject are superimposed and represented by different colours; black traces correspond to the grand average. Lower panel: graphs representing intra-individual differences in the three main outcome variables (pain threshold, RIII reflex threshold, RIII reflex size amplitude) before and after secondary hyperalgesia induction. AUC: area under the curve as measured with arbitrary units.

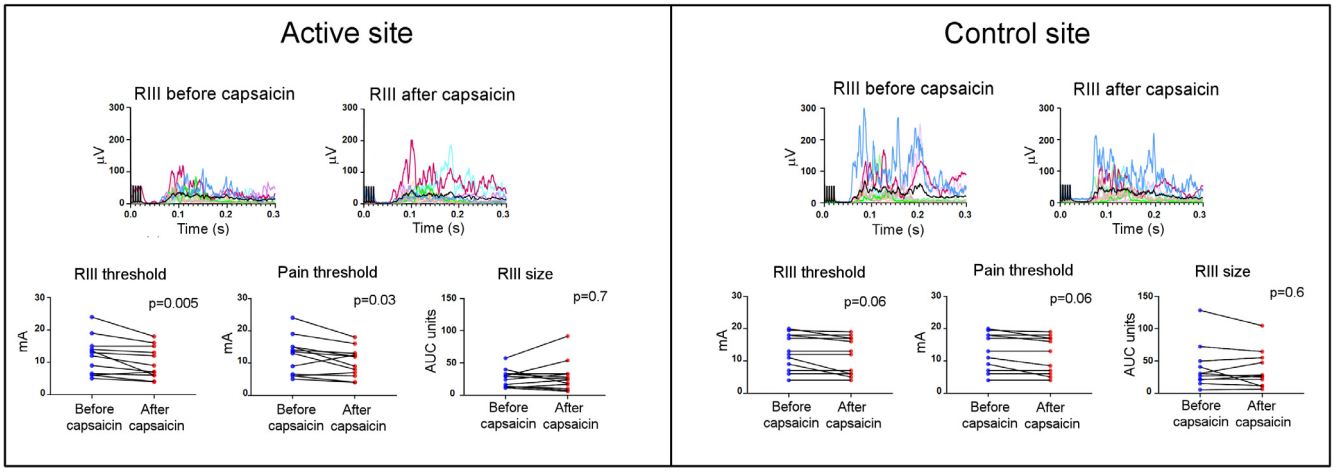


**Fig. 3.** RIII reflex modulation induced by capsaicin. Upper panel: RIII reflex recording before and after capsaicin-induced secondary hyperalgesia. The different averaged recordings for each subject are superimposed and represented by different colours; black traces correspond to the grand average. Lower panel: graphs representing intra-individual differences in the three main outcome variables (pain threshold, RIII reflex threshold, RIII reflex size amplitude) before and after secondary hyperalgesia induction. AUC: area under the curve as measured with arbitrary units.

**Table 2**
Capsaicin modulation of RIII reflex variables.

| | Active side | | | | Control side | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | Post | p | Effect size* | Baseline | Post | p | Effect size* |
| Reflex Threshold (mA) | 11.9 (5.8)<br>12.5 (6.1;14.8) | 9.8 (4.8)<br>8 (6;14.5) | **0.005** | 0.39 | 12.9 (5.6)<br>12.5 (7.5;18) | 11.7 (5.7)<br>12.5 (6;17) | 0.062 | - |
| Pain Threshold (mA) | 12.2 (5.9)<br>13.25 (6.1;15) | 10.1 (4.5)<br>10.5 (6.3;12.9) | **0.033** | 0.4 | 12.9 (5.9)<br>13 (7;18) | 11.9 (5.8)<br>13 (6;17) | 0.062 | - |
| NRS Pain** (0–100) | 47.2 (11.4)<br>45.7 (38.7;55.8) | 47.6 (23.5)<br>48 (29.1;64.3) | 0.764 | - | 48.1 (15.7)<br>46.5 (34.1;62.2) | 45.9 (9.7)<br>47.08 (41;49) | 0.622 | - |
| AUC (AUC units) | 26.2 (13.9)<br>26.6 (13.6;33.1) | 28.9 (23.7)<br>24.6 (11.7;33.0) | 0.733 | - | 40.2 (34.41)<br>30.48 (20.8;49.8) | 36.5 (29.4)<br>26.8 (11.66;55) | 0.577 | - |
| MPS (0–100) | 1.4 (2.2)<br>0.5 (0.23;1.9) | 4.0 (5.8)<br>2.3 (0.49;3.3) | **0.001** | 0.59 | - | - | - | - |
| DMA (0–100) | 0.12 (0.09)<br>0.1 (0.1;0.1) | 5.3 (5.79)<br>3.38 (0.1;15.84) | **0.015** | 1.26 | - | - | - | - |

Each value is expressed as Mean (SD) (1st line) and Median (25;75 IRQ) (2nd line).
NRS: Numerical Rating Scale; AUC: area under the curve as measured with arbitrary units; MPS: mechanical pain sensitivity; DMA: dynamic mechanical allodynia.
* Cohen's effect size.
** Pain to electrical stimulation.

have suggested that widespread effects of the endogenous modulatory system might interfere with the facilitation effect of HFS and capsaicin on RIII (Manresa et al., 2010). In this study we found that HFS and capsaicin did not reduce the perception of the heterotopic noxious heat stimuli on the contralateral volar forearm thus indicating that these two experimental pain models did not trigger any inhibitory effect of the endogenous modulatory system. This finding is further supported by the lack of effect on the RIII reflex variables in the control lower limb. Conversely, we found that after HFS the perception of the heterotopic noxious heat stimuli was slightly increased, thus suggesting that HFS might have a facilitatory effect on pain processing. This hypothesis is in line with a previous study that demonstrated that limb HFS has a complex effect on pain processing, including remote facilitatory processes of trigeminal nociceptive responses (Vo and Drummond, 2014). Conversely, in our study, capsaicin failed to induce a diffuse facilitation. We may hypothesize that HFS triggers facilitatory effects due to a massive short-lasting nociceptor activation (conversely, capsaicin activates peptidergic fibres only). However, the low capsaicin cream concentration and the topical application we used probably affected the capsaicin efficacy. Therefore, we believe that a reliable comparison of differences in diffuse facilitatory and inhibitory effects of the two experimental models is hardly tenable.

Although RIII reflex size did not change during HFS- and capsaicin-induced central sensitization, the pain threshold and the RIII reflex threshold were significantly reduced, thus indicating that these two RIII reflex variables consistently reflect dorsal horn excitability changes associated with central sensitization. Therefore, the RIII reflex might be reliably used to detect central sensitization in human studies and possibly investigate how medicinal products affect central sensitization. Since many analgesic drugs have failed when tested in clinical populations and since the associated costs of these translational failures are extremely high (Clark, 2016), the use of the RIII reflex in preliminary pharmacological trials might facilitate the identification and selection of promising drug candidates for chronic pain. Admittedly, the relatively small sample size of this study and the homogeneous age range of participants might hamper the generalizability of our findings. Further studies on a larger and heterogeneous cohort of subjects are needed to confirm our data.

We found that HFS induced a larger secondary hyperalgesia area than capsaicin, thus raising the possibility that HFS produces stronger central sensitization in the dorsal horn than topical capsaicin application. However, the two experimental pain models similarly modulated the different RIII reflex variables. This previously unreported finding might be useful in future pain research investigations since it provides evidence that the two experimental pain models may be used alternatively to investigate the effect of central sensitization. It is worth mentioning that although the topical capsaicin model is a less painful and more tolerable procedure, the HFS procedure is more reproducible and may therefore be more appropriate in multicentre studies.

Alternative neurophysiological techniques might be used to test central sensitization. Previous studies have demonstrated pinprick-evoked potential amplitude changes after stimulation of the secondary hyperalgesia area (Iannetti et al., 2013; van den Broeke and Mouraux, 2014, 2015, 2019). However, the RIII reflex is an easy-to-record neurophysiological response that is more suitable in lower limb investigations than the pinprick-evoked potentials.

Our study aiming at testing whether the descending modulatory system may affect RIII variables reflecting central sensitization might concur to improve our understanding on how endogenous modulatory system impairment in patients with chronic pain is associated with central sensitization. Previous studies showed that in different conditions central sensitization is accompanied by concurrent endogenous pain modulatory system abnormalities (Leone and Truini, 2019). Accordingly, patients with widespread pain, exhibiting hyperalgesia and decreased inhibition have a "pro-nociceptive" pattern of pain modulation (Di Stefano et al., 2016; Leone et al., 2020). In line with these clinical observations, in our study we found that HFS induced central sensitization and triggered a diffuse "pro-nociceptive" facilitation, thus possibly mimicking chronic pain conditions.

## 5. Conclusions

In this neurophysiological study, we showed that HFS induced- and topical capsaicin-induced central sensitization similarly modulate the RIII reflex. While pain threshold and RIII reflex threshold consistently reflected dorsal horn excitability changes during central sensitization, RIII reflex size was unaffected. We found no evidence that the endogenous modulatory system triggered by HFS and capsaicin may have concomitant inhibitory effects on the central-sensitization-induced RIII reflex changes. Our findings support the use of the RIII reflex in investigating central sensitization in humans.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2021.08.018.

## References


Andersen OK, Gracely RH, Arendt-Nielsen L. Facilitation of the human nociceptive reflex by stimulation of Aβ-fibres in a secondary hyperalgesic area sustained by nociceptive input from the primary hyperalgesic area. Acta Physiol Scand 1995;155:87–97.

Andersen OK, Felsby S, Nicolaisen L, Bjerring P, Jensen TS, Arendt-Nielsen L. The effect of Ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin – a double-blind, placebo-controlled, human experimental study: Pain 1996;66:51–62.

Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, Wells C, Bouhassira D, Mohr Drewes A. Assessment and manifestation of central sensitisation across different chronic pain conditions. Eur J Pain 2018;22(2):216–41.

Arendt-Nielsen L, Brennum J, Sindrup S, Bak P. Electrophysiological and psychophysical quantification of temporal summation in the human nociceptive system. Eur J Appl Physiol Occup Physiol 1994;68(3):266–73.

Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, Curatolo M. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. Pain 2004;107:7–15.

Benrath J, Brechtel C, Stark J, Sandkühler J. Low dose of S+-ketamine prevents long-term potentiation in pain pathways under strong opioid analgesia in the rat spinal cord in vivo. Br J Anaesth 2005;95(4):518–23.

Clark JD. Preclinical Pain Research: Can We Do Better? Anesthesiology 2016;125:846–9.

Coffin B, Bouhassira D, Sabaté JM, Barbe L, Jian R. Alteration of the spinal modulation of nociceptive processing in patients with irritable bowel syndrome. Gut 2004;53:1465–70.

Dahl JB, Brennum J, Arendt-Nielsen L, Jensen TS, Kehlet H. The effect of pre- versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin. Pain 1993;53:43–51.

Desmeules JA, Cedraschi C, Rapiti E, Baumgartner E, Finckh A, Cohen P, Dayer P, Vischer TL. Neurophysiologic Evidence for a Central Sensitization in Patients with Fibromyalgia. Arthritis Rheum 2003;48(5):1420–9.

Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. The effect of systemic lidocaine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Anesth Analg 2000;91(4):967–72.

Di Stefano G, Celletti C, Baron R, Castori M, Di Franco M, La Cesa S, Leone C, Pepe A, Cruccu G, Truini A, Camerota F. Central sensitization as the mechanism underlying pain in joint hypermobility syndrome/Ehlers-Danlos syndrome, hypermobility type. Eur J Pain 2016;20(8):1319–25.

García-Larrea L, François M. Electrophysiological Assessment of Nociception in Normals and Patients: The Use of Nociceptive Reflexes. Electroencephalogr Clin Neurophysiol Suppl 1990;41:102–18.

Grönroos M, Pertovaara A. Capsaicin-Induced Central Facilitation of a Nociceptive Flexion Reflex in Humans. Neurosci Lett 1993;159(1-2):215–8.

Henrich F, Magerl W, Klein T, Greffrath W, Treede R-D. Capsaicin-Sensitive C- and A-Fibre Nociceptors Control Long-Term Potentiation-like Pain Amplification in Humans. Brain 2015;138(9):2505–20.

Iannetti GD, Baumgärtner U, Tracey I, Treede RD, Magerl W. Pinprick-Evoked Brain Potentials: A Novel Tool to Assess Central Sensitization of Nociceptive Pathways in Humans. J Neurophysiol 2013;110(5):1107–16.

Jankowska E. Interneuronal relay in spinal pathways from proprioceptors. Prog Neurobiol 1992;38(4):335–78.

Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: a systematic review. Pain 2016;157(11):2410–9.

Klein T, Magerl W, Hopf HC, Sandkühler J, Treede RD. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. J Neurosci 2004;24:964–71.

Koltzenburg M, Lundberg LE, Torebjörk HE. Dynamic and static components of mechanical hyperalgesia in human hairy skin. Pain 1992;51:207–19. https://doi.org/10.1016/0304-3959(92)90262-a. Erratum. In: Pain 1993;53:363.

LaMotte RH, Shain CN, Simone DA, Tsai EF. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 1991;66(1):190–211.

Leone CM, Celletti C, Gaudiano G, Puglisi PA, Fasolino A, Cruccu G, Camerota F, Truini A. Pain due to Ehlers-Danlos Syndrome Is Associated with Deficit of the Endogenous Pain Inhibitory Control. Pain Med 2020;21:1929-1935.

Leone C, Truini A. The CPM Effect: Functional Assessment of the Diffuse Noxious Inhibitory Control in Humans. J Clin Neurophysiol 2019;36:430-436.

Leroux A, Bélanger M, Boucher JP. Pain effect on monosynaptic and polysynaptic reflex inhibition. Arch Phys Med Rehabil 1995;76(6):576–82.

Liu X-G, Sandkühler J. Characterization of long-term potentiation of C-fiber-evoked potentials in spinal dorsal horn of adult rat: essential role of NK1 and NK2 receptors. J Neurophysiol 1997;78(4):1973–82.

Manresa JAB, Mørch CD, Andersen OK. Long-term facilitation of nociceptive withdrawal reflexes following low-frequency conditioning electrical stimulation: A new model for central sensitization in humans. Eur J Pain 2010;14:822–31.

Meinck H-M, Piesiur-Strehlow B, Koehler W. Some principles of flexor reflex generation in human leg muscles. Electroencephalogr Clin Neurophysiol 1981;52(2):140–50.

Quesada C, Kostenko A, et al. Human surrogate models of central sensitization: A critical review and practical guide. European Journal of pain 2021;25(7):1389–428. https://doi.org/10.1002/ejp.1768.

Raja SN, Campbell JN, Meyer RA. Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. Brain 1984;107(4):1179–88.

Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. The lower limb flexion reflex in humans. Prog Neurobiol 2005;77(6):353–95.

Shahani BT, Young RR. Human flexor reflexes. J Neurol Neurosurg Psychiatry 1971;34(5):616–27.

Simone DA et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991;66(1):228–46. https://doi.org/10.1152/jn.1991.66.1.228.

Treede R-D, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 1992;38(4):397–421.

van den Broeke EN, Mouraux A. High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. J Neurophysiol 2014;111(8):1564–73.

van den Broeke EN, Mouraux A, Groneberg AH, Pfau DB, Treede R-D, Klein T. Characterizing pinprick-evoked brain potentials before and after experimentally induced secondary hyperalgesia. J Neurophysiol 2015;114(5):2672–81.

van den Broeke EN, Van den Bergh O. Central sensitization in humans: Popular phrase or useful concept? J Psychosom Res 2019;119:51–2. https://doi.org/10.1016/j.jpsychores.2019.02.007.

Vo L, Drummond PD. Coexistence of ipsilateral pain-inhibitory and facilitatory processes after high-frequency electrical stimulation. Eur J Pain 2014;18(3):376–85.