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NEUROSYSTEMS

The effect of heterotopic noxious conditioning stimulation on Aδ-, C- and Aβ-fibre brain responses in humans

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Abstract

Human studies have shown that heterotopic nociceptive conditioning stimulation (HNCS) applied to a given body location reduces the percept and brain responses elicited by noxious test stimuli delivered at a remote body location. It remains unclear to what extent this effect of HNCS relies on the spinal–bulbar–spinal loop mediating the effect of diffuse noxious inhibitory controls (DNICs) described in animals, and/or on top-down cortical mechanisms modulating nociception. Importantly, some studies have examined the effects of HNCS on the brain responses to nociceptive input conveyed by Aδ-fibres. In contrast, no studies have explored the effects of HNCS on the responses to selective nociceptive C-fibre input and non-nociceptive Aβ-fibre input. In this study, we measured the intensity of perception and event-related potentials (ERPs) to stimuli activating Aδ-, C- and Aβ-fibres, before, during and after HNCS, obtained by immersing one foot in painful cold water. We observed that (i) the perceived intensity of nociceptive Aδ- and C-stimuli was reduced during HNCS, and (ii) the ERPs elicited by Aδ- and Aβ- and C-stimuli were also reduced during HNCS. Importantly, because Aβ-ERPs are related to primary afferents that ascend directly through the dorsal columns without being relayed at spinal level, the modulation of these responses may not be explained by an influence of descending projections modulating the transmission of nociceptive input at spinal level. Therefore, our results indicate that, in humans, HNCS should be used with caution as a direct measure of DNIC-related mechanisms.

Introduction

Pain can be modulated by inhibitory and facilitatory mechanisms acting at both spinal and supra-spinal levels (Millan, 2002; Porreca *et al.*, 2002). These modulatory mechanisms are increasingly considered to play an important role in the individual susceptibility to pain.

In animals, modulatory mechanisms can involve a spinal–bulbar–spinal loop in which noxious stimuli applied to a given body part inhibit, at the level of the dorsal horn, transmission of nociceptive input originating from all other body parts (reviewed by Le Bars, 2002). The neural substrates of this mechanism, termed 'diffuse noxious inhibitory control' (DNIC), remain to be fully understood. Lesions of the periaqueductal grey do not modify DNIC. In contrast, the mechanism is disrupted by lesions of the subnucleus reticularis dorsalis in the caudal medulla (Bouhassira *et al.*, 1992; Villanueva & Le Bars, 1995). Recent data also suggest that, contrary to previous proposals, lesions of the rostral ventromedial medulla, including the nucleus raphe magnus, can affect DNIC (Chebbi *et al.*, 2014). Furthermore, the parabrachial nucleus would also be involved in triggering DNIC (Lapirot *et al.*, 2009). Finally, a role of descending dopaminergic controls has been recently identified (Lapirot *et al.*, 2011).

Human studies have shown that nociceptive conditioning stimuli applied to a given body location reduce the percept and brain responses elicited by test stimuli delivered at a remote location (De Broucker *et al.*, 1990; Willer *et al.*, 1990; Bouhassira *et al.*, 1993). Furthermore, human studies have shown that heterotopic noxious conditioning stimulation (HNCS) inhibits the spinal RIII reflex, suggesting that at least part of the effect of HNCS on pain perception may be due to descending inhibitory influences on spinal transmission (Willer *et al.*, 1984, 1989; Roby-Brami *et al.*, 1987; De Broucker *et al.*, 1990; Danziger *et al.*, 1998; Scheuren *et al.*, 2014). Therefore, the effects of HNCS have often been considered as a direct human correlate for DNIC (see Pud *et al.*, 2009).

Importantly, descending modulatory effects are thought to operate predominantly at the level of wide dynamic range (WDR) neurons, onto which nociceptive C-fibres, Aδ-fibres and Aβ-fibres converge. Using laser-evoked potentials, studies have demonstrated that HNCS

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can reduce the magnitude of the brain responses to nociceptive input conveyed by Aδ-fibres (Plaghki *et al.*, 1994; Watanabe *et al.*, 1996).

So far, no studies have examined whether HNCS affects the brain responses to inputs conveyed selectively by nociceptive C-fibres. One of the reasons for this lack of knowledge is that selective activation of C-fibres is difficult to achieve. However, we (Jankovski *et al.*, 2013; van den Broeke & Mouraux, 2014a) and other groups (Magerl *et al.*, 1999) have shown that laser-evoked brain responses and percepts (Churyukanov *et al.*, 2012) related to the selective activation of C-fibres can be obtained using a temperature-controlled infrared CO_2 laser stimulator to selectively activate heat-sensitive C-fibre afferents that have a lower thermal activation threshold than Aδ-fibre afferents (Jankovski *et al.*, 2013; van den Broeke & Mouraux, 2014a). Therefore, the first objective of our study was to assess the effects of HNCS on the perception and brain responses to selective C-fibre input.

Furthermore, no study has yet examined whether HNCS also affects the perception and event-related potentials (ERPs) to sensory input conveyed by non-nociceptive Aβ-fibres (somatosensory evoked potentials, SEPs). Given that these brain responses are related to Aβ-fibre primary afferents which are not relayed in the spinal cord but ascend directly through the dorsal columns to reach second-order neurons in the dorsal column nuclei, one would expect that these responses would not be under the influence of descending spinal modulatory mechanisms. Therefore, the second objective of our study was to assess the effects of HNCS on the perception and brain responses to Aβ-fibre input, to examine whether the effects of HNCS can be attributed to changes occurring exclusively at spinal level and/or to changes also occurring at supra-spinal level.

Methods

Experiment 1

Participants

Experiment 1 was performed on eight healthy volunteers recruited among students and staff of the Université catholique de Louvain (three female and five male; all right-handed, aged 23–35 years). The phase of the menstrual cycle in the main experiment was not taken into consideration in the participating women, although previous studies have suggested that during the ovulatory phase, HNCS may trigger a greater modulation of nociceptive responses (Tousignant-Laflamme & Marchand, 2009). Participants had no history of neurological, psychiatric, dermatological or chronic pain disorders, and no recent history of psychotropic or analgesic drug use. During a preliminary session, volunteers were given a fully detailed explanation concerning the experimental procedures and were familiarized with the experimental setup and task, the nociceptive stimuli and the rating procedures. Participants were comfortably seated in a chair with forearms resting on the armrests. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Université catholique de Louvain (B40320096449).

Experimental design

Each participant underwent three blocks: before, during and after the HNCS conditioning procedure. Within each block, behavioural (reaction times, intensity of perception) and electrophysiological (event-related brain potentials) responses to 20 infrared CO_2 laser stimuli activating nociceptive C- and Aδ-fibre free nerve endings (Aδ-stimulus, C-stimulus) and 20 transcutaneous electrical stimuli activating non-nociceptive Aβ-fibres (Aβ-stimulus) were recorded. The laser stimuli were applied to the right-hand dorsum. The electrical stimuli were applied over the superficial branch of the right radial nerve. Within each block, the two types of stimuli were presented in separate runs, whose order was randomized across participants.

Thermal CO_2 laser stimulation (Aδ- and C-stimulus)

Thermal stimuli were applied to the right-hand dorsum using a CO_2 laser in which the power of the laser beam is regulated continuously during stimulation using an online measurement of skin temperature optically in line with the laser beam. This feedback control of power output allowed us to define specific skin temperature heating profiles (LSD, SIFEC, Ferrières, Belgium). Because the CO_2 laser has a wavelength of 10.6 μm, 99% of the delivered energy was confined within the most superficial layer of the skin (< 100 μm beneath the skin surface), i.e. where most of the free nerve endings of nociceptors terminate (Meyer *et al.*, 1976). The laser beam was conducted through a 10-m optical fibre. An optical fibre vibrator was used to introduce a small amount of variable lateral or angular displacement in the fibre, altering internal reflections and thereby resulting in a homogeneous spatial distribution of power within the stimulated area. At the end of the fibre, optics collimated the beam to 6 mm diameter at the target site.

An approach similar to that of Magerl *et al.* (1999) was used to temporally dissociate the activation of nociceptive C- and Aδ-fibres and thereby allow the recording of behavioural and electrophysiological responses related to the activation of each fibre type. The procedure is represented in Fig. 1B. First, skin temperature was brought to 34 °C using a 1-s heating ramp and maintained at that temperature for 3 s (baseline skin temperature). Second, the skin

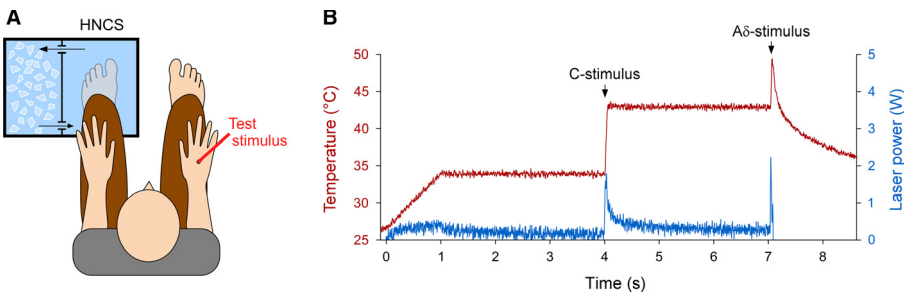

FIG. 1. Experimental set-up. (A) Twenty thermal stimuli activating successively C- and Aδ-fibres and 20 electrical stimuli activating Aβ-fibres were applied on the right-hand dorsum before, during and after applying a heterotopic noxious conditioning stimulus (HNCS) to the left foot (immersion in a 5 °C circulating water bath). The order of administration of the test stimuli was counterbalanced across participants. (B) Details of how activation of C- and Aδ-fibres was obtained with the laser stimulator. The ordinate represents skin surface temperature, the abscissa represents time.

temperature was increased to 43 °C in 10 ms. In a previous study, we showed that such temperatures are above the thermal activation threshold of C-fibres but below the thermal activation threshold of Aδ-fibres (Churyukanov *et al.*, 2012). Hence, this first step increase activated C-fibres selectively (C-stimulus). Finally, after maintaining the skin temperature at 43 °C for 3 s, the skin temperature was increased to 49 °C in 10 ms, and maintained at that temperature for 40 ms before interrupting laser power output. This second target temperature activated Aδ-fibres. After each trial, the target of the laser was displaced to a random position on the hand dorsum, to avoid nociceptor sensitization and/or habituation. The inter-trial interval varied randomly between 5 and 10 s.

Transcutaneous electrical stimulation (Aβ-stimulus)

Transcutaneous electrical stimuli consisted of constant current square-wave electrical pulses (0.5-ms duration; DS7A, Digitimer Ltd, Welwyn Garden City, UK) delivered through a pair of feltpad skin electrodes (0.5 cm in diameter, 2-cm inter-electrode distance) placed at the right wrist, over the superficial branch of the radial nerve. Prior to the experimental session, the intensity of the electrical stimulus was set to twice the detection threshold (2.30 ± 0.80 mA). The inter-trial interval varied randomly between 5 and 10 s.

Heterotopic noxious conditioning stimulation

During the first block (before HNCS), participants immersed their left foot into a water bath containing water at 30.9 ± 0.8 °C, that was perceived as neither warm nor cold. During the second block (during HNCS), participants immersed their foot in another water bath of circulating water whose temperature was set to 5 °C (measured at 5.1 ± 0.3 °C at the beginning of the block and 5.7 ± 0.3 °C at the end of the block). This procedure elicited a perception of pain after about 10–15 s, which peaked after about 30–45 s and then stabilized. The intensity of the elicited pain was just below the limit of tolerance for most subjects. During the third block (after HNCS), participants re-immersed the foot in the water bath of the first block (temperature 30.0 ± 0.8 °C). In each block, recordings began 30 s after immersing the foot in the water bath. A 5-min rest period separated each block. The foot was dried and covered with a towel during the rest period.

Behavioural measures

Reaction times. During nociceptive stimulation, participants were asked to respond as quickly as possible by pressing a button held in the left hand when perceiving the first (43 °C) and second (49 °C) step-increase in skin temperature, corresponding to the C- and Aδ-stimulus, respectively. Reaction times were measured relative to the onset of the step-increase. Based on the large difference between the conduction velocities of Aδ- and C-fibres, responses ≤ 650 ms were considered related to the detection of Aδ-fibre input, whereas responses > 650 ms were considered related to the detection of C-fibre input (Churyukanov *et al.*, 2012). Only trials with a reaction time between 650 and 2000 ms were included in the analysis of C-fibre ERPs. This represented 85 ± 3% of the total number of trials. Only trials with a reaction time < 650 ms were included in the analysis of Aδ-fibre ERPs, representing 91 ± 5% of the total number of trials. During non-nociceptive stimulation, participants were also asked to respond as quickly as possible by pressing the same button used to detect nociceptive stimuli.

Intensity of perception. After each stimulus, participants were asked to rate the intensity of the perception elicited by C-, Aδ- and Aβ-fibre stimuli using a numerical rating scale (NRS) presented at eye level, approximately 1.5 m in front of the participant. The extremities of the scale were annotated with the words 'no detection' (NRS = 0) and 'maximum pain' (NRS = 100). An anchor at the middle of the scale (NRS = 50) defined the borderline between the non-painful and painful domains of perception. Participants provided a verbal report of the perceived intensity of perception. Only ratings to perceived stimuli (> 0) were included in the analysis.

Electrophysiological measures

The EEG was recorded using 64 Ag-AgCl electrodes placed on the scalp according to the international 10–20 system (Waveguard64 cap, Cephalon A/S, Nørresundby, Denmark). Scalp signals were recorded using an average reference. Impedance was kept below 10 kΩ. Ocular movements and eye blinks were recorded using two surface electrodes placed at the upper-left and lower-right sides of the right eye. All signals were amplified and digitized at a 1 kHz sampling rate (64-channel ASA-LAB EEG/ERP system, Advanced Neuro Technologies, The Netherlands).

Continuous EEG signals were band-pass filtered (0.5–30 Hz) using a Butterworth zero-phase filter and segmented into 3-s epochs ranging from −1 to +2 s relative to the onset of the stimulus (C-, Aδ- and Aβ-stimulus). Artefacts produced by eye blinks or eye movements were subtracted using a validated method based on an Independent Component Analysis (ICA) (Jung *et al.*, 2000). Baseline correction was performed by subtracting the average voltage of the prestimulus interval ranging from −1 to 0 s. In addition, epochs with amplitude values exceeding ± 100 μV (i.e. epochs likely to be contaminated by an artefact) were rejected.

For each type of stimulus (C-, Aδ- and Aβ-stimulus), separate average waveforms were computed for each block (before, during and after), resulting in three average waveforms for the C-stimulus (C-LEP), three average waveforms for the Aδ-stimulus (Aδ-LEP) and three average waveforms for the Aβ-stimulus (Aβ-SEP). In the C-LEP waveforms, no significant signal deflection could be identified, and the signals were not further analysed using this approach (see below). In the Aδ-LEP waveforms, three distinct ERP components were identified as follows. The Aδ-N2 and Aδ-P2 waves were identified at electrode Cz (average reference) as the most negative and positive deflections occurring between 100 and 600 ms after stimulus onset or step-increase in skin temperature. The Aδ-N1 wave was identified at electrode C3 re-referenced to electrode Fz, as the first negative deflection preceding the Aδ-N2 wave. In the Aβ-ERP waveforms, three distinct ERP components were identified as follows. The Aβ-N2 and Aβ-P2 waves were identified at electrode Cz (average reference) as the most negative and positive deflections occurring between 100 and 350 ms after stimulus onset. The Aβ-N1 was identified at electrode C3 re-referenced to electrode Fz and defined as the first negative deflection preceding the Aβ-N2.

Experiment 2

As we could not obtain reliable C-fibre responses with the experimental paradigm used in experiment 1, we conducted a second experiment in which we tested selectively the effects of HNCS on C-LEPs. This experiment was performed on eight healthy volunteers recruited among students and staff of the Université catholique de Louvain (two female and six male; one left handed,

aged 22–37 years). We recruited mainly men to avoid potentially confounding effects of the phase of the menstrual cycle. Participants had no history of neurological, psychiatric, dermatological or chronic pain disorders, and no recent history of psychotropic or analgesic drug use. During a preliminary session, volunteers were given a fully detailed explanation concerning the experimental procedures. Participants were comfortably seated in a chair with forearms resting on the armrests. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Université catholique de Louvain.

Experimental design

As in experiment 1, each participant underwent three blocks: before HNCS, during HNCS and after HNCS. Water temperature was 30.5 ± 0.9 °C before HNCS, 5.9 ± 0.5 °C during HNCS and 30.3 ± 0.8 °C after HNCS.

Thermal CO_2 laser stimulation (C-stimulus)

Thermal stimuli were applied using the same device as in experiment 1. The laser beam was 6 mm. The stimuli consisted of 100 ms of radiant heat, of which 10 ms corresponded to the rapid heating ramp during which the skin was brought to the target temperature, and 90 ms corresponded to the plateau of the target temperature. To increase the number of trials in which the thermal stimulus activated C-fibres selectively, we used the approach recently proposed by van den Broeke & Mouraux (2014a). At the beginning of the experiment, the thermal detection threshold of Aδ- and C-fibres was determined using two staircase algorithms. Reactions times > 650 ms were considered compatible with the detection of input conveyed by C-fibres, and reaction times ≤ 650 ms were considered compatible with the detection of input conveyed by faster Aδ-fibres (Churyukanov *et al.*, 2012). Participants were asked to respond as soon as they perceived a stimulus. The staircase procedure used to estimate the C-fibre thermal detection threshold started at 41 °C. The temperature was then increased by 1 °C when the stimulus was not detected, and decreased by 1 °C when the stimulus was detected. The staircase procedure used to estimate the Aδ-fibre thermal detection threshold started at 46 °C. The temperature was increased by 1 °C when the stimulus was detected with a reaction time > 650 ms, and decreased when the stimulus was detected with a reaction time ≤ 650 ms. The staircases were interrupted after the occurrence of three reversals. The target temperature of the test stimulus was defined as the average of the C- and Aδ-fibre thresholds. This ensured that the test stimulus was above the threshold of C-fibres, but below the threshold of Aδ-fibres (i.e. that the test stimulus activated C-fibres selectively). In each block, the test stimuli were repeated until we obtained at least 20 trials in which the stimulus was detected with a reaction time > 650 ms, with a maximum of 40 trials. After each trial, the target of the laser was displaced to a random position on the hand dorsum, to avoid nociceptor sensitization and/or habituation. The inter-trial interval varied randomly between 5 and 10 s.

Intensity of perception. After each stimulus, participants were asked to rate the intensity of the perception elicited by the stimulus using an NRS. The extremities were described as 'no detection' (NRS = 0) and 'maximum pain' (NRS = 100). An anchor at the middle of the scale (NRS = 50) defined the transition between the non-painful and painful domains of perception. As in experiment 1, only ratings to perceived stimuli (> 0) were included in the analysis.

Electrophysiological measures

Previous studies showed that N2 and P2 peaks of the C-LEP are maximal over the midline centro-parietal electrodes. Therefore, we recorded the EEG using five channels (Cz, Cpz, Pz, M1, M2) of a 64 Ag-AgCl cap (Waveguard64 cap, Cephalon A/S). Impedance was kept below 10 kΩ. Ocular movements and eye blinks were recorded using two surface electrodes placed at the upper-left and lower-right sides of the right eye. All signals were amplified and digitized at a 4-kHz sampling rate (64-channel ASA-LAB EEG/ERP system, Advanced Neuro Technologies).

Continuous EEG signals were band-pass filtered (0.5–30 Hz) using a Butterworth zero-phase filter and segmented into 3-s epochs ranging from −1 to +2 s relative to the onset of the stimulus. The Gratton–Coles method was used to correct eye-movements and blinks (Gratton *et al.*, 1983). Contrary to ICA, this method can be applied also with a limited number of channels. Baseline correction was performed by subtracting the average voltage of the prestimulus interval ranging from −1 to 0 s. In addition, epochs with amplitude values exceeding ±70 μV (i.e. epochs likely to be contaminated by an artefact) were rejected. The signal was re-referenced to the mastoids. The maximal peak was observed at Cz in six subjects and in Cpz in two subjects.

Statistical analyses

To assess possible differential effects of HNCS on the intensity of perception and ERPs elicited by each of the three types of stimuli, we used non-parametric repeated measures Friedman's tests. *Post-hoc* analyses with Wilcoxon signed-rank tests were conducted to follow up significant effects. All statistical analyses were performed in SPSS 19 (IBM, Armonk, NY, USA).

Results

Experiment 1

Intensity of perception

The Friedman test revealed a significant effect of 'block' (before, during and after HNCS) on the perception of Aδ- and C-stimuli (Aδ-NRS: $\chi^2 = 14.2$, $P = 0.001$; C-NRS: $\chi^2 = 12.2$, $P = 0.002$) but not Aβ stimuli (Aδ-NRS: $\chi^2 = 4.2$, $P = 0.122$). *Post-hoc* analyses with Wilcoxon tests showed that Aδ-NRS ratings were reduced during HNCS as compared with before HNCS ($z = -2.5$, $P = 0.012$), but also as compared with after HNCS ($z = -2.5$, $P = 0.012$) (Table 1). Aδ-NRS ratings after HNCS were also significantly reduced as compared with before HNCS ($z = -2.3$, $P = 0.017$). C-NRS ratings were reduced during HNCS as compared with before HNCS ($z = -2.5$, $P = 0.012$), and returned to baseline level after HNCS (during vs. after HNCS: $z = -2.5$, $P = 0.012$; after vs. before HNCS: $z = -1.2$, $P = 0.208$) (Table 1).

ERPs elicited by C-, Aδ- and Aβ-stimuli

Group-level average waveforms are shown in Figs 3 and 4. As explained in the Methods, C-stimuli did not elicit consistent ERPs, and were not further analysed.

N2 waves elicited by Aδ- and Aβ-stimuli. There was a significant effect of 'block' on the vertex negative peak of the Aδ-ERP (Aδ-N2: $\chi^2 = 9.7$, $P = 0.008$), which was reduced during HNCS as

TABLE 1. Perceived intensity and ERP magnitude

		Post-hoc comparisons (Wilcoxon)		
	Friedman test	Before vs. during	During vs. after	Before vs. after
Ratings				
Aδ	$P = 0.001$	$P = 0.012$	$P = 0.012$	$P = 0.017$
C (experiment 1)	$P = 0.002$	$P = 0.012$	$P = 0.012$	$P = 0.208$
C (experiment 2)	$P = 0.036$	$P = 0.012$	$P = 0.208$	$P = 0.050$
Aβ	n.s.			
ERPs				
Aδ-N2	$P = 0.008$	$P = 0.017$	n.s.	$P = 0.012$
Aδ-P2	n.s.			
Aβ-N2	$P = 0.021$	n.s.	0.012	n.s.
Aβ-P2	$P = 0.030$	$P = 0.017$	$P = 0.036$	$P = 0.484$
C-N2	$P = 0.034$	$P = 0.012$	n.s.	$P = 0.036$
C-P2	n.s.			

Statistical values of the non-parametric analysis on the perceived intensity and on the magnitude of the ERPs. *Post-hoc* comparisons (Wilcoxon test) are reported when the overall analysis (Friedman test) achieved the significance threshold.

compared with before HNCS ($z = -2.3$, $P = 0.017$), and remained reduced after HNCS (after vs. during HNCS: $z = -0.280$, $P = 0.779$; after vs. before HNCS: $z = -2.5$, $P = 0.012$) (Table 1). The magnitude of the Aβ-N2 was also influenced by 'block' ($\chi^2 = 7.7$, $P = 0.021$). However, this effect was mainly driven by an enhancement of the negative peak after HNCS, rather than by a reduction during HNCS (before vs. during HNCS: $z = -1.1$, $P = 0.263$; before vs. after HNCS: $z = -1.2$, $P = 0.208$; during vs. after HNCS: $z = -2.5$, $P = 0.012$).

P2 waves elicited by Aδ- and Aβ-stimuli. No significant effects of 'block' were observed for the vertex positive peak of the Aδ-ERP ($\chi^2 = 3.2$, $P = 0.197$). In contrast, the magnitude of the Aβ-P2 was significantly modulated by 'block' ($\chi^2 = 7$, $P = 0.030$). *Post-hoc* comparisons showed that the Aβ-P2 was significantly reduced during HNCS as compared with before HNCS ($z = -2.3$, $P = 0.017$). Importantly, the amplitude of the Aβ-P2 returned to baseline levels after HNCS (during vs. after HNCS: $z = -2.1$, $P = 0.036$; after vs. before HNCS: $z = -0.7$, $P = 0.484$) (Table 1).

N1 waves elicited by Aδ- and Aβ-stimuli. The amplitude of the Aδ-N1 did not change as a function of 'block' ($\chi^2 = 4$, $P = 0.135$). In contrast, the Aβ-N1 was significantly modulated by 'block' ($\chi^2 = 6.7$, $P = 0.034$). However, and in line with the effect of 'block' on the magnitude of the negative vertex component, this effect was not driven by a significant reduction during HNCS, but by a significant enhancement after HNCS (before vs. during HNCS: $z = -1.6$, $P = 0.093$; during vs. after HNCS: $z = -0.840$ $P = 0.401$; before vs. after HNCS: $z = -2.5$ $P = 0.012$).

Experiment 2

Intensity of perception

As in experiment 1, the Friedman test revealed a significant effect of 'block' (before, during and after HNCS) on the perception of C-stimuli (C-NRS: $\chi^2 = 7.750$, $P = 0.021$). *Post-hoc* analyses with Wilcoxon tests showed that C-NRS ratings were reduced during HNCS as compared with before HNCS ($z = -2.1$, $P = 0.036$), but remained on average lower after HNCS (during vs. after HNCS: $z = -1.2$, $P = 0.208$; before vs. after HNCS: $z = -1.9$, $P = 0.050$) (Table 1 and Fig. 2).

ERPs elicited by C-stimuli

The group-level average waveforms obtained in experiment 2 are shown in Fig. 5.

N2 waves elicited by C-stimuli. There was a significant effect of 'block' on the vertex negative peak of the C-LEP (C-N2: $\chi^2 = 6.750$, $P = 0.034$), which was reduced during HNCS as compared with before HNCS ($z = -2.5$, $P = 0.012$), and remained reduced after HNCS (after vs. during HNCS: $z = -0.28$, $P = 0.779$; after vs. before HNCS: $z = -2.1$ $P = 0.036$).

P2 waves elicited by C-stimuli. No significant effect of 'block' was observed for the vertex positive peak of the C-LEP ($\chi^2 = 1$, $P = 0.607$).

Discussion

In this study, we investigated the effects of HNCS on the perception and ERPs elicited by the stimulation of nociceptive Aδ- and C-fibre afferents, and non-nociceptive Aβ-fibre afferents.

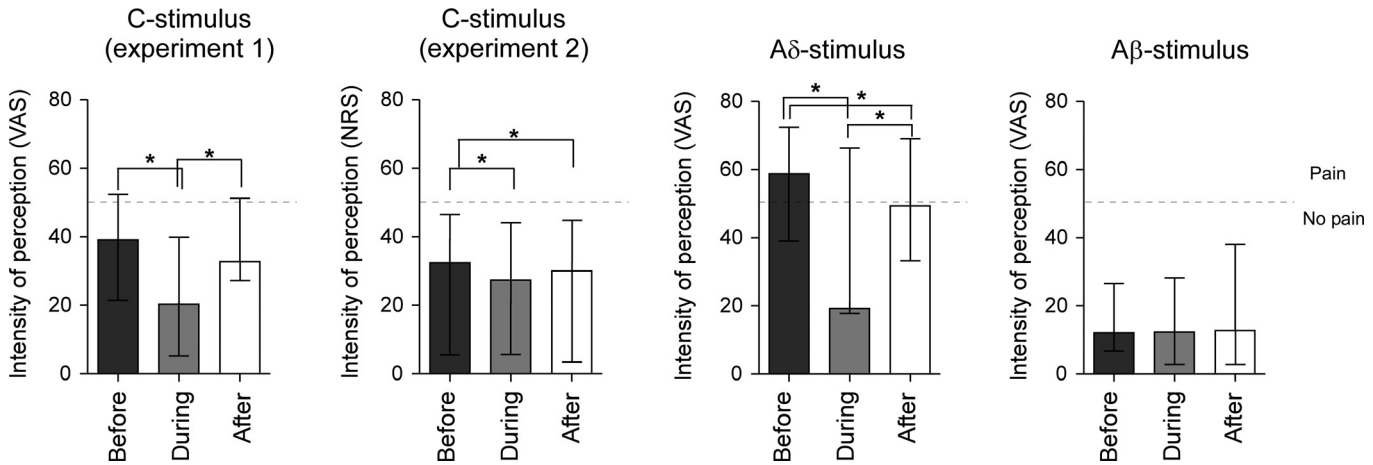

FIG. 2. Perceptual ratings. Median intensity of perception ratings for C-, Aδ- and Aβ-stimuli presented before, during and after HNCS. Bars represent the interquartile range. Asterisks indicate significant differences between conditions at $P < 0.05$.

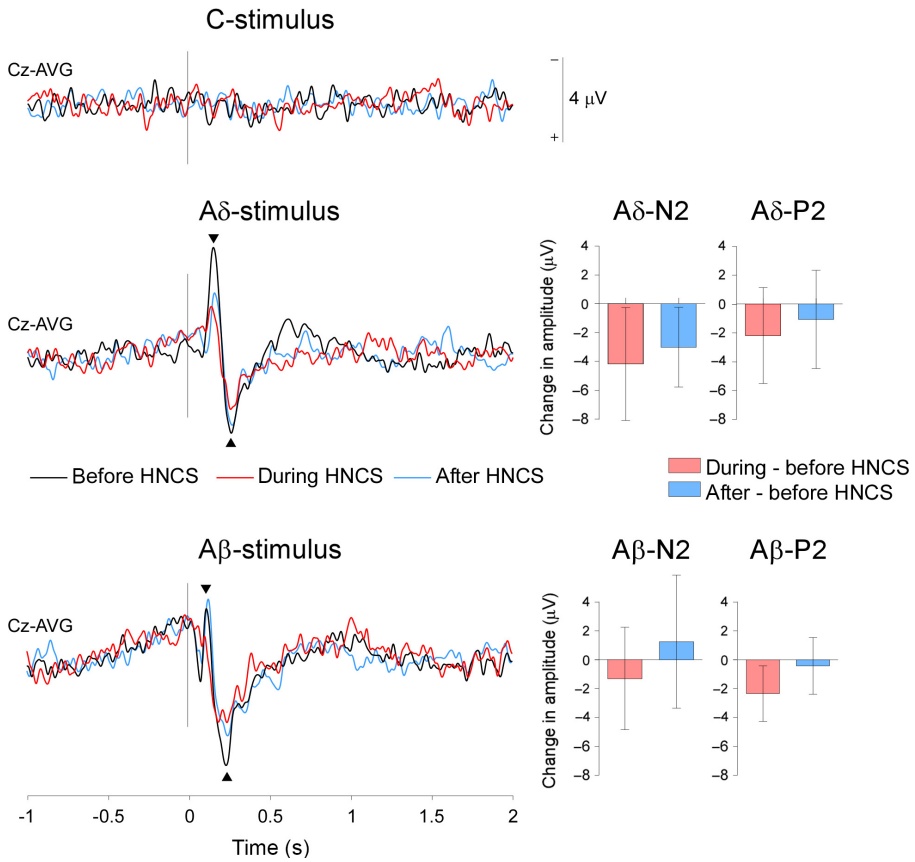

FIG. 3. Event-related potentials (N2-P2). Grand-average waveforms of the vertex ERPs elicited by C-, Aδ- and Aβ-stimuli. Note that the magnitude of both the Aδ-N2 and Aβ-P2 evoked responses was reduced during HNCS as compared with before and after HNCS. In contrast, no clear ERP could be identified following C-fibre stimulation. The bar graphs represent the difference in amplitude between conditions. Negative changes in amplitude correspond to a decrease in amplitude as compared with before HNCS.

We observed that HNCS reduced the perceived intensity of both Aδ- and C-fibre nociceptive inputs. In addition, HNCS reduced the magnitude of the Aδ-N2, the C-N2 and the Aβ-P2. Importantly, this last effect could not be attributed only to habituation as the amplitude of the Aβ-P2 decreased during HNCS, but returned to baseline levels after HNCS.

Notably, the ERPs elicited by Aβ-stimuli are primarily related to inputs that are not relayed in the spinal cord but ascend directly through the dorsal columns to reach second-order neurons in the dorsal column nuclei. Therefore, by showing that HNCS modulates the brain responses to non-nociceptive Aβ-fibre inputs, our results indicate that the effects of HNCS should be cautiously solely attributed to descending projections modulating transmission at spinal level.

HNCS modulates perception of C- and Aδ-stimuli

In human studies, it has been shown that HNCS is able to modulate consistently the perceived intensity of noxious test stimuli, irrespective of the nociceptive conditioning procedure and the nature of the nociceptive test stimulus (for reviews see Pud *et al.*, 2009; van Wijk & Veldhuijzen, 2010). This effect has been attributed to descending modulatory projections which are thought to operate predominantly at the level of WDR neurons of the dorsal horn, onto which nociceptive C- and Aδ-fibres, as well as non-nociceptive Aβ-fibres, converge (Le Bars, 2002).

We observed that HNCS reduced the pain evoked by both nociceptive C-fibre input and nociceptive Aδ-fibre input. As in previous studies (Treister *et al.*, 2010), the reduction in perception was stronger during HNCS as compared with after HNCS, indicating that the reduction observed during HNCS was not solely due to perceptual habituation. Indeed, had this been the case, one would have expected the intensity of the percepts elicited after HNCS to be further reduced. Importantly, the finding that the intensity of the percept elicited by Aδ- and C-fibre input remained lower 5–10 min after HNCS could be due to perceptual habituation, but also to a long-lasting after-effect of HNCS. Indeed, Willer *et al.* (1990) showed that the RIII reflex can be suppressed up to 6–9 min after HNCS.

Contrasting with the effects of HNCS on the perception of nociceptive Aδ- and C-fibre inputs, we observed no effect of HNCS on the perception of non-nociceptive Aβ-fibre input. This lack of modulation should be interpreted with caution, as it could be explained by different factors. Indeed, several studies including the present one have observed that experimental manipulations can modulate the brain responses elicited by transcutaneous stimulation of non-nociceptive Aβ-fibres without modulating subjective reports of intensity of perception (Torta *et al.*, 2013, 2015; van den Broeke & Mouraux, 2014b). This could be explained by the fact that transcutaneous electrical stimulation elicits a robust percept, identical from trial to trial. Hence, the modulatory effect of HNCS on the processing of Aβ-fibre input could be insufficient to result in a noticeable change of perception.

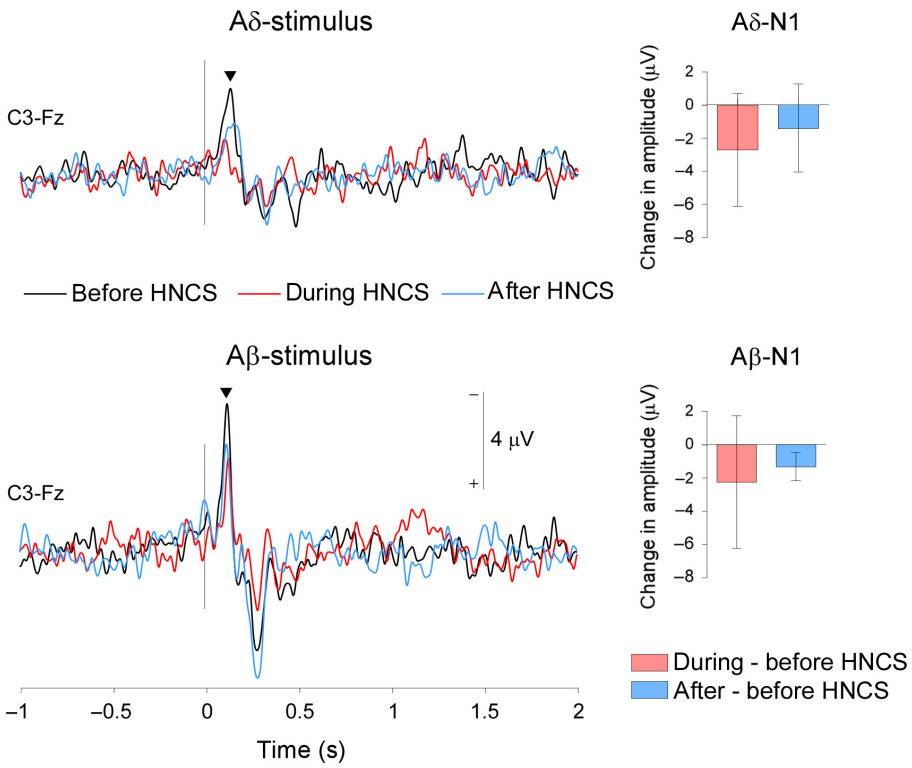

FIG. 4. Event-related potentials (N1). Grand-average waveforms of the ERPs recorded at the contralateral central electrode (C3) following Aδ- and Aβ-stimulation (N1). Both stimuli elicited an early-latency negative wave (N1). The magnitude of the Aβ-N1 but not the Aδ-N1 was reduced significantly during HNCS. The bar graphs represent the difference in amplitude between conditions. Negative changes in amplitude correspond to a decrease in amplitude as compared with before HNCS.

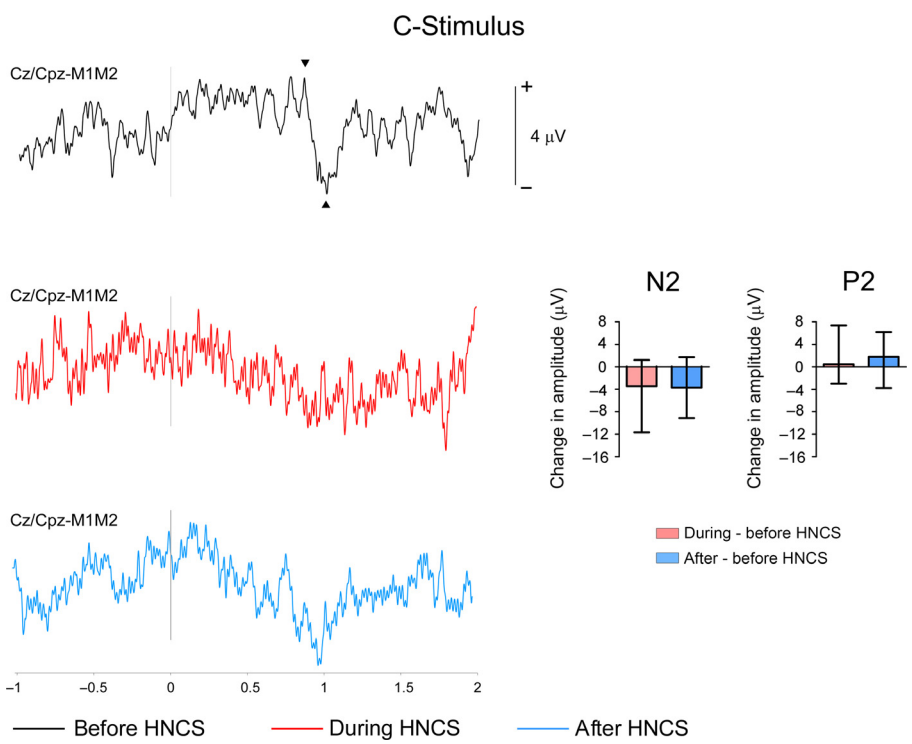

FIG. 5. Event-related potentials elicited by selective C-fibre stimulation. Grand-average waveforms of the vertex ERPs elicited by selective C-stimuli. There was a significant reduction of the vertex negative peak of the C-LEP during and after HNCS as compared with before HNCS. No significant effect of 'block' was observed for the vertex positive peak of the C-ERP. The bar graphs represent the difference in amplitude between conditions. Negative changes in amplitude correspond to a decrease in amplitude as compared with before HNCS.

HNCS modulates ERPs elicited by Aδ-, Aβ- and C-stimuli

In line with previous studies showing a reduction of the vertex negativity (Aδ-N2) after CO_2 laser stimulation (Plaghki *et al.*, 1994) and of the N2-P2 vertex component elicited by contact-heat stimulation (Moont *et al.*, 2011), we found that HNCS reduces the magnitude of the ERPs elicited by nociceptive Aδ-fibre input (experiment 1), as well as the ERPs elicited by C-fibre input (experiment 2). However, because this reduction remained present after HNCS, the observed reduction could be explained by a sustained effect of HNCS, or by an unspecific effect of habituation.

Most notably, we observed that HNCS also modulates the ERPs elicited by non-nociceptive Aβ fibre input. Indeed, the Aβ-P2 was significantly more reduced during HNCS as compared with after HNCS, indicating that the reduction of Aβ-P2 amplitude observed during HNCS was not simply due to response habituation (Fig. 3).

The finding that HNCS exerts a similar effect on the brain responses to nociceptive inputs conveyed by Aδ- and C-fibres and non-nociceptive input conveyed by Aβ-fibres is important. Indeed, the ERPs elicited by non-nociceptive Aβ-fibre input reflect activity that is not mainly relayed in the dorsal horn of the spinal cord, but ascends predominantly through the dorsal columns to reach second-order neurons in the dorsal column nuclei. Therefore, the effect of HNCS on the brain responses to Aβ-fibre input may not be explained by mechanisms involving descending spinal modulatory projections, in particular the spinal–bulbar–spinal loop underlying DNIC. It has to be considered that WDR neurons of the dorsal horn also receive input from non-nociceptive Aβ-fibre afferents, and that the ascending Aβ information through the dorsal column also has, in animals, an indirect components (postsynaptic dorsal column) with relay in the spinal cord. However, the contribution of these Aβ-fibre projections onto the spinothalamic tract to the middle-latency N1, N2 and P2 waves of Aβ-ERPs is probably negligible. This is demonstrated by the results of studies showing that lesions of the spinothalamic tract lead to the dissociated observation of (i) altered or absent Aδ-ERPs and (ii) preserved Aβ-ERPs (Kakigi *et al.*, 1991; Treede *et al.*, 1991; Iannetti *et al.*, 2001, 2013).

What then are the mechanisms that could explain the effects of HNCS on the evoked brain responses to both nociceptive and non-nociceptive inputs? Using functional magnetic resonance imaging (fMRI), Sprenger *et al.* (2011) found that HNCS leads to a tonic increase of blood oxygen level-dependent (BOLD) signal in the anterior cingulate cortex, as well as to increased functional coupling between the cingulate cortex and brainstem regions hypothesized to be involved in the control of descending spinal pain-modulatory pathways. Interestingly, using fMRI of the spinal cord, Sprenger *et al.* (2012) also showed that mental distraction can reduce the BOLD response to nociceptive stimuli at the level of the dorsal horn. Therefore, the effects of HNCS on the brain responses to nociceptive and non-nociceptive inputs might also be explained by top-down cognitive-attentional mechanisms distinct from DNIC. Regarding nociceptive input, there is convincing evidence that at least part of these effects is related to a top-down modulation occurring already at the level of the first relay in the dorsal horn. Whether similar corticofugal mechanisms could modulate the transmission of non-nociceptive somatosensory input at the level of dorsal column nuclei should be investigated. Supporting this hypothesis, it has been shown that corticofugal projections can modulate the responses of dorsal column nuclei to tactile stimuli (Nunez & Malmierca, 2007; Malmierca *et al.*, 2014). This effect has been suggested to contribute to the mechanisms of selective attention. Obviously, the fact that top-down mechanisms triggered by HNCS may modulate the transmission of nociceptive and non-nociceptive somatosensory inputs at the level of their first synaptic relay in the dorsal horn or brainstem does not preclude the fact that somatosensory processing may be further modulated at higher-order relays such as the thalamus and cortex (Dockstader *et al.*, 2010).

Brain responses related to the selective activation of C-fibres

In the first experiment we were unable to identify consistent ERPs related to the activation of C-fibres, in contrast to the results obtained by Magerl *et al.* (1999). This could be due to the fact that assessing the effects of HNCS on the responses elicited by Aβ-, Aδ- and C-stimuli required collecting EEG responses within very limited time periods. Therefore, we were able to collect only a small number of trials, and this could explain why no reproducible C-LEPs were identified in the average waveforms (van den Broeke & Mouraux, 2014a). To overcome this issue, we conducted a second experiment, which focused exclusively on C-LEPs. In this second experiment, we were able to increase the number of trials in which the stimulus selectively activated C fibres, and we were able to collect more C-LEPs in a more reliable fashion.

Conclusion

Our results suggest that HNCS inhibits the responses to nociceptive Aδ- and C-fibre inputs, but also to non-nociceptive Aβ-fibre input. This has important clinical implications as it indicates that the effects of HNCS on the perception or brain responses to noxious stimuli should be used cautiously as a direct measure of DNIC mechanisms and their possible involvement in patients with chronic pain.

Conflict of interest

The authors have no conflict of interest.

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Abbreviations

BOLD, blood oxygen level-dependent; DNIC, diffuse noxious inhibitory control; ERP, event-related potential; fMRI, functional magnetic resonance imaging; HNCS, heterotopic noxious conditioning stimuli; NRS, numerical rating scale; SEP, somatosensory evoked potential; WDR, wide dynamic range.

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