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Low intensity intra-epidermal electrical stimulation can activate Aδ-nociceptors selectively

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ABSTRACT

In the past 30 years, the study of nociception has relied mostly on thermal stimulation to activate nociceptors selectively. However, thermal stimulation suffers from some important limitations. For this reason, investigators have proposed intra-epidermal electrical stimulation (IES) as an alternative method to activate nociceptors selectively. This method relies on the fact that nociceptors are located mainly in the epidermis, while non-nociceptive fibres terminate more deeply in the dermis. Therefore, provided that the difference in receptor depth is sufficient, electric currents spatially restricted to the epidermal layers might activate nociceptors selectively. Here, we examined whether or not IES provides a fully selective nociceptive input. In a first experiment, we used capsaicin to induce a selective denervation of capsaicin-sensitive nociceptors, and thereby test whether the responses to IES are mediated by this population of afferent fibres. We found that capsaicin abolishes both the behavioural and the electrophysiological responses to IES applied at twice the perceptual threshold. In a second experiment, we applied a nerve pressure block to the superficial radial nerve to induce a temporally dissociated impairment of Aβ-, Aδ- and C-fibre afferents, and thereby determine the fibre populations contributing to the responses elicited by IES. We found that the time course of the blockade of the responses to IES follows closely the time course of the blockade of Aδ-fibres, but not of Aβ-fibres. Taken together, our results provide converging evidence that Aδ-nociceptors can be activated selectively using IES, provided that low intensities of stimulation are used.

1. Introduction

In the past 30 years, investigators have relied mostly on thermal stimulation to study nociception and pain perception. Indeed, heating the skin allows activating heat-sensitive Aδ- and C-fibre-free nerve endings selectively [31]. In particular, laser stimulators have been used extensively because they can generate very steep heating ramps and thus elicit time-locked cortical responses (laser-evoked potentials, LEPs) [2,6,13]. In fact, LEPs are currently considered the best available tool to diagnose dysfunctions within nociceptive pathways [8].

However, laser stimulation suffers from some important limitations. First, long inter-stimulus intervals (usually 5–20 s) must separate two stimuli applied at the same location to avoid skin overheating, nociceptor habituation and/or sensitization [31]. Second, currently available stimulators do not offer control of the target skin temperature, which is not determined solely by the physical properties of the stimulus, but also by the biothermal properties of the skin [31]. Therefore, the risk of inducing a skin lesion is not negligible. Third, because of the additional time required for heat conduction and transduction into a neural impulse, the heat-evoked afferent volley is not as synchronous as the afferent volley produced by the direct electrical activation of nerve fibres.

For all these reasons, several investigators have suggested the use of intra-epidermal electrical stimulation (IES) as an alternative method to activate nociceptors selectively [3,14,17]. The method relies on the fact that nociceptive-free nerve endings are located mainly in the epidermis [19], while non-nociceptive fibres terminate more deeply, in the dermis [25]. Therefore, provided that the difference in receptor depth is sufficient, electric currents spatially restricted to the epidermal layers might activate nociceptors selectively.

However, before IES can be used to explore nociception, it is crucial to ensure that this type of somatosensory stimulation is truly selective for nociceptors. So far, a conclusive demonstration of this selectivity is lacking, although several arguments have been put forward to suggest it. It has been shown, for example, that the

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latency of the responses to IES is compatible with the conduction velocity of Aδ-fibres [15]. However, because differences in latency resulting from differences in the conduction velocity of Aδ- and Aβ-fibres are very small, and because the range of velocities within these fibres is very high, this observation does not rule out the possibility that responses to IES are conveyed by Aβ-fibres. Second, it has been shown that topical anaesthetics (e.g. lignocaine) may alter the responses to IES [17]. However, these results are difficult to interpret because these anaesthetics block sodium channels in both nociceptive and non-nociceptive neurons.

Here, we aimed to test whether IES provides a fully selective nociceptive input. In a first experiment, capsaicin was used to induce a selective denervation of capsaicin-sensitive nociceptors [27], and thereby test whether the responses elicited by IES are mediated by this population of afferent fibres. Second, a nerve pressure block was used to induce a temporally dissociated impairment of Aβ-, Aδ- and C-afferents [26,40], and thus determine the fibre populations contributing to the responses elicited by IES.

2. Methods

2.1. Participants

All experimental procedures were approved by the local Ethics Committee. Written informed consent was obtained from all participants. Eleven healthy volunteers took part in the study. Six (six males, aged 22–34 years, median 26 years) participated in the first experiment. Five (three males and two females, aged 22–44 years, median 29 years) participated in the second experiment. Each participant was familiarized with the experimental setup, and exposed to a small number of test stimuli (5–10 stimuli for each stimulus type).

2.2. Experimental design

2.2.1. Experiment 1

Capsaicin cream (Axsain 0.075%, Cephalon, UK) was applied continuously to the postero-lateral side of the right calf (10 cm above the lateral malleolus). A layer of cream (3–5 mm thick) was applied inside a 5×5 cm square window, cut inside a 10×10 cm elasto-gel occlusive dressing (Duoderm, Convatec, UK). The dressing was then covered by a thin plastic adhesive film (Tegaderm, 3 M Health Care, Germany). The dressing prevented the spread of capsaicin outside the boundaries of the treated area. The cream was replaced every 24 h, and applied continuously for a total of 72 h. This topical treatment induces a selective, localized and reversible degeneration of capsaicin-sensitive nociceptors located in the epidermis [27].

Assessments were performed immediately after the end of the treatment, on both the treated (right) and the untreated (left) calves. Aβ-fibre function was assessed by testing the ability of the participant to detect a 4-mN Semmes–Weinstein filament [26]. Aδ-fibre function was assessed by testing the ability of the participant to detect the cold sensation elicited by touching the skin with a 7-mm diameter metal rod cooled to approximately 10 °C [26]. Both types of stimuli were repeated five times on the treated skin and five times on the untreated skin. The thresholds for perceiving intra-epidermal electrical stimuli (IES), non-nociceptive transcutaneous electrical stimuli (ES) and nociceptive CO_2 laser stimuli (LS) were then estimated using an adaptive staircase procedure detailed in the following section. Finally, the EEG was recorded while each type of stimulus (IES, ES and LS) was applied 40 times on the treated skin and 40 times on the untreated skin, as detailed in the following section. The order of presentation of the different stimuli was balanced across the participants and the experimental sessions.

2.2.2. Experiment 2

A selective conduction block of myelinated nerve fibres was achieved by acompression of the left *nervus radialis superficialis*. The right hand rested on a soft padding with the wrist in neutral position. The participants were instructed to hold a fixed vertical handle bar, to prevent pronation or supination of the hand and forearm. A 2-cm wide rubber band was positioned across the forearm, approximately 7 cm proximal to the wrist joint. A 750-g weight was attached at each end of the rubber band. This focal nerve compression procedure has been shown to block preferentially the conduction in myelinated A-fibres [26,40].

Just before starting the nerve compression procedure, the perceptual threshold of IES, ES and LS was estimated using the same adaptive staircase procedure performed in Experiment 1. Immediately after applying the nerve pressure, the progress of the block was monitored by repeating the following sequence, which lasted ~10 min and was separated by a 5-min pause. Aβ-fibre function was assessed by testing the ability of the participant to detect a 4-mN Semmes–Weinstein filament, and Aδ-fibre function was assessed by testing the ability of the participant to detect the cold sensation elicited by a 7-mm diameter metal rod cooled to approximately 10 °C. Both types of stimuli were repeated 5 times. The EEG was then recorded while IES, ES and LS were applied sequentially to the same skin area, as detailed in the following section. Each stimulus type was repeated 15 times. The experiment was interrupted 2 h after the beginning of the block procedure, or once the participants failed to perceive more than half of ES and half of LS with reaction times compatible with the conduction velocity of myelinated nerve fibres (i.e., reaction time <650 ms) [22,26].

Skin temperature was measured throughout both experiments, using an infrared thermometer (Tempett, SENSELab, Sweden), to ensure that observed differences were not due to differences in baseline skin temperature.

2.3. Sensory stimuli

An auditory warning signal was given 2–5 s before the onset of each stimulus. The inter-stimulus interval was 10–15 s (rectangular distribution).

Intra-epidermal electrical stimuli (IES) consisted of two rapidly succeeding constant-current square-wave pulses as described in [16]. The inter-pulse interval was 10 ms. Each pulse lasted 50 μs (Digitimer DS7, Digitimer, UK). Stimuli were delivered using a stainless steel concentric bipolar needle electrode developed by Inui et al. [14,16], consisting of a needle cathode (length: 0.1 mm, Ø: 0.2 mm) surrounded by a cylindrical anode (Ø: 1.4 mm). By gently pressing the device against the skin, the needle electrode was inserted into the epidermis (Fig. 1). In Experiment 1, three different intensities were used: (1) twice the perceptual threshold at the untreated calf (0.18 ± 0.15 mA, mean ± SD), (2) 0.25 mA and (3) 2.5 mA. In Experiment 2, a single intensity was used, corresponding to twice the perceptual threshold estimated on the hand dorsum immediately before starting the nerve compression procedure (0.08 ± 0.01 mA).

Non-nociceptive transcutaneous electrical stimuli (ES) consisted of a single current square-wave electrical pulse (50 μs duration) delivered through a pair of round felt-tip electrodes soaked with electrolyte and applied against the skin (Ø: 0.5 cm, 1-cm inter-electrode distance) (Fig. 1). In Experiment 1, the intensity of the electrical stimulus corresponded to twice the perceptual threshold at the untreated calf (2.9 ± 0.9 mA). In Experiment 2, the intensity of the electrical stimulus corresponded to twice the perceptual threshold estimated on the hand dorsum before starting the nerve compression procedure (1.5 ± 0.3 mA). In all the participants, the stimulus elicited a clear non-painful paresthesia (most often reported as a tingling sensation). Importantly, transcutaneous electrical stimuli delivered at these low intensities may be considered

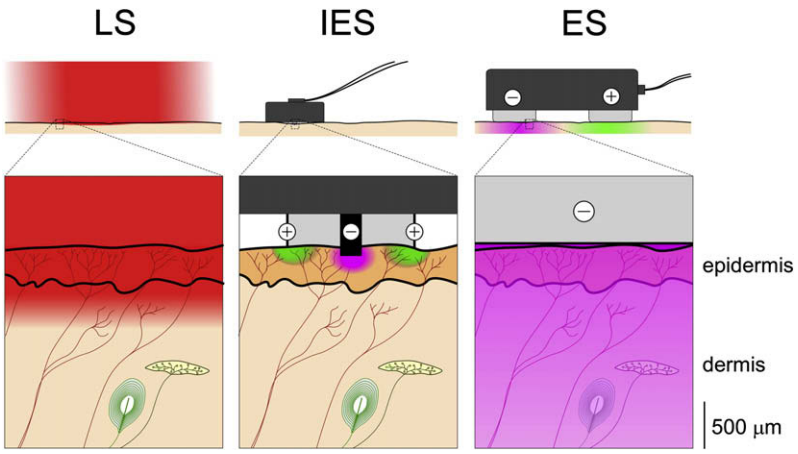

Fig. 1. Schematic representation of non-nociceptive laser stimulation (LS), intra-epidermal electrical stimulation (IES) and transcutaneous electrical stimulation (ES) of the hairy skin. Only Aδ and C nociceptive-free nerve endings (shown in dark red) can be found in the most superficial layers of the epidermis. Non-nociceptive myelinated endings are located deeper in the dermis (e.g. Ruffini and Pacini corpuscules, shown in yellow and green). *Left panel:* the increase in skin temperature generated by LS activates heat-sensitive nociceptive afferents located in the epidermis selectively. *Middle panel:* because the electric current generated by IES is spatially restricted to the epidermis, it is hypothesized to selectively activate nociceptive-free nerve endings located in the epidermis. *Right panel:* the electric current generated by ES activates deeper large-diameter non-nociceptive myelinated nerve fibres preferentially as these have a lower electrical activation threshold than small-diameter nociceptive afferents.

to be well below the electrical activation threshold of nociceptive Aδ- and C-fibres [5].

Nociceptive laser stimuli (LS) consisted of radiant heat pulses generated by a CO_2 laser (wavelength: 10.6 μm) designed and built in the Department of Physics of the Université catholique de Louvain [30] (Fig. 1). Stimulus duration was 40 ms. Beam diameter at target site was 10 mm. To avoid skin overheating and minimize nociceptor sensitization or habituation, the target of the laser beam was slightly displaced between each trial using a mirror set on a two-axis computer-controlled device. In Experiment 1, the energy of the laser stimulus corresponded to 1.5 times the perceptual threshold of Aδ-fibre input at the untreated calf, with a maximum of 9.5 mJ/mm^2 (8.9 ± 0.8 mJ/mm^2). In Experiment 2 the energy of the laser stimulus corresponded to 1.5 times the perceptual threshold of Aδ-fibre input estimated on the hand dorsum before starting the nerve compression procedure, with a maximum of 9.5 mJ/mm^2 (8.2 ± 0.9 mJ/mm^2). Measurement of stimulus energy density was performed at the beginning and end of each experiment using an optical energy meter (13PEM001, Melles Griot, The Netherlands).

2.4. Behavioural measures

Detection thresholds for IES, ES and LS were estimated using an adaptive staircase procedure. For LS, two distinct thresholds were estimated: the threshold for absolute detection, and the threshold for detection with reaction times (RT) < 650 ms [24]. Because RTs < 650 ms are compatible with the conduction velocity of small-diameter myelinated Aδ-fibres, but not with the slower conduction velocity of unmyelinated C-fibres, and because the thermal activation threshold of C-nociceptors is lower than that of Aδ-nociceptors, the absolute detection threshold reflected the detection threshold of C-fibre input (i.e. "second pain"), while threshold for detection with RTs < 650 ms reflected the detection threshold of Aδ-fibre input (i.e. "first pain"). The initial stimulus intensity in the adaptive staircase was 0.2 mA for IES, 1 mA for ES and 500 mJ for LS, and the initial step sizes were 0.1 mA, 0.5 mA, and 200 mJ, respectively. After the first staircase reversal (i.e. when the stimulus was detected if previously undetected, or when the stimulus was undetected if previously detected), step size was reduced to 0.02 mA, 0.1 mA, and 50 mJ, respectively. The procedure was interrupted after the occurrence of four staircase reversals at final step size. Thresholds were estimated by averaging the intensity/energy of the stimuli at which these four reversals occurred.

Reaction times. In both experiments, the participants were instructed to push a button held in the right hand as soon as they perceived the stimulus. A digital chronometer with a 1-ms resolution was used to measure the time elapsed between the onset of the stimulus and the button press.

Quality and intensity of perception. In the first experiment, the participants were asked to describe verbally the quality of perception by selecting one or more items from the following list of seven descriptors: "light touch", "touch", "shock", "tingling", "warm", "pricking" and "burning" [26]. The participants were then asked to report verbally the intensity of perception using a numerical rating scale (NRS) ranging from 0 to 100 [26]. Extremities of the scale were defined as "no detection" (NRS = 0) and "maximum pain" (NRS = 100). An anchor at the middle of the scale (NRS = 50) marked the borderline between non-painful and painful domains of sensation.

2.5. Electrophysiological measures

Experiments were conducted in a dim, silent, temperature-controlled room. The participants laid semi-supine in a comfortable armchair, and were instructed to keep their gaze fixed on a black cross (3 × 3 cm) placed centrally in front of them, at a distance of approximately 2 m, 30° below eye level. The electroencephalogram (EEG) was recorded using four Ag–AgCl electrodes placed on the scalp at positions Fz, Cz, T3 and T4 (International 10–20 system). Linked earlobes (A1A2) were used as reference. Ocular movements and eye-blinks were recorded using two surface electrodes placed at the upper-left and lower-right sides of the right eye. Signals were amplified and digitized using a sampling rate of 167 cps (PL-EEG, Walter Graphtek, Germany). Continuous EEG recordings were segmented into 2.5 s-long epochs (−0.5 to +2.0 s relative to stimulus onset) and band-pass filtered (0.5–30 Hz). After baseline-correction (reference interval −0.5 to 0 s), epochs with amplitude values exceeding ± 100 μV (i.e. epochs likely to be contaminated by an artefact) were rejected. These epochs constituted 2.3 ± 1.6% of the total number of epochs. Separate average ERP waveforms were computed for each participant, stimulus type, and experimental condition. For each waveform, the negative and positive peaks of the ERP were measured as follows. The negative peak was defined as the most

negative deflection occurring between 50 and 350 ms following stimulus onset, while the positive peak was defined as the most positive deflection occurring between 100 and 500 ms following stimulus onset. All EEG processing steps were carried out using Letswave (amouraux.webnode.com/letswave) [23] and Matlab (The MathWorks, USA).

2.6. Statistical analyses

Statistical analyses were carried out using Prism v5.0 (GraphPad Software, USA) and Matlab (The MathWorks, USA).

3. Results

3.1. Experiment 1: capsaicin

3.1.1. Skin temperature

The skin temperatures measured at the treated site (31.7 ± 1.0 °C) and at the control site (31.4 ± 1.4 °C) were not significantly different ($p = 0.630$, paired two-sample t-test).

3.1.2. Psychophysical results

Detection rates and reaction times. The detection rate of light touch was unaffected by capsaicin treatment: detection rates were normal (defined as >50% detection rate) both at the control site (97 ± 8% detection) and at the treated site (93 ± 10% detection) ($p = 0.363$). In contrast, the detection rate of cold stimuli was markedly affected by capsaicin: detection rates were normal at the control site (97 ± 8% detection), while none of the participants were able to detect a single cold stimulus applied to the treated site (0% detection) ($p < 0.0001$).

The threshold for detecting ES was unaffected by capsaicin: detection thresholds were similar at the control site (1.4 ± 0.5 mA) and at the treated site (1.5 ± 0.5 mA) ($p = 0.747$). In contrast, the thresholds for detecting Aδ-related "first pain" and C-related "second pain" elicited by LS were both markedly affected by capsaicin: while the thresholds for detecting Aδ- and C-fibre inputs were 842 ± 49 mJ and 542 ± 116 mJ at the control site, LS were no longer detected at the treated site, even when stimulus energies were increased to 1100 mJ (i.e., the energy set as an upper limit to avoid skin lesions). Such as LS, the threshold for detecting IES was markedly affected by capsaicin: the threshold for detecting IES was significantly higher at the treated site (0.60 ± 0.45 mA) than at the control site (0.09 ± 0.07 mA) ($p = 0.043$). At the treated site, the threshold for detecting IES was, on average, 16.3 times greater than the threshold for perceiving the same stimulus applied to the control site.

The frequency distributions of RTs are shown in Fig. 2. The detection rate of ES was 100% in all participants, both at the treated site and at the control site. Furthermore, mean RTs were similar at both sites (274 ± 55 vs. 283 ± 47 ms; $p = 0.814$). In contrast, while the detection rate of LS applied at the control site was 100% in all participants, the detection rate of LS applied at the treated site was greatly reduced (7 ± 10%; $p < 0.0001$). On few occasions where LS was detected at the treated site, the RT was greatly increased as compared to the control site (1514 ± 278 vs. 504 ± 105 ms; $p < 0.0001$). Similarly, while the detection rate of IES applied at the control site at twice the perceptual threshold was 100% in all participants, the detection rate of the same stimulus applied at the treated site was drastically reduced (3 ± 8%; $p < 0.0001$). The detection rate of IES applied at 0.25 mA was reduced at the treated site (56 ± 50%) as compared to the control site (100%), but this difference was not significant across the participants ($p = 0.082$). RTs were not significantly different (462 ± 67 ms vs. 374 ± 51 ms; $p = 0.153$). Finally, the detection rate of IES applied at 2.5 mA was 100% in all participants, both at the control site and at the treated site, and RTs were comparable across the two sites (274 ± 29 ms vs. 289 ± 58 ms; $p = 0.582$).

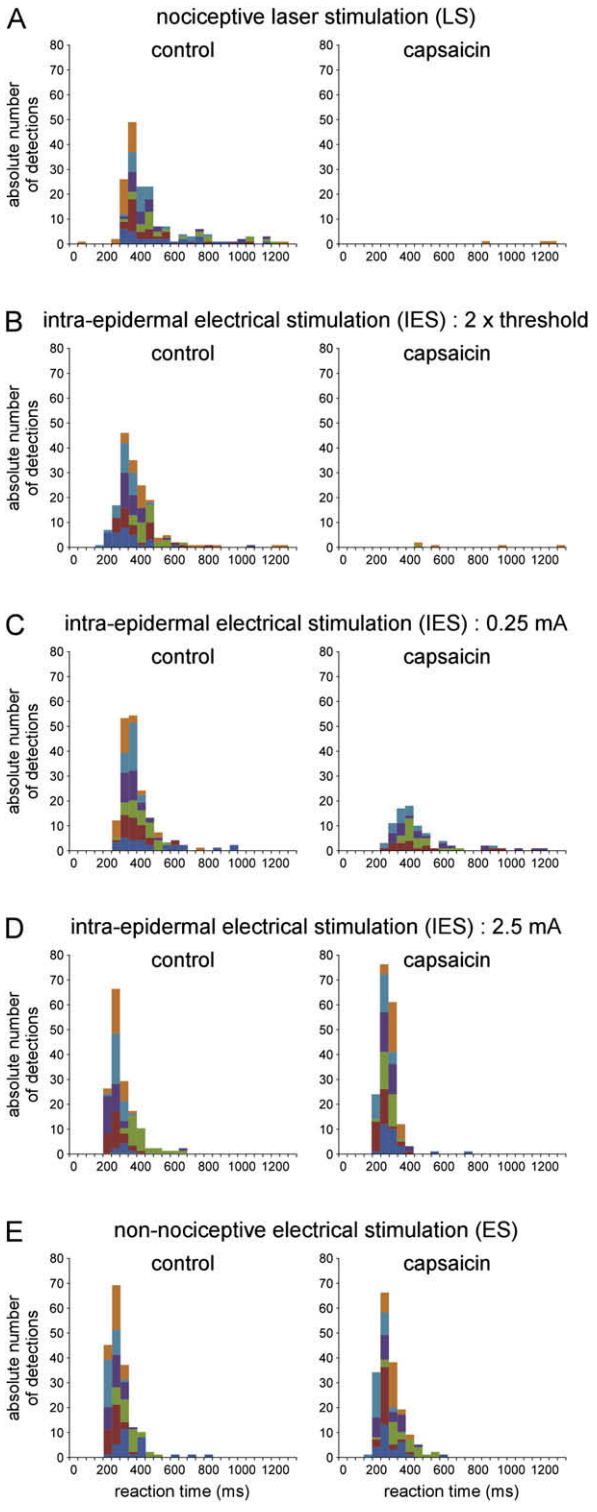

Fig. 2. Frequency distribution of reaction times to nociceptive laser stimuli (LS) (A), intra-epidermal electrical stimuli (IES) delivered at twice the perceptual threshold (B), at 0.25 mA (C) and at 2.5 mA (D), and non-nociceptive electrical stimuli (ES) (A) (Experiment 1). In all panels, stimuli applied to the control site are shown in the left graph, while those applied to the capsaicin-desensitized site are shown in the right graph. x axis, reaction time categorized in 50-ms bins. y axis, absolute number of detections. The different colours represent different participants. Note how capsaicin alters profoundly the detection of LS and IES delivered at twice the perceptual threshold. Also note that capsaicin does not affect the detection of ES and IES delivered at 2.5 mA.

Quality of perception. Capsaicin did not affect the quality of the percept elicited by ES. Indeed, regardless of whether the stimulus was applied at the control site or at the treated site, the percept was most often described as a "shock", "touch" or "tingling" sensation (Fig. 3). In contrast, capsaicin had a profound effect on the quality of the percept elicited by LS. While LS applied at the control site were most often described as "pricking", "burning" or "tingling", in the few instances where LS applied at the treated site were perceived (7 ± 10% of the total number of stimuli), they were mostly described as "warm" or "light touch". Similarly, the quality of the sensations elicited by IES applied at twice the perceptual threshold and at 0.25 mA was markedly affected by capsaicin. Indeed, these stimuli were most often described as "pricking" at the control site, but not at the treated site. Finally, the quality of the sensations elicited by IES applied at 2.5 mA were unaffected by capsaicin.

3.2. Electrophysiological results

At the control site, all stimuli elicited a clear vertex potential, constituted by a negative–positive biphasic wave (Fig. 4 and Table 1).

The effect of capsaicin treatment on the negative–positive (*N–P*) complex elicited by ES, LS and IES is detailed in Fig. 4 and Table 2. Capsaicin did not affect the *N–P* complex elicited by ES. Indeed, the magnitude (*N–P* amplitude: 28.8 ± 13.6 μV at control site, 24.9 ± 11.5 μV at treated site; $p = 0.394$) and latency (*N*-wave latency: 135 ± 13 ms at control site and 128 ± 53 ms at treated site; $p = 0.766$; *P*-wave latency: 293 ± 85 ms at control site and 306 ± 84 ms at treated site; $p = 0.649$) of the *N–P* complex were similar at control and treated sites. In contrast, capsaicin markedly affected the *N–P* complex elicited by LS: although an *N–P* complex was identified clearly in every single participant when LS were delivered at the control site (*N–P*: 36.4 ± 18.4 μV; *N*: 273 ± 35 ms; *P*: 399 ± 57 ms), not a single *N–P* complex was identified when LS were delivered at the treated site. Likewise, capsaicin treatment abolished completely the *N–P* complex evoked by IES: although an *N–P* complex was identified clearly in every single participant when IES was applied at twice the perceptual threshold at the treated site (*N–P*: 24.6 ± 12.7 μV; *N*: 199 ± 47 ms; *P*: 369 ± 83 ms), not a single *N–P* complex was identified when the same stimulus was applied at the treated site. Similarly, capsaicin treatment reduced markedly the magnitude of the *N–P* complex elicited by IES applied at 0.25 mA (*N–P*: 22.7 ± 5.7 μV at control site and 5.7 ± 9.0 μV at treated site; $p = 0.036$), and increased its peak latency (*N*-wave: 159 ± 39 ms at control site and 211 ± 14 ms at treated site; $p = 0.045$; *P*-wave: 340 ± 111 ms at control site and 386 ± 76 ms at treated site; $p = 0.669$). In contrast, both the amplitude (*N–P*: 27.1 ± 10.7 μV at control site, 35.2 ± 15.8 μV at treated site; $p = 0.130$) and the latency (*N*-wave: 145 ± 75 ms at control site and 163 ± 33 ms at treated site; $p = 0.564$; *P*-wave: 312 ± 67 ms at control site and 311 ± 69 ms at treated site; $p = 0.978$) of the *N–P* complex following IES applied at 2.5 mA were entirely unaffected by capsaicin.

3.3. Experiment 2: nerve pressure block

3.3.1. Skin temperature

The skin temperatures measured before (31.4 ± 1.7 °C) and at the end (30.7 ± 1.2 °C) of the nerve compression procedure were not significantly different ($p = 0.441$, paired two-sample *t*-test).

3.3.2. Psychophysical results

Before the nerve compression procedure, the detection rate of tactile and cold stimuli was 100% in all participants. Impairment of light touch detection (defined as a detection rate < 50%) appeared 52 ± 7 min after the onset of the nerve compression procedure, while impairment of cold detection appeared 46 ± 14 min after the onset of the nerve compression procedure. In other words, the nerve compression appeared to impair the detection of light touch and the detection of cold at very similar latencies ($\Delta t = 6 \pm 8$ min; $p = 0.180$).

Before the nerve compression procedure, the detection rate of ES and LS was close to 100% in all participants (Fig. 5A), and both ES and LS were detected with RTs compatible with the conduction velocity of myelinated nerve fibres (RT < 650 ms). Impairment of the detection of Aβ-fibre input conveyed by ES (detection rate < 50%) appeared 74 ± 27 min after compression onset, in all but one participant. Impairment of the detection of Aδ-fibre input conveyed by LS (detection rate < 50% with RT < 650 ms) appeared 51 ± 11 min after compression onset. In other words, the nerve compression appeared to impair the detection of Aβ-fibre input conveyed by ES only after the impairment of the detection of Aδ-fibre input conveyed by LS ($\Delta t = 23 \pm 18$ min; $p = .0484$).

Similar to what was observed for ES and LS, before the nerve compression procedure, the detection rate of IES applied at twice the detection threshold was close to 100% (Fig. 5A). In all participants, impairment of the detection of IES appeared simultaneously to the impairment of the detection of Aδ-fibre input conveyed by LS (i.e., 51 ± 11 min after block onset; $\Delta t = 0$ min), and, hence,

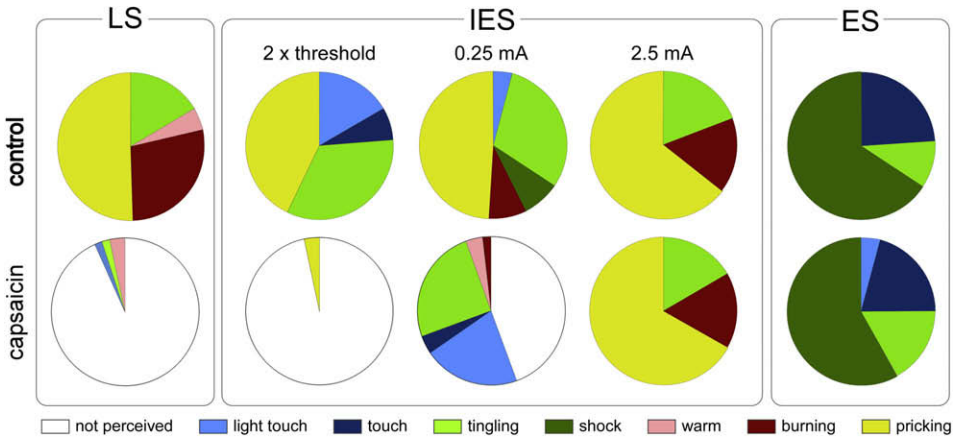

Fig. 3. Quality of perception elicited by nociceptive laser stimuli (LS, left panel), intra-epidermal electrical stimuli (IES) delivered at twice the perceptual threshold, 0.25 mA and 2.5 mA (middle panel), and non-nociceptive electrical stimuli (IES, right panel), applied at the control site (upper graphs) or at the capsaicin-desensitized site (lower graphs). The pie charts represent the number of times each descriptor was chosen, expressed as percentage of the total number of reports (group-level average).

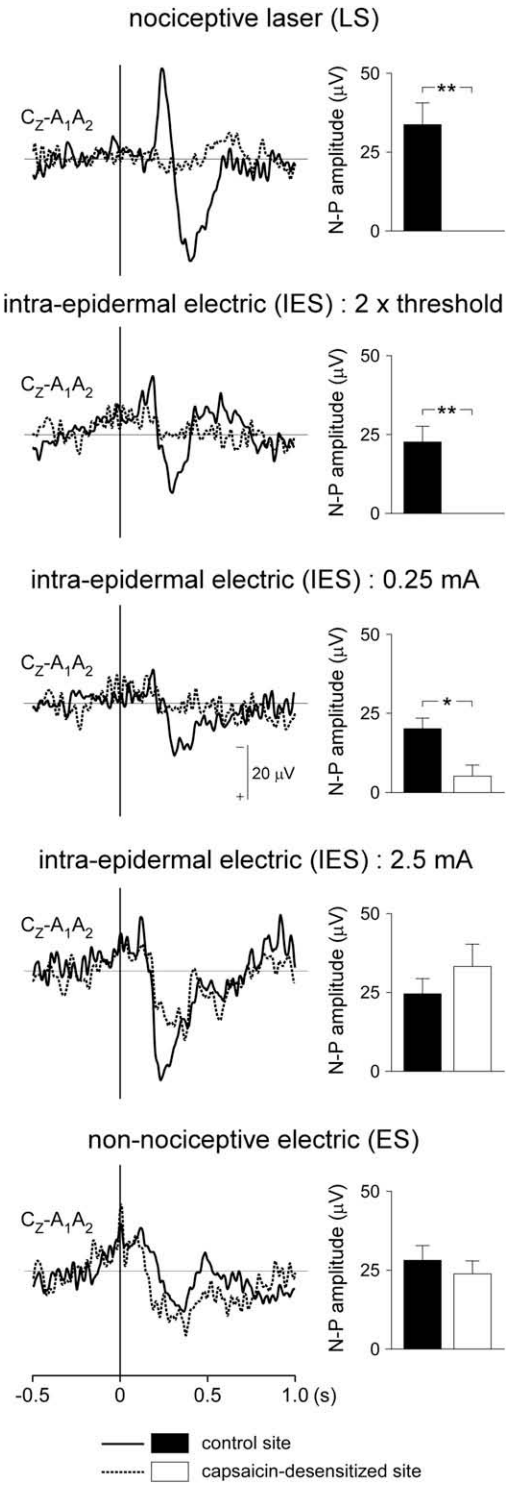

Fig. 4. Event-related potentials (ERPs) elicited by nociceptive laser stimuli (LS), intra-epidermal electrical stimuli (IES) delivered at twice the perceptual threshold, at 0.25 mA and at 2.5 mA, and non-nociceptive electric stimuli (ES), applied either to the control site or to the capsaicin-desensitized site. ERP waveforms are from one representative subject (signals recorded from C_Z vs. A_1A_2; *x* axis, time in s; *y* axis, amplitude in μV). Bar graphs represent the group-average *N*–*P* peak-to-peak amplitudes (*y* axis, mean ± SD amplitude, in μV). Note that the ERPs elicited by LS are abolished by capsaicin, while the ERPs elicited by ES are unaffected by capsaicin. Most importantly, note that the ERPs elicited by IES delivered at twice the perceptual threshold are also abolished by capsaicin. Finally, note that the ERPs elicited by IES delivered at 2.5 mA are unaffected by capsaicin. $^{*}p < .05$, $^{**}p < .005$ (paired two-sample *t*-test).

Table 1
Latencies of the negative–positive (*N*–*P*) complex elicited by non-nociceptive electric stimuli (ES), nociceptive laser stimuli (LS) and intra-epidermal electric stimuli (IES) delivered at the control site in Experiment 1.

	ES	LS	IES	
N latency	134 ± 13	274 ± 35	2 × threshold	199 ± 47
			0.25 mA	159 ± 39
			2.5 mA	145 ± 75
P latency	293 ± 85	399 ± 57	2 × threshold	369 ± 83
			0.25 mA	340 ± 111
			2.5 mA	312 ± 67

N and *P* latencies are expressed in milliseconds (mean ± SD). The shorter latency of the *N*–*P* complex elicited by IES vs. LS is most probably due to the additional time required for LS to reach the thermal activation threshold of Aδ-nociceptors, and the additional time required for the transduction of LS into a neural impulse. It could also be due to the fact that type I AMH, which have a faster conduction velocity than type II AMH, contributed to the responses elicited by IES, but not to the responses elicited by LS (see also [37,38]).

before the impairment of the detection of Aβ-fibre input conveyed by ES (Δt = 23 ± 18 min; p = .0484) (Fig. 5B).

Once the detection of Aδ-fibre input conveyed by LS was impaired, the participants were still able to detect LS (detection rate: 87 ± 14%), but with delayed RTs (974 ± 255 ms) compatible only with the slower conduction velocity of unmyelinated C-fibres (RT > 650 ms). In contrast, the participants were no longer able to detect IES at all (detection rate: 7 ± 12%). In other words, spared C-fibre conduction appeared to allow the detection of LS, but not the detection of IES.

3.3.3. Electrophysiological results

Because the time course of the nerve conduction block was different for each participant, to perform a group-level comparison, all EEG epochs were categorized according to whether or not Aβ-fibre detection was impaired (detection rate < 50% to ES), and whether or not Aδ-fibre detection was impaired (detection rate < 50% to LS with RT < 650 ms). Because, in all participants, the impairment of Aδ-fibre detection preceded the impairment of Aβ-fibre detection, this led to three trial categories: Aβ- and Aδ-fibres preserved, Aβ-fibres preserved but Aδ-fibres blocked and Aβ- and Aδ-fibres blocked. The obtained average waveforms are shown in Fig. 6.

At the beginning of the block, when both Aβ- and Aδ-fibre detections were still preserved, all types of stimuli elicited a clear negative–positive complex: ES (*N*–*P* amplitude: 66 ± 24 μV; *N*-wave latency: 108 ± 41 ms; *P*-wave latency: 295 ± 31 ms), LS (*N*–*P*: 32 ± 17 μV; *N*: 193 ± 48 ms, *P*: 314 ± 74 ms) and IES (*N*–*P*: 35 ± 12 μV; *N*: 147 ± 48 ms, *P*: 240 ± 66 ms) (Fig. 6, left panel). Once Aδ-fibre detection was impaired, ES still elicited a clear *N*–*P* complex in all participants (*N*–*P*: 26 ± 6 μV; *N*: 85 ± 40 ms, *P*: 237 ± 92 ms), while LS and IES did not elicit any identifiable response (Fig. 6, middle panel). Once both Aδ- and Aβ-fibre detections were impaired, ES elicited an identifiable *N*–*P* complex in only 1 of 5 participants (*N*–*P*: 26.7 μV; *N*: 201 ms, *P*: 326 ms), while LS and IES did not elicit any identifiable response (Fig. 6, right panel).

4. Discussion

The present study provides converging experimental evidence that Aδ-nociceptors can be activated selectively using IES, provided that low intensities of stimulation are used (e.g. not above twice the perceptual threshold). Indeed, in Experiment 1, we show that capsaicin desensitization abolishes both the behavioural and the electrophysiological responses elicited by LS and IES applied at twice the perceptual threshold, while it spares entirely the

Table 2
Latencies and amplitude of the negative–positive (*N–P*) complex elicited by non-nociceptive electric stimuli (ES), nociceptive laser stimuli (LS) and intra-epidermal electric stimuli (IES) delivered at the control and capsaicin-desensitized sites (Experiment 1).

Stimulus type		*N* peak latency (ms)		*P* peak latency (ms)		*N–P* amplitude (μV)		
		Control	Capsaicin	Control	Capsaicin	Control	Capsaicin	
ES		134 ± 13	128 ± 53	293 ± 85	306 ± 84	28.8 ± 13.6	24.9 ± 11.5	
LS		274 ± 35	–	399 ± 57	–	36.3 ± 18.4	(0)	**
IES	2 × threshold	199 ± 47	–	369 ± 83	–	24.6 ± 12.6	(0)	**
	0.25 mA	159 ± 39	210 ± 14*	340 ± 111	386 ± 76	22.7 ± 8.4	5.7 ± 9.0	*
	2.5 mA	145 ± 75	162 ± 33	312 ± 67	311 ± 69	36.1 ± 18.0	26.4 ± 12.9	

N and *P* latencies are expressed in milliseconds (mean ± SD). $^{*}p < .05$; $^{**}p < .005$ (paired *t*-test comparison between control and treated sites).

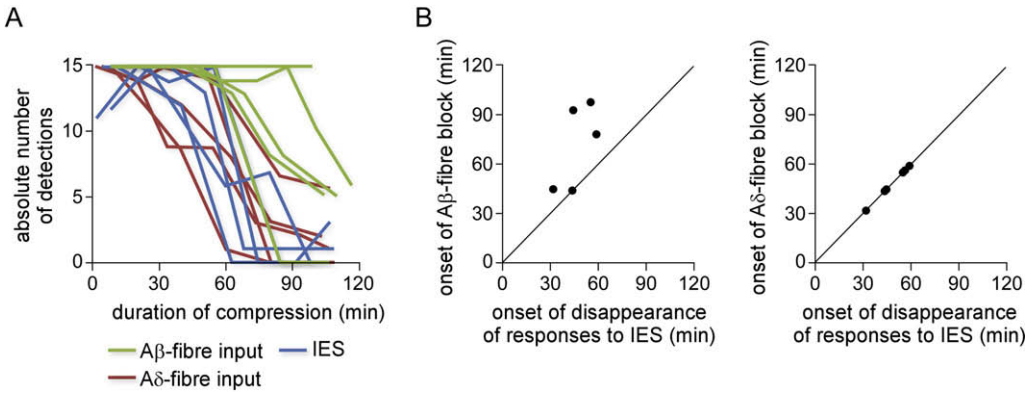

Fig. 5. Left panel: detection rate of Aβ-fibre input conveyed by non-nociceptive electrical stimuli (ES, in green), Aδ-fibre input conveyed by nociceptive laser stimuli (LS, in red), and intra-epidermal electric stimuli applied at twice the perceptual threshold (IES, in blue) as a function of the duration of the nerve compression procedure. Each line represents the time course of a single participant. *y* axis, number of detections with a reaction time compatible with the conduction velocity of myelinated nerve fibres (RT < 650 ms), *x* axis, time relative to the onset of the nerve compression. Note that the blockade of Aβ-fibres appeared after the blockade of Aδ-fibres. Most importantly, note that the time course of the blockade of IES is very similar to the time course of the blockade of Aδ-fibres. Right panel. The onsets of the block to IES (detection rate < 50%) are plotted against the onsets of the Aβ-fibre block (left graph) and Aδ-fibre block (right graph). Each point represents a single participant. Note that the sensory input elicited by IES was blocked before the blockade of Aβ-fibre input, and simultaneously to the blockade of Aδ-fibre input.

responses elicited by ES. This indicates that the bulk of the responses elicited by IES delivered at twice the perceptual threshold are mediated by capsaicin-sensitive Aδ-fibre afferents. In Experiment 2, using a nerve pressure block, we show that the time course of the blockade of the responses elicited by IES closely follows the time course of the blockade of the Aδ-related responses elicited by LS. This further indicates that the responses elicited by IES delivered at twice the perceptual threshold are conveyed by Aδ-fibres, and not by Aβ-fibres. Furthermore, we show that when myelinated Aδ- and Aβ-fibres are blocked, LS still elicits responses related to the activation of C-fibres, while IES does not, thus indicating that C-fibres do not contribute significantly to the percept elicited by IES.

4.1. Effect of capsaicin on IES

The vanilloid capsaicin excites selectively the subgroup of nociceptive afferents that express the vanilloid receptor 1 (TRPV-1), which is also activated by noxious heat and acids, and is thought to play a major role in pain sensation and hyperalgesia [7]. In addition to this excitatory effect, capsaicin can also exert a neurotoxic effect on the same population of afferent fibres: the prolonged topical application of capsaicin leads to a near-complete elimination of epidermal-free nerve endings [18,27,32], resulting in heat analgesia [1,18,27] and disappearance of heat-evoked potentials [1]. Although this "excitotoxic" effect of capsaicin is not clearly understood, it has been suggested to result from excessive intracellular calcium and induction of proteases [7]. Several studies have indicated that capsaicin induces a dissociated impairment of nociception, affecting profoundly Aδ- and C-fibre-mediated heat nociception, but affecting only moderately Aδ-fibre-mediated mechanical nociception [1,10,21,34,35]. This observation has been interpreted as reflecting the fact that nociceptors involved more specifically in mechanical nociception (type I Aδ mechano-heat nociceptors [AMH] and high-threshold mechano-receptors [HTM]) are devoid of TRPV-1 receptors and are thus insensitive to capsaicin [33,36].

As expected, we observed that the behavioural and electrophysiological responses to LS, which are mediated by the activity of capsaicin-sensitive type II AMHs [38], were entirely abolished by capsaicin, while the perceptual and electrophysiological responses to ES, which are mediated by the activity of capsaicin-insensitive, non-nociceptive Aβ fibres, were entirely unaffected by capsaicin. Therefore, the finding that the behavioural and electrophysiological responses to IES presented at twice the perceptual threshold were entirely abolished by capsaicin constitutes strong evidence that these responses reflect mainly the activation of capsaicin-sensitive nociceptive fibres.

The observation that the responses to IES presented at twice the perceptual threshold were completely abolished by capsaicin is thus surprising. Indeed, IES is expected to bypass transduction by activating axonal fibres directly. Hence, if we accept that the psychophysical responses to high-intensity punctuate mechanical stimuli are preserved after capsaicin treatment because they are conveyed by capsaicin-insensitive nociceptive afferents (type I AMH) [21], one would also expect a residual psychophysical and/or electrophysiological response to IES consequent to the activation of this spared population of nociceptive fibres. One possibility, although speculative, could be that the capsaicin-resistant responses to high-intensity punctate mechanical stimuli may be related not to the activation of type I AMH but to the activation of non-nociceptive low-threshold mechano-receptors. Alternatively,

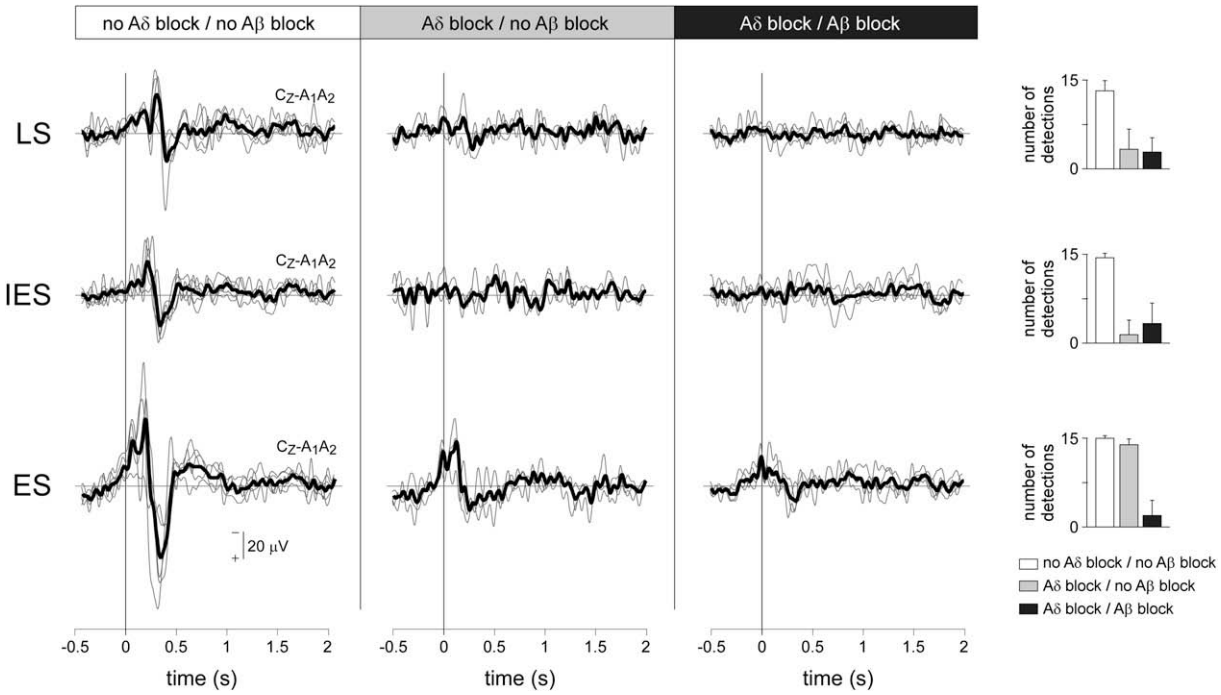

Fig. 6. Event-related potentials (ERPs) elicited by nociceptive laser stimuli (LS), intra-epidermal electrical stimuli applied at twice the perceptual threshold (IES) and non-nociceptive electric stimuli (ES) during the three different stages of the nerve pressure block: (1) when Aβ- and Aδ-fibre conductions are still preserved, (2) when Aβ-fibre conduction is preserved but Aδ-fibre conduction is impaired and (3) when both Aβ- and Aδ-fibre conductions are impaired. Thin grey waveforms represent single-subject ERPs while the thick black waveform represents the group-level average ERP (signals recorded from C_Z vs. A_1A_2; *x* axis, time in s; *y* axis, amplitude in μV). Detection rates to LS, IES and ES are shown in the right graphs. Preserved Aβ-fibre conduction was defined as >50% detection rate for ES while preserved Aδ-fibre conduction was defined as >50% detection rate for LS with reaction times <650 ms (i.e. reaction times compatible with the conduction velocity of myelinated A-fibres). Note that once Aδ-fibre detection is impaired, ES still elicits a clear ERP while LS and IES elicit no identifiable ERP.

one should consider that IES activates capsaicin-sensitive type II AMH nociceptors but not capsaicin-insensitive HTM and type I AMH nociceptors.

While the responses to IES applied at twice the perception threshold were completely abolished by capsaicin, the responses to IES applied at 0.25 mA were only reduced (Fig. 4). Furthermore, the responses to IES applied at 2.5 mA were completely unaffected by capsaicin. These findings indicate that the selectivity of IES for nociceptive afferents is lost completely as soon as the intensity of the stimulation is increased, most probably because the spread of the electric current generated by the more intense stimulus becomes sufficient to activate the more deeply-located non-nociceptive afferents.

4.2. Effect of nerve pressure block on IES

There is a large body of evidence indicating that focal compression of a peripheral nerve induces, within the first hours of the procedure, a differential impairment of the function of myelinated and unmyelinated fibres, impairing the function of myelinated fibres while preserving the function of unmyelinated fibres [4,9,12,26,37,39,40]. For example, in humans, it has been shown that applying pressure to a peripheral nerve for 20–60 min abolishes tactile sensations related to Aβ-fibres and cold sensations related to Aδ-fibres, while C-fibre-mediated thermal sensations are preserved up to 2 h after the onset of the compression [4,26,37,39]. Furthermore, it has been suggested that the time required to block the conduction of small-diameter Aδ-fibres is greater than the time required to block the conduction of large-diameter Aβ-fibres [9,20]. Indeed, some investigators have shown that Aδ-fibre sensations evoked by noxious thermal or mechanical stimuli disappear after a significantly longer delay than light-touch sensations [20,26,40]. The mechanisms underlying the effect of nerve compression on nerve conduction remain speculative (e.g. nerve dysfunction due to focal ischemia or to direct mechanical nerve deformation) [11,28], and may be largely dependent on the specific method used to produce the block. Hypothesized mechanisms include cleavage and displacement of the myelin sheath, which could explain why myelinated fibres are more sensitive to pressure than unmyelinated fibres.

Our results confirm the notion that unmyelinated C-fibres are more resistant to compression than myelinated Aδ- and Aβ-fibres. However, although we observed that the ability to detect light touch related to the activation of Aβ-fibres by the 4 mN Semmes–Wenstein filament disappeared before the ability to detect "first pain" related to the activation of Aδ-fibres by LS, our results question the notion that small-myelinated Aδ-fibres are more resistant to compression than large-myelinated Aβ-fibres. Indeed, we found that the behavioural and electrophysiological responses to the activation of Aβ-fibres by ES disappeared significantly later than the behavioural and electrophysiological responses to the activation of Aδ-fibres by LS. In fact, these observations suggest that differences between the latency of the blockade of the responses elicited by the activation of Aβ-fibres (detection of light touch: 52 ± 7 min, detection of ES: 74 ± 27 min) and Aδ-fibres (detection of LS: 51 ± 11 min) result from differences in the temporal and spatial recruitment of nerve fibres by the different stimuli, rather than from differences in the relative resistance to pressure of myelinated Aδ- and Aβ-fibres, as suggested previously [9,20]. For example, when Aβ-fibre conduction is only partially impaired, the ability to detect light touch but not ES could be explained by the fact that the spatial and temporal recruitment of nerve fibres by ES is stronger than the spatial and temporal recruitment of nerve fibres by light touch.

Nevertheless, we found that the time course of the blockade of the responses to IES followed more closely the time course of the blockade of the Aδ-fibre responses to LS than the time course of

the blockade of the Aβ-fibre responses to ES. This finding provides additional evidence that the responses to IES, when applied at twice the perception threshold, are conveyed by nociceptive Aδ-fibres.

Interestingly, once myelinated Aβ- and Aδ-fibres were blocked entirely, LS were still able to elicit a perception of "second pain", related to the activation of spared unmyelinated C-fibres. In contrast, IES were no longer perceived, thus indicating that, unlike LS, IES did not activate C-fibres in a manner sufficient to elicit a sensation. Because, at the level of the epidermis, both Aδ- and C-fibre-free nerve endings are unmyelinated, this observation is unlikely to be due to differences in the electrical activation threshold of Aδ- and C-fibre-free nerve endings. A more plausible explanation is that eliciting consistent C-fibre responses requires a greater spatial recruitment and/or a more sustained recruitment of nociceptive afferents than what is required to elicit a consistent Aδ-fibre response. In agreement with this interpretation, a recent study showed that by increasing the spatial recruitment through the use of multiple stimulation electrodes and by increasing the temporal recruitment through the use of a longer pulse duration, IES may be able to elicit C-fibre ERPs [29].

5. Conclusion and perspectives

Here we provide converging evidence that when IES are applied at twice their perceptual threshold, they elicit behavioural and electrophysiological responses related exclusively to the activation of epidermal Aδ-fibres. Thus, our results indicate that IES constitutes an interesting and viable alternative to laser stimulation to study nociception in healthy humans.

Crucially, we also show that when the intensity of IES is increased, the stimulus looses its selectivity for epidermal nociceptors, and elicits behavioural and electrophysiological responses related to the activation of deeper, non-nociceptive, Aβ-fibre afferents.

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