Clinical Neurophysiology 156 (2023) 28–37

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Modulation of the spinal N13 SEP component by high- and low-frequency electrical stimulation. Experimental pain models matter

C. Leone [a,*], G. Di Pietro [a], Y. Salman [b], E. Galosi [a], G. Di Stefano [a], O. Caspani [c], L. Garcia-Larrea [d], A. Mouraux [b], R.-D. Treede [c], A. Truini [a]

[a] Department of Human Neuroscience, Sapienza University of Rome, Italy
[b] Université Catholique de Louvain, Institute of Neuroscience (IoNS), Faculty of Medicine, Bruxelles, Belgium
[c] Department of Neurophysiology, Center for Biomedicine and Medical Technology Mannheim (CBTM), Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany
[d] Lyon Neurosciences Center Research Unit Inserm U 1028, Pierre Wertheimer Hospital, Hospices Civils de Lyon, Lyon 1 University, Lyon, France

HIGHLIGHTS

- High and low frequency stimulation have a different effect on the N13 SEP component.
- Dorsal horn excitability changes induced by low frequency stimulation can modulate the N13.
- Different experimental models of central sensitization may induce dorsal horn excitability changes through different mechanisms.

ARTICLE INFO

Article history:
Accepted 31 August 2023
Available online 6 October 2023

Keywords:
Central sensitization
Pain biomarker
Neuropathic pain
Experimental pain models

ABSTRACT

Objective: The N13 component of somatosensory evoked potential (N13 SEP) represents the segmental response of cervical dorsal horn neurons. Neurophysiological studies in healthy participants showed that capsaicin-induced central sensitization causes an increase of the N13 SEP amplitude. Consequently, in human research, this spinal component may serve as a valuable readout of central sensitization.

In this study, we wanted to verify if the sensitivity of the N13 SEP for detecting central sensitization is consistent across different experimental pain models inducing central sensitization and secondary hyperalgesia, namely high and low-frequency electrical stimulation (HFS and LFS).

Methods: In 18 healthy participants, we recorded SEP after bilateral ulnar nerve stimulation before and after secondary hyperalgesia was induced through HFS and LFS applied on the ulnar nerve territory of the hand of one side. The area of secondary hyperalgesia was mapped with a calibrated 128-mN pinprick probe, and the mechanical pain sensitivity with three calibrated 16–64-256-mN pinprick probes.

Results: Although both HFS and LFS successfully induced secondary hyperalgesia only LFS increased the amplitude of the N13 SEP.

Conclusions: These findings suggest that the sensitivity of the N13 SEP for detecting dorsal horn excitability changes may critically depend on the different experimental pain models.

Significance: Our results indicate that LFS and HFS could trigger central sensitization at the dorsal horn level through distinct mechanisms, however this still needs confirmation by replication studies.

1. Introduction

Central sensitization is 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/resources/terminology/).

Although central sensitization is thought to be a key mechanism underlying many chronic pain conditions, it cannot be directly measured in humans but only assessed by indirect proxies (Arendt-Nielsen et al., 2018).

Many attempts have been made to find a reliable neurophysiological measure of the spinal cord dorsal horn excitability changes induced by secondary hyperalgesia models (Andersen et al., 1996, 1995; Biurrun Manresa et al., 2014; Grönroos and Pertovaara, 1993; Leone et al., 2021; Linde et al., 2021; Ellrich and Treede 1998; Guekos et al., 2022; Manresa et al., 2010; Di Lionardo et al., 2021) with conflicting results. The heterogeneity of evidence

* Corresponding author at: Department of Human Neuroscience, Sapienza University of Rome, Italy.
E-mail addresses: caterinamaria.leone@gmail.com, caterina.leone@uniroma1.it (C. Leone).

https://doi.org/10.1016/j.clinph.2023.08.022
1388-2457/

provided by neurophysiological studies on this topic might be attributable to the different human experimental pain models used for inducing central sensitization and secondary hyperalgesia, which may act differently on dorsal horn excitability (Quesada et al. 2021; Manresa et al. 2010).

The lone instance when both spinal neurons' input and output have been documented in the same model is the phenomenon of secondary hyperalgesia induced by intradermal capsaicin, which fulfils the definition of central sensitization (Treede et al., 2016). However, at present we have more than a dozen available experimental pain models of secondary hyperalgesia to indirectly investigate central sensitization (Quesada et al. 2021). All of these experimental pain models are known to cause second order neurons in the dorsal horn to become sensitized, which results in a zone of secondary hyperalgesia surrounding the primary hyperalgesic area. The onset, duration, and magnitude of the area of secondary hyperalgesia, and the pharmacological profile, on the other hand, are different between these experimental pain models (Quesada et al. 2021). Therefore, they may affect dorsal horn excitability through different mechanisms.

Previous studies on the modulation of the nociceptive flexion reflex size provided evidence in support of this theory, showing that it is not equally modulated by spinal excitability induced by different experimental pain models of secondary hyperalgesia, namely high (100 Hz, 1 sec, 20x detection threshold) and low frequency electrical stimulation (1 Hz, 1000 sec, 10x detection threshold) (HFS and LFS) (Andersen et al., 1996; Manresa et al., 2010), and leading to the conclusion that a stimulation frequency within the primary afferents' physiological firing range, as is the case for LFS, is probably required to modulate the reflex size.

The N13 cervical component of somatosensory evoked potentials (SEP), mediated by non-nociceptive Aβ fibres and reflecting the response of dorsal horn neurons to non-noxious inputs (Desmedt and Cheron 1980; Mauguiere and Courjon 1981) has been indicated as a promising biomarker of central sensitization. The N13 SEP is sensitive to heterotopic noxious stimuli, and thus presumably reflects the activity of wide dynamic range neurons (Di Pietro et al., 2022); additionally, this spinal component is modulated by capsaicin-induced central sensitization (Di Lionardo et al., 2021). It follows that this neurophysiological measure might be used to quantify central sensitization in human studies.

However, it is still unclear whether the N13 SEP modulation is consistent across the various experimental pain models used for inducing central sensitization in humans. Having information on the N13 SEP consistency for detecting dorsal horn excitability changes might increase its reliability and usefulness as a central sensitization biomarker within the dorsal horn of the human spinal cord, thus concurring with the identification of a robust biomarker for more efficient analgesic drug development, in accordance with the standards provided by the European Medicines Agency for the clinical development of pharmaceuticals used to relieve pain.

In this neurophysiological study in healthy humans, our goal was to determine whether the N13 SEP is responsive to different experimental pain models that cause secondary hyperalgesia and central sensitization. To do so, we tested if the dorsal horn excitability changes induced by high- and low-frequency electrical stimulations modulate the amplitude of the N13 SEP.

2. Methods

2.1. Participants

We enrolled 18 healthy participants (7 M, aged 25 ± 4 years); none of them had a history of chronic pain disorders, peripheral or central nervous system illnesses, other medical conditions, drug use within the previous two weeks, jet lag, irregular work schedules, sleep deprivation within the previous week, or history of drug abuse. Of the 18 participants enrolled, ten underwent the two experimental sessions at least a week apart from each other; the other eight participants were equally split over one of the two sessions (4 HFS and 4 LFS).

All participants gave their informed consent. This study was approved by the local review board and was carried out in accordance with the Declaration of Helsinki governing the use of humans in experimental studies.

2.2. Experimental design

The study consisted of two experimental sessions (HFS and LFS), each lasting approximately 80 minutes. During each session, we recorded SEP after ulnar nerve stimulation of the right and left side before and after secondary hyperalgesia induction with HFS/LFS applied on the ulnar nerve territory of the hand of one side, namely a baseline and a post-HFS/LFS recording. The order of recordings and the side of secondary hyperalgesia induction were pseudo-randomized between participants. The two experimental sessions were designed taking into account the different time courses of the two experimental pain models (Klein et al. 2004; Manresa et al. 2010). HFS has a duration of 40 seconds and, after 10–15 minutes, induces an area of secondary hyperalgesia, which is maintained for at least 60 minutes (Klein et al. 2004). Conversely, LFS is a long-lasting continuous stimulation, with a duration of 16 minutes; immediately after the end of stimulation, it induces an area of secondary hyperalgesia, which rapidly decreases in 10 minutes (Manresa et al. 2010). In the HFS session, we tested the area of secondary hyperalgesia, and we recorded SEP 14 minutes after the electrical stimulation; in the LFS session, we recorded SEP immediately after electrical stimulation and tested the area of secondary hyperalgesia at the end of the SEP recording (Fig. 1).

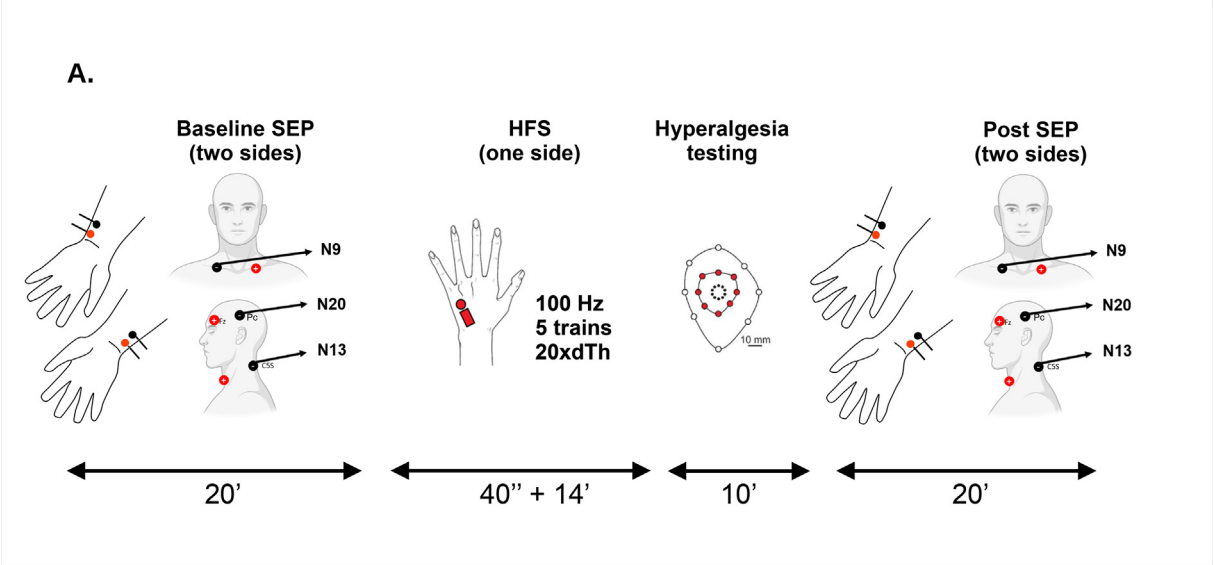
2.3. SEP recording

We recorded somatosensory evoked potentials after electrical stimulation of the ulnar nerve at the wrist (surface recording bar electrode; stimulus duration: 0.1 ms; stimulation frequency: 4 Hz; high-pass filter 2 Hz, low-pass filter 2 kHz; analysis time base: 50 ms; sampling rate: 2000 Hz).

The cathode was positioned over the distal ulnar nerve, 2 cm proximal to the wrist crease and the anode was positioned on the wrist crease. Intensity was kept constant between baseline and post-HFS/LFS recordings (7.9 ± 1.9 mA on the right side and 7.8 ± 1.8 on the left side) corresponding to the threshold for inducing a muscle twitch in ulnar nerve-innervated hand muscles.

Two blocks of 650 trials were collected, superimposed (to test reproducibility), and averaged. The participants were instructed to rest in a supine position while lying on a medical cot. By providing the greatest level of comfort, muscle artefacts were prevented. Occasionally occurring high-amplitude transients (>100 μV) were removed using automatic artefact rejection.

To record and measure the different SEP components we followed the International Federation of Clinical Neurophysiology guidelines. To record the peripheral (N9) component, electrodes (disposable surface electrodes 20x25) were placed over the Erb point bilaterally, 2–3 cm above the clavicle, within the angle created by the posterior border of the clavicular head of the sternocleidomastoid muscle and the clavicle. To record the dorsal horn N13 component we placed the recording surface electrode over the sixth cervical spinous process (C6), with an anterior cervical electrode serving as a reference on the midline skin surface of the supraglottic region (disposable surface electrodes 20x25mm). According to the 10–20 international system, N20 and P25 compo-



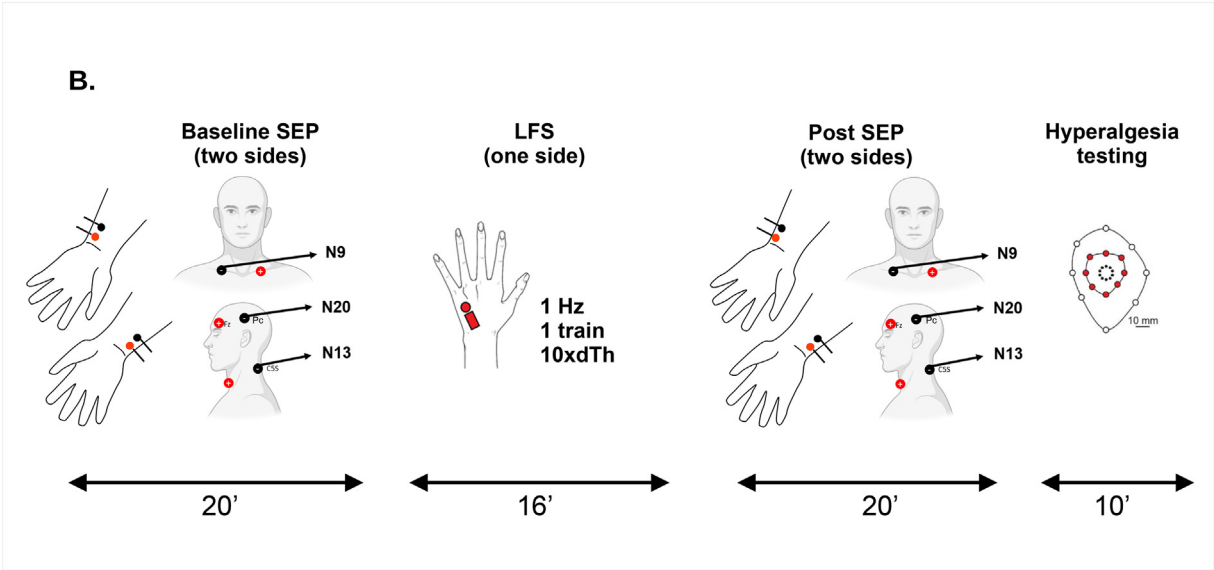


Fig. 1. Experimental design. High frequency stimulation (HFS) session (1A) and low frequency stimulation (LFS) session (1B). 1A) The HFS consisted of five trains of electrical pulses delivered at 100 Hz (pulse width: 2 ms). Stimulation intensity was set to 20x the detection threshold. Each train lasted 1 s, with a 10-s interval between each train (overall duration of stimulation around 40 seconds). Hyperalgesia was tested 14 minutes after stimulation. 1B) The LFS consisted of a single train of 1000 pulses (pulse width: 2 ms), delivered at 1 Hz (overall duration of stimulation around 16.7 min). Stimulation intensity was set to 10x the detection threshold. Hyperalgesia was tested at the end of somatosensory evoked potentials recording.

nents were recorded with a parietal scalp electrode (Pc) contralateral to the stimulation, placed 5 cm posterior to Cz and 7 cm lateral to the midline, with reference on Fz (AgAgCl cup electrodes). A wet velcro ground was placed on the stimulated arm. Impedance was kept below 3000 Ω. Epochs were averaged after automatic artefact rejection. The individual waveforms' amplitudes of the various SEP components were manually extracted: N9 amplitude was measured from zero volts baseline to peak; N13 was measured between the peaks of N13 and the preceding positivity (P9) and N20-P25 was measured between the peaks of N20 and the following positivity (P25) (Restuccia and Mauguiere 1991).

Both stimulating and recording electrodes were left in place for the entire duration of the experiment.

2.4. Secondary hyperalgesia induction: Electrode positioning

Secondary hyperalgesia was induced using two different experimental pain models: HFS and LFS. In both experimental pain models, the electrical stimulation was elicited with a constant current electrical stimulator (DS5, Digitimer, UK) using a customized electrode (HFS Electrode "EPS-P10", MRC Systems GmbH, Heidelberg, Germany). The cathode of this electrode consists of 10 blunt tungsten pins which are arranged on a circle with a diameter of 5 mm. Each pin has a diameter of 250 μm and protrudes by 0.8 mm from the base. The electrode has a rectangular anode measuring 24x20 mm2. The anode was covered by a gel cushion. HFS and LFS were applied to the lateral side of the hand dorsum in the ulnar nerve

territory. We determined the electrical detection threshold, i.e., the lowest intensity at which participants detected a single square wave electrical pulse. The first stimulus was applied with the intensity of 0,05 mA and if the participant did not feel anything, progressively stronger stimuli, increased by 0.05 mA, were applied. The first value that the participant felt was recorded, and the intensity of the next stimuli was reduced by 0.02 mA, until the participant didn't feel anything. Then, the intensity of the following stimuli was increased by 0.02 mA until the positive answer. The detection threshold was the geometric mean of three suprathreshold and three subthreshold values. The device's position was immediately changed if participants reported any radiating sensation along their hand, which would indicate that the electrode had been placed directly above nerve branches. Similar to that, blood vessel route was avoided.

2.5. Secondary hyperalgesia induction: HFS

We induced secondary hyperalgesia in 14 participants using HFS. The HFS was made up 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. Participants were asked to evaluate each individual train on a 0–100 numerical rating scale (NRS) with the stimulation intensity set to 20x the detection threshold (Leone et al. 2021). The subjects were pseudo-randomly assigned to the electrical stimulation side.

2.6. Secondary hyperalgesia induction: LFS

We induced secondary hyperalgesia in 14 participants using LFS. The LFS was made up of a single train of 1000 pulses, 2 ms pulse width, delivered at 1 Hz, i.e., with a total duration of around 16.7 min (Manresa et al. 2010). Stimulation intensity was set to 10x the detection threshold. Three times during the conditioning process, namely at the beginning, after 8 minutes of stimulation, and at the conclusion, participants were asked to evaluate the pain intensity on a 0–100 numerical rating scale (NRS). The participants were pseudo-randomly assigned to the electrical stimulation side.

2.7. Mapping and rating hyperalgesia

A calibrated 128-mN pinprick probe (MRC Systems GmbH, Heidelberg, Germany) was used to map the region of increased sensory perception on the electrical stimulation side. Participants were instructed to close their eyes and describe any changes in the pinprick feeling, i.e. more intense or coupled with a burning sensation. Eight radii of pinprick stimulation were delivered, starting from the neutral areas, and moving toward the centre of the primary hyperalgesia area (corresponding to the conditioning electrode's cathode). We measured the radii in cm, marked the area, and determined the geometric mean of the eight radii. Given the heterogeneous spreading of the area, the geometric mean compensates for the large fluctuations of the radii.

To assess the magnitude of pinprick hyperalgesia, we tested mechanical pain sensitivity with three weighted pinprick probes (16, 64, and 256 mN, flat contact area of 250 μm diameter) randomly applied on both test and contralateral side (contralateral site mirrored to the test site). We used a 0–100 NRS to rate the pain induced by pinpricks in relation to each stimulation. We provided the participants with the following instructions: "Please rate the painfulness of each stimulus by giving a number between "0" and "100". Any "sharp", "pricking", "stinging" or "burning" sensation should be defined as being painful and given a rating value above "0". You may also use decimals. "0" meaning: no pain, no "sharp", "pricking", "stinging" or "burning" sensation. "100" meaning: Most intense pain sensation imaginable."

We tested the presence of dynamic mechanical allodynia with tactile 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 evaluated stimulus intensity with a 0–100 NRS. Pinprick stimuli were interspersed with light touch stimuli in a balanced test sequence.

To calculate the hyperalgesia score, we added 0.1 to all ratings, then took the log 10. We then averaged together the log10-ratings obtained with the three different pinpricks (16, 64 and 256 mN) to obtain the arithmetic mean of each site, both test and contralateral, and subtracted the arithmetic mean of the contralateral site from the test site. A positive value indicated hyperalgesia.

The same procedure was separately applied to the mechanical allodynia score.

2.8. Statistical methods

Based on the effect size of interest (the N13 SEP amplitude change after secondary hyperalgesia induction, corresponding to Cohen's d 1.8; G-Power 3.0 α 0.05; β 0.95), we calculated the sample size of n = 14 participants needed to detect a difference in the N13 amplitude, comparable to that described in a previous study (Di Lionardo et al. 2021).

For each condition (HFS/LFS), descriptive summaries for the latencies and amplitudes of each SEP component (i.e., N9, N13, N20-P25) are shown as mean ± standard deviation (SD) (Table 1). Cohen's d is used to calculate effect sizes. The Shapiro-Wilk test was performed to evaluate the data distribution, and the results showed that our variables had a gaussian distribution.

One-way repeated measures analysis of variances (ANOVA) was used to compare pain ratings at the first minute, minute eight and at the end of LFS conditioning.

Two-way ANOVA, and post hoc multiple comparisons with Sidak's correction when significant, were used to assess the effect of time (before and after secondary hyperalgesia induction) and side (active vs. control) and their interaction on the amplitude of SEP components, separately for the two different human models of secondary hyperalgesia used.

T-test for unpaired data was used to compare the percentage of change pre vs post conditioning between HFS and LFS, to test if the two experimental pain models differently modulated the N13.

To test the intrinsic variability of the N13, we calculated the percentage of change in the two consecutive blocks of 650 stimuli. To test if the difference observed due to the conditioning model outclassed the intrinsic variability of the N13, we performed a t-test between the percentage of change between blocks and the percentage of change pre vs post conditioning.

A paired analysis was performed in the 10 participants who performed both sessions. We used the t-test to compare the area and the score of secondary hyperalgesia induced by the two experimental pain models.

All the tests were two-sided, and a p-value of 0.05 was considered statistically significant. All statistical analysis and plotting of data were performed in Prism 8.0 (GraphPad, CA, USA).

3. Results

The mean intensity of HFS and LFS was 7.8 ± 2 mA and 2.8 ± 0.7 mA, respectively. The pain rating for HFS was 80.14 ± 17.9; the pain ratings for LFS were 33.6 ± 12.3 at the first minute, 37.8 ± 12.8 at minute eight and 39.3 ± 18 at the end of the stimulation, with no significant difference across the three measures (ANOVA, F (1.603, 20.84) = 0.6966; p = 0.2974).

In all participants, HFS and LFS induced an area of secondary hyperalgesia. In the 18 participants, the mean radius of the area

Table 1
Descriptive summary of somatosensory evoked potential components pre and post conditioning.

	HFS				LFS			
	Active side		Control side		Active side		Control side	
	Baseline	After Conditioning	Baseline	After Conditioning	Baseline	After Conditioning	Baseline	After Conditioning
N9 latency (ms)	10.1 ± 0.7	10.2 ± 0.7	10.2 ± 0.7	10.1 ± 0.9	10.0 ± 0.9	10.1 ± 0.9	10.0 ± 0.9	9.5 ± 2.7
N13 latency (ms)	13.3 ± 1.0	13.3 ± 1.1	13.4 ± 1.1	13.5 ± 1.1	13.4 ± 1.2	13.4 ± 1.1	13.2 ± 1.2	13.4 ± 1.3
N20-P25 latency (ms)	19.3 ± 1.2	19.3 ± 1.3	19.2 ± 1.3	19.3 ± 1.2	19.4 ± 1.4	19.8 ± 1.6	19.1 ± 1.3	19.3 ± 1.3
N9 amplitude (μV)	2.15 ± 1.1	2.4 ± 1.0	1.89 ± 1.0	2.08 ± 0.8	1.96 ± 1.1	1.69 ± 0.8	1.54 ± 1.1	1.72 ± 1.2
N13 amplitude (μV)	1.32 ± 0.4	1.37 ± 0.4	1.24 ± 0.6	1.26 ± 0.3	1.14 ± 0.3	1.36 ± 0.4	1.0 ± 0.3	1.31 ± 0.3
N20-P25 amplitude (μV)	2.71 ± 0.9	2.77 ± 0.8	2.68 ± 0.8	2.67 ± 0.9	2.85 ± 0.7	2.92 ± 0.7	2.46 ± 0.8	2.32 ± 0.6

Data are expressed as mean ± SD.
HFS: High Frequency Stimulation; LFS: Low Frequency Stimulation.
Amplitude calculation: N9 baseline to peak; N13 peak to peak (P9-N13); N20 peak to peak (N20-P25).

Table 2
F-test p-values of the two-way repeated measures ANOVA for the amplitude of Somatosensory evoked potentials components for High and Low Frequency Stimulation.

HFS	N9 p-value	N13 p-value	N20-P25 p-value
Time	0.3694	0. 7113	0.8036
Treatment	0.3399	0.5217	0.8336
Time* Treatment	0.9040	0.9184	0.6962
LFS			
Time	0.7976	**<0. 0001***	0.7369
Treatment	0.6115	0.5294	0.0637
Time* Treatment	0.1616	0.5597	0.2838

HFS: High Frequency Stimulation; LFS: Low Frequency Stimulation.

was 3.09 ± 0.8 cm for HFS and 2.98 ± 1.18 cm for LFS and the hyperalgesia score was 0.64 ± 0.42 for HFS and 0.32 ± 0.26 for LFS. The analysis performed on paired data of 10 participants showed a significantly larger radius (3.29 ± 1.1 cm for HFS and 2.8 ± 1.1 cm for LFS) and a higher score (0.62 ± 0.45 for HFS and 0.23 ± 0.25 for LFS) of secondary hyperalgesia for the HFS compared to the LFS ($p = 0.037$, Cohen's d 0.436; $p = 0.048$, Cohen's d 1.06).

The results of the two-way repeated measures ANOVA are summarised in Table 2 and Fig. 2. When we analysed changes of the N13 SEP amplitude we found that the two-way repeated measures ANOVA showed a significant effect of time for the LFS model ($p < 0.0001$; T0-T1 post-hoc comparisons with Sidak's correction, active: $p = 0.0065$, Cohen's d 0.65; control: $p = 0.0008$, Cohen's d 0.99), no effect for the HFS model (Table 2) (Figs. 2 and 3), and no interaction between the two factors (Table 2). Individual traces are shown in Figs. 4 and 5. Neither LFS nor HFS modulated the amplitude of the other SEP components (N9 and N20-P25) (Table 2) (Fig. 2).

The unpaired t-test of the N13 percentage of change between HFS and LFS showed a different effect of the two experimental pain models, though only approaching significance ($p = 0.051$, Cohen's d 0.77).

The paired t-test between the N13 percentage of change between blocks and pre vs post conditioning, showed that the increase induced by LFS was significantly higher than the intrinsic variability of the N13 amplitude (t-test of pooled data active/control, $n = 28$; $p < 0.007$, Cohen's d 0.61). No significant difference was found for HFS (t-test of pooled data active/control, $p = 0.54$).

4. Discussion

In this study on human neurophysiology, we demonstrated that both HFS and LFS successfully induced secondary hyperalgesia; LFS increased the amplitude of the N13 SEP, while HFS failed to modulate the N13 SEP amplitude. These findings indicate that the sensitivity of N13 SEP for detecting dorsal horn excitability changes may critically depend on the different experimental pain model.

To learn more on the effect of central sensitization on the N13 SEP we used HFS and LFS, two widely agreed experimental pain models to induce secondary hyperalgesia in humans. We delivered HFS and LFS with an electrode composed of 10 blunt stainless-steel pins designed to specifically activate Aδ and C-fibres. Both experimental pain models are supposed to induce modification of synaptic strength at the dorsal horn level, which underlies central sensitization (Quesada et al., 2021; Klein et al., 2004). The choice to use different timing to assess secondary hyperalgesia in the two sessions was dictated by the different time courses of the two experimental pain models in inducing secondary hyperalgesia (see Methods). Neither of the two models induced pain to non-nociceptive stimulation (mechanical allodynia) nor pain outlasting the cessation of the stimulus. Both HFS and LFS were perceived as painful by all the participants. However, the hyperalgesia score was higher, and the area of secondary hyperalgesia was larger with HFS than LFS.

The larger area and the higher pain score of secondary hyperalgesia associated with HFS in comparison to those associated with LFS might reflect a different effect of the two experimental pain models at the dorsal horn level. We hypothesise that HFS, due to the high intensity of stimulation, may activate more intensely the high-threshold neurones population in the superficial laminae than LFS. In animals, these neurons are known to contribute to secondary hyperalgesia to pinprick stimuli, as testified by their significantly increased responses to a pinprick stimulation in the area of capsaicin-induced secondary hyperalgesia (Simone et al., 1991).

We found that while LFS significantly modulated the amplitude of the N13 SEP, HFS failed to change the amplitude of this spinal component of SEP. This finding is in line with a previous neurophysiological study that investigated the conditioning effect of HFS and LFS on the nociceptive withdrawal reflex and found that only LFS modulated the amplitude of this spinal reflex (Manresa et al., 2010). We have no simple explanations for the differential effect of HFS and LFS on the N13 SEP amplitude. We may speculate that LFS is a long-lasting continuous stimulation, which plausibly mimics the actual low-frequency, persistent nociceptive afferent barrage triggered by inflammation. These specific stimulation parameters (1000 stimuli at 1 Hz; Price 1972) may induce two different amplification phenomena affecting the N13 SEP. Besides inducing the dorsal horn excitability changes evidenced by the presence of secondary hyperalgesia, this experimental pain model may also trigger a concurring wind-up phenomenon due to its specific stimulation frequency. These two phenomena are independent (Magerl et al., 1998) and may then concur to the N13 SEP modulation. Conversely, HFS stimulus parameters are able to induce synaptic strength modifications at dorsal horn level, but they are not suitable for the induction of a wind-up phenomenon.

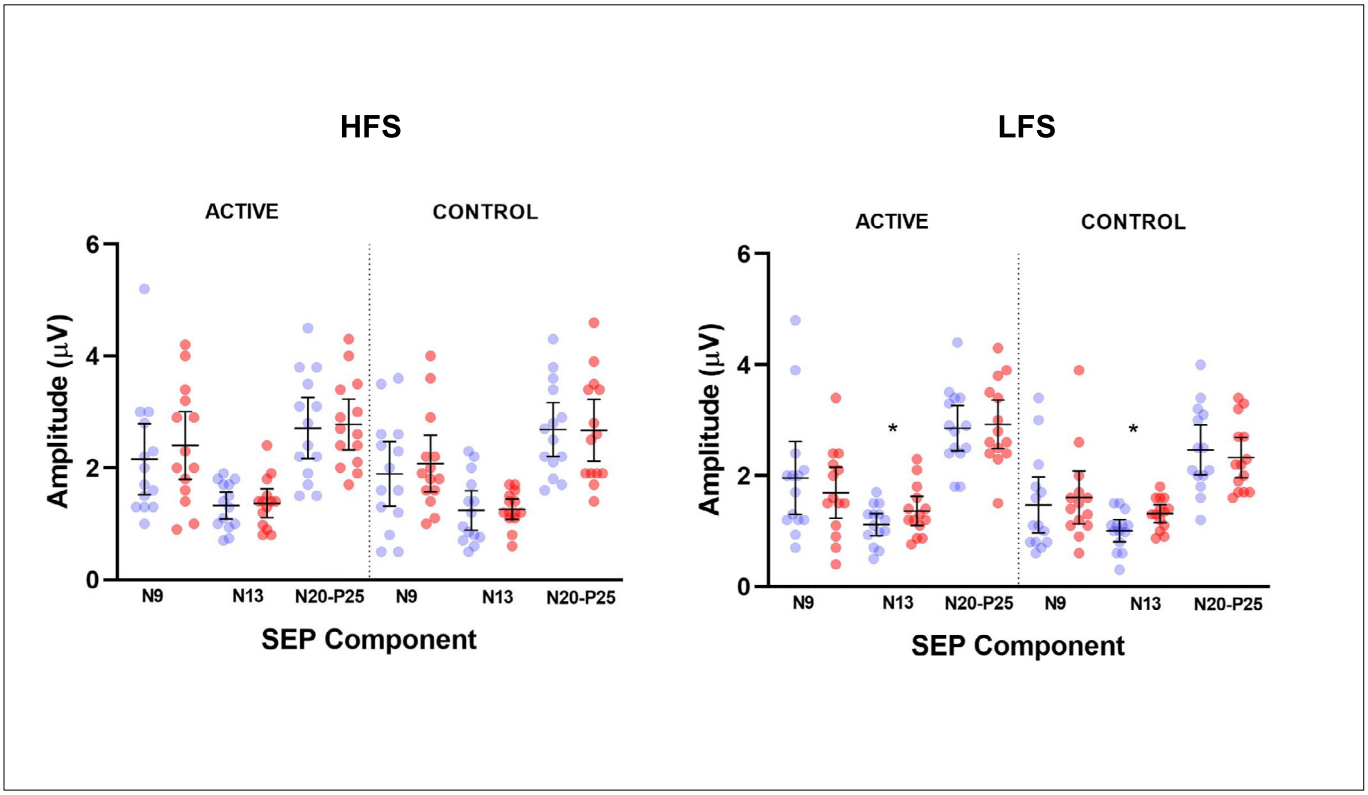


Fig. 2. Modulation of the N13 somatosensory evoked potential (SEP) induced by high frequency (HFS) and low frequency stimulation (LFS). Individual values of the amplitudes of the somatosensory evoked potentials components (N9, N13, N20-P25) before (in blue) and after (in red) high frequency and low frequency stimulation (HFS and LFS) for the active and the control side. Black lines represent mean and 95% Confidence Interval, dots represent individual subjects. Asterisks indicate significance vs baseline ($p < 0.05$, Sidak's corrected p value).

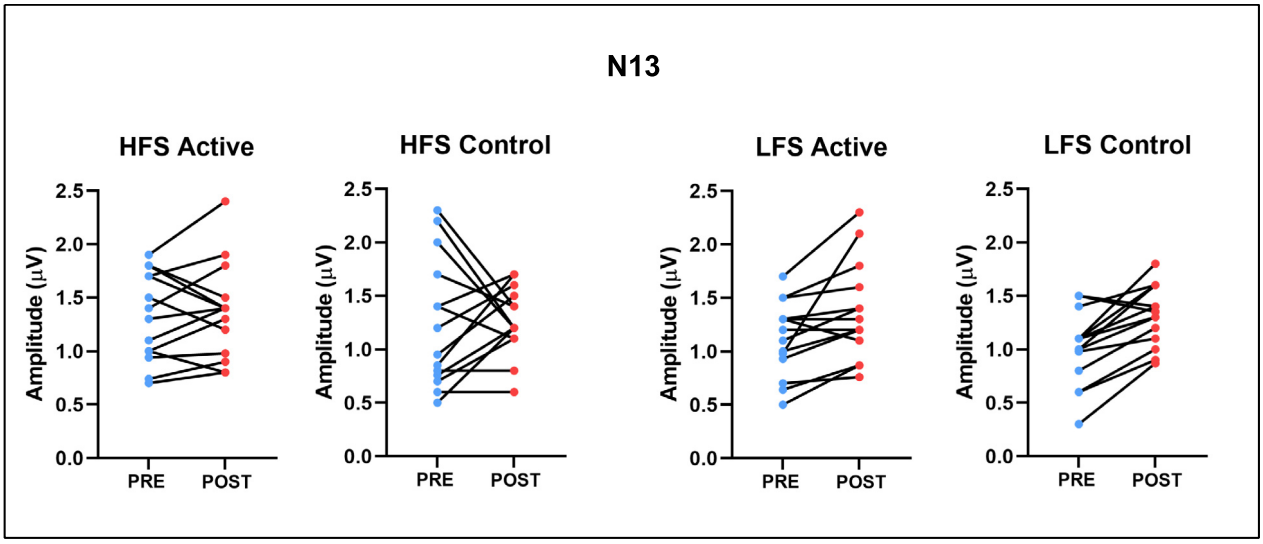


Fig. 3. Intra-individual modulation of N13 somatosensory evoked potential (SEP) induced by high frequency (HFS) and low frequency stimulation (LFS). Intra-individual differences in the N13-somatosensory evoked potential amplitude before (blue) and after (red) high frequency stimulation (HFS on the left) and low frequency stimulation (LFS on the right) for the active and the control side. Each dot represents a participant.

The rapid decrease of LFS modulation at dorsal horn level (Manresa et al. 2010) further support our hypothesis since the effect of wind-up is known to last a few minutes (Liu and Sandkühler 1997).

Admittedly, we cannot exclude alternative explanations for the differential effect on N13 SEP of HFS and LFS. The two experimental pain models may trigger distinct activation of nociceptive Aδ and C-fibres. This hypothesis is consistent with a previous study showing that LFS changes C-fibre mediated cutaneous blood flow, while HFS does not (Manresa et al., 2010). Alternatively, HFS and LFS may differ in descending pain modulatory system activation, thus affecting the N13 SEP modulation differently (Manresa et al.,

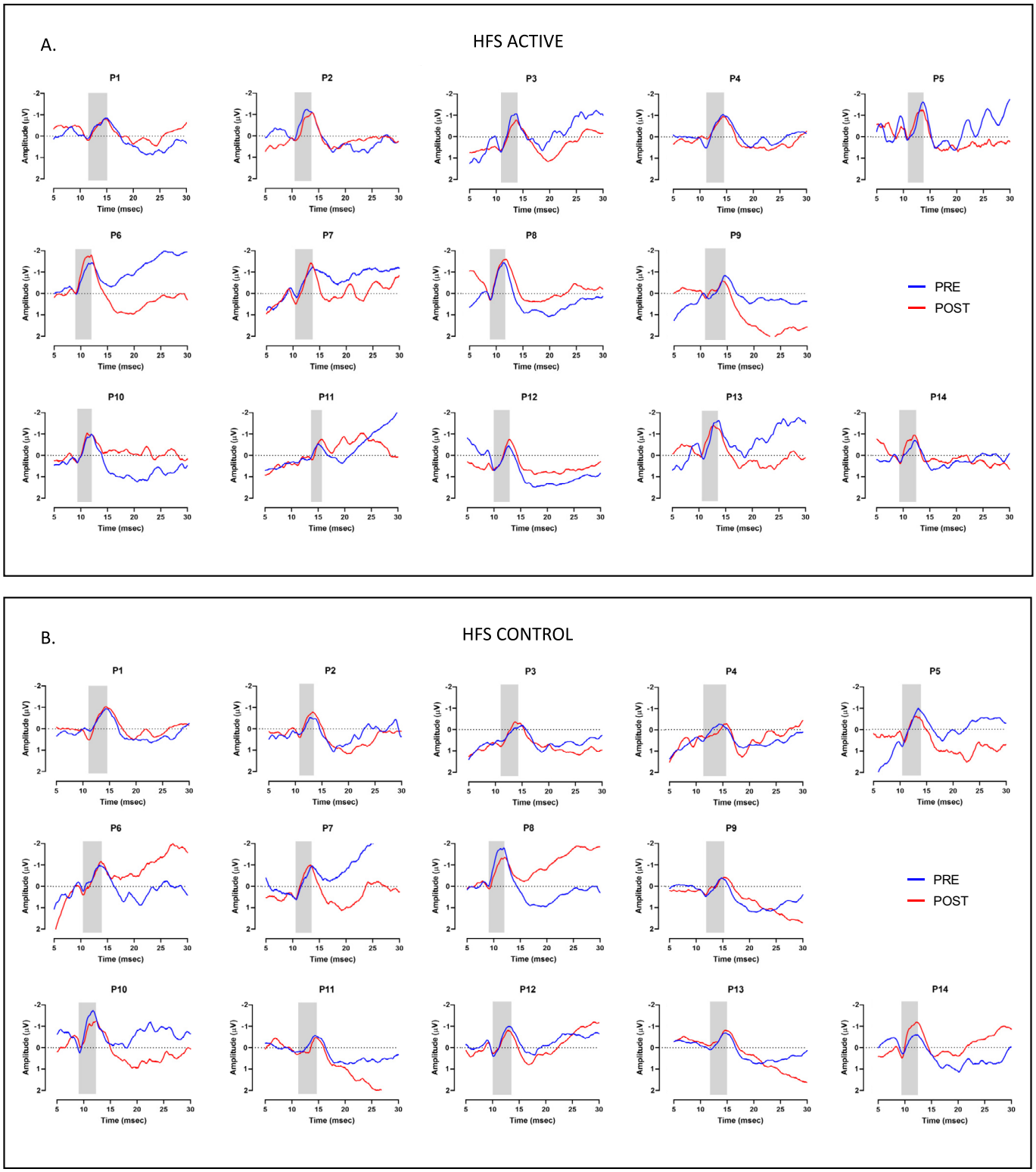


Fig. 4. Individual traces for the N13 somatosensory evoked potential (SEP) in the high frequency stimulation (HFS) session. Individual average N13-somatosensory evoked potential before (blue) and after (red) high frequency stimulation (HFS). 4A) active side; 4B) control side. Amplitude is expressed in microvolts and time in milliseconds. The grey rectangle indicates the P9-N13 interval.

2010). However, a previous neurophysiological study in healthy humans (Leone et al., 2021), showing that HFS does not significantly affect a conditioning pain modulation protocol, argues against this possibility.

We found that LFS applied on one side modulated the N13 SEP elicited after stimulation of both sides. The bilateral LFS effect is consistent with animal studies showing that unilateral nerve compression induces mechanical dynamic allodynia bilaterally (Chacur et al., 2001; Hubbard et al., 2008). Clinical studies also showed that in patients with mononeuropathy, neuropathic pain is associated with pinprick and thermal hyperalgesia and wind-up phenomena on both sides, presumably reflecting central sensitization phenom-

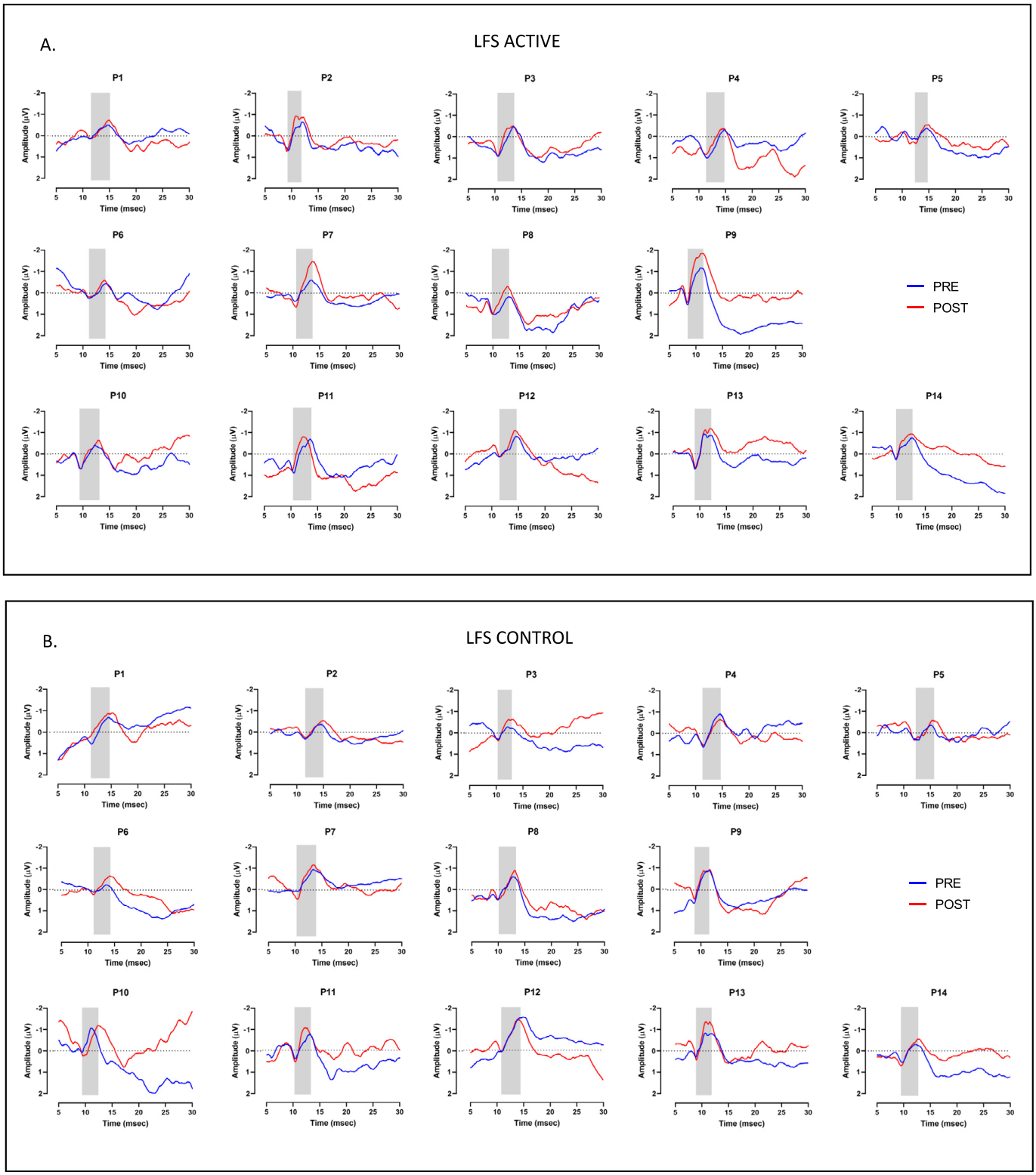


Fig. 5. Individual traces for the N13 somatosensory evoked potential (SEP) in the low frequency stimulation (LFS) session. Individual average N13-somatosensory evoked potential before (blue) and after (red) low frequency stimulation (LFS). 4A) active side; 4B) control side. Amplitude is expressed in microvolts and time in milliseconds. The grey rectangle indicates the P9-N13 interval.

ena affecting dorsal horn bilaterally (Enax-Krumova et al., 2017-2021a,b; Konopka 2012). Although the mechanisms underlying bilateral central sensitization are still an open issue, the involvement of commissural interneurons or nerve growth factors and spinal pro-inflammatory cytokines have been suggested (Koltzenburg et al., 1999; Milligan et al., 2003).

As expected, HFS and LFS did not affect the peripheral N9 SEP and the cortical N20-P25 SEP. The unchanged N9 SEP shows that the peripheral input to the spinal cord did not follow the same trend as the N13 (Supplementary Figs. 1-2) and remained quite stable. The N20-P25 SEP reflects a postsynaptic potential "generated in the geometrically coherent dendrites of cortical pyramids"

(Eccles 1951) in the primary somatosensory cortex by activating large, myelinated fibres, whose collaterals activate dorsal horn neurons generating N13 SEP. Therefore, the observation that LFS does not change cortical N20-P25 SEP lends further support to the evidence that N13 SEP precisely reflects dorsal horn excitability changes.

4.1. Limitations

Admittedly our study has a few limitations. A reliable evaluation of LFS-induced N13 SEP modulation at single subject level is likely prevented by the modest amplitude of ulnar-nerve-mediated N13 SEP and the slight amplitude increase following LFS.

Attention might concur with the differential effect of LFS and HFS on the N13 SEP amplitude changes we found. Prior research demonstrated that nociceptive responses, such as the nociceptive flexion reflex, that are mediated by the dorsal horn, are modulated by attention (Lannon et al., 2021). We cannot exclude that HFS and LFS might differentially trigger attention and top-down modulation of N13 SEP.

The subjects submitted to both experiments were not the same (10 out of 14), hence we cannot rule out a subject-dependent effect.

Lastly, the comparison between the effect of HFS and LFS was calculated through an unpaired t-test, though part of the data were paired.

5. Conclusions

Our findings showing that LFS modulated N13 SEP unlike HFS, indicate that how this spinal SEP component reflects central sensitization phenomenon, may critically depend on the experimental pain model used. This information might be useful in the selection of reliable biomarkers in the analgesic drug development experiments. Although our study suggests that LFS and HFS may trigger central sensitization at the dorsal horn level through distinct mechanisms, this finding still needs confirmation by replication studies.

Conflict of interest and Acknowledgements

The authors declare no conflict of interest regarding this work, which has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No [777500]. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

The statements and opinions presented here reflect the author's view and neither IMI nor the European Union, EFPIA, or any Associated Partners are responsible for any use that may be made of the information contained therein. https://www.imi-paincare.eu; https://www.imi.europa.eu.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2023.08.022.

References

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. https://doi.org/10.1016/0304-3959(96)02995-8.

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. https://doi.org/10.1111/j.1748-1716.1995.tb09951.x.

Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. Eur J Pain 2018;22:216–41. https://doi.org/10.1002/ejp.1140.

Biurrun Manresa JA, Finnerup NS, Johannesen IL, Biering-Sørensen F, Jensen TS, Arendt-Nielsen L, et al. Central sensitization in spinal cord injured humans assessed by reflex receptive fields. Clin Neurophysiol 2014;125:352–62. https://doi.org/10.1016/j.clinph.2013.06.186.

Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, et al. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain 2001;94:231–44. https://doi.org/10.1016/S0304-3959(01)00354-2.

Desmedt JE, Cheron G. Central somatosensory conduction in man: Neural generators and interpeak latencies of the far-field components recorded from neck and right or left scalp and earlobes. Electroencephalogr Clin Neurophysiol 1980;50:382–403. https://doi.org/10.1016/0013-4694(80)90006-1.

Di Lionardo A, Di Stefano G, Leone C, Di Pietro G, Sgro E, Malara E, et al. Modulation of the N13 component of the somatosensory evoked potentials in an experimental model of central sensitization in humans. Sci Rep 2021;11:20838. https://doi.org/10.1038/s41598-021-00313-7.

Di Pietro G, Di Stefano G, Leone C, Di Lionardo A, Sgro E, Blockeel AJ, et al. Erratum to "The N13 spinal component of somatosensory evoked potentials is modulated by heterotopic noxious conditioning stimulation suggesting an involvement of spinal wide dynamic range neurons". Neurophysiol Clin 2022;52:410–1. https://doi.org/10.1016/j.neucli.2022.09.001.

Eccles JC. Interpretation of action potentials evoked in the cerebral cortex. Electroencephalogr Clin Neurophysiol 1951;3:449–64.

Ellrich J, Treede R-D. Convergence of nociceptive and non-nociceptive inputs onto spinal reflex pathways to the tibialis anterior muscle in humans. Acta Physiol Scand 1998;163:391–401. https://doi.org/10.1046/j.1365-201X.1998.t01-1-00392.x.

Enax-Krumova EK, Pohl S, Westermann A, Maier C. Ipsilateral and contralateral sensory changes in healthy subjects after experimentally induced concomitant sensitization and hypoesthesia. BMC Neurol 2017;17:60. https://doi.org/10.1186/s12883-017-0839-9.

Enax-Krumova E, Attal N, Bouhassira D, Freynhagen R, Gierthmühlen J, Hansson P, et al. Contralateral Sensory and Pain Perception Changes in Patients With Unilateral Neuropathy. Neurology 2021a;97:e389–402.

Enax-Krumova E, Baron R, Treede RD, Vollert J. Contralateral sensitisation is not specific for complex regional pain syndrome. Br J Anaesth 2021b;127:e173–6.

Restuccia D, Mauguière F. The contribution of median nerve SEPs in the functional assessment of the cervical spinal cord in syringomyelia: a study of 24 patients. Brain 1991;114:361–79. https://doi.org/10.1093/brain/114.1.

Grönroos M, Pertovaara A. Capsaicin-induced central facilitation of a nociceptive flexion reflex in humans. Neurosci Lett 1993;159:215–8. https://doi.org/10.1016/0304-3940(93)90837-B.

Guekos, A., Grata, A.C., Hubli, M., Schubert, M., Schweinhardt, P., 2022. Are Changes in Nociceptive Withdrawal Reflex Magnitude a Viable Central Sensitization Proxy? Implications of a Replication Attempt. Clin Neurophysiol. https://doi.org/10.1016/j.clinph.2022.09.011.

Hubbard RD, Quinn KP, Martínez JJ, Winkelstein BA. The role of graded nerve root compression on axonal damage, neuropeptide changes, and pain-related behaviors. Stapp Car Crash J 2008;52:33–58. https://doi.org/10.4271/2008-22-0002.

Klein T, Magerl W, Hopf H-C, Sandkühler J, Treede R-D. Perceptual Correlates of Nociceptive Long-Term Potentiation and Long-Term Depression in Humans. J Neurosci 2004;24:964–71. https://doi.org/10.1523/JNEUROSCI.1222-03.2004.

Koltzenburg M, Wall PD, McMahon SB. Does the right side know what the left is doing? Trends Neurosci 1999;22:122–7. https://doi.org/10.1016/S0166-2236(98)01302-2.

Konopka K-H, Harbers M, Houghton A, Kortekaas R, van Vliet A, Timmerman W, et al. Bilateral sensory abnormalities in patients with unilateral neuropathic pain; a quantitative sensory testing (QST) study. PLoS One 2012;7:e37524.

Lannon EW, Jure FA, Andersen OK, Rhudy JL. Does Threat Enlarge Nociceptive Reflex Receptive Fields? J Pain 2021;22:487–97. https://doi.org/10.1016/j.jpain.2020.10.006.

Leone C, Di Lionardo A, Di Pietro G, Di Stefano G, Falco P, Blockeel AJ, et al. How different experimental models of secondary hyperalgesia change the nociceptive flexion reflex. Clin Neurophysiol 2021;132:2989–95. https://doi.org/10.1016/j.clinph.2021.08.018.

Linde LD, Bent LR, Dickey JP, Kumbhare DA, Srbely JZ. Exploring the effect of capsaicin-induced central sensitization on the upper limb nociceptive withdrawal reflex threshold. Exp Brain Res 2021;239:3405–15. https://doi.org/10.1007/s00221-021-06216-3.

Liu X, 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;78:1973–82. https://doi.org/10.1152/jn.1997.78.4.1973.

Magerl W, Wilk SH, Treede R-D. Secondary hyperalgesia and perceptual wind-up following intradermal injection of capsaicin in humans. Pain 1998;74:257–68. https://doi.org/10.1016/S0304-3959(97)00177-2.

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. https://doi.org/10.1016/j.ejpain.2009.12.008.

Mauguière F, Courjon J. The origins of short-latency somatosensory evoked potentials in humans: Short-latency Evoked Potentials. Ann Neurol 1981;9:607–11. https://doi.org/10.1002/ana.410090616.

Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, et al. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. J Neurosci 2003;23:1026–40. https://doi.org/10.1523/JNEUROSCI.23-03-01026.2003.

Price DD. Characteristics of second pain and flexion reflexes indicative of prolonged central summation. Exp Neurol 1972;37:371–87.

Quesada C, Kostenko A, Ho I, Leone C, Nochi Z, Stouffs A, et al. Human surrogate models of central sensitization: a critical review and practical guide. Eur J Pain 2021;25:1389–428. https://doi.org/10.1002/ejp.1768.

Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991;66:228–46. https://doi.org/10.1152/jn.1991.66.1.228.

Treede R-D. Gain control mechanisms in the nociceptive system. Pain 2016;157:1199–204. https://doi.org/10.1097/j.pain.0000000000000499.