

**Thermal detection thresholds of A $\delta$ - and C-fibre afferents activated by brief CO<sub>2</sub> laser pulses applied onto the human hairy skin.**

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**ABSTRACT**

(245 words)

Brief high-power laser pulses applied onto the hairy skin of the distal end of a limb generate a double sensation related to the activation of A $\delta$ - and C-fibres, often referred to as first and second pain. However, neurophysiological and behavioural responses related to the activation of C-fibres can be studied reliably only if the concomitant activation of A $\delta$ -fibres is avoided. Here, using a CO<sub>2</sub> laser stimulator able to deliver constant-temperature heat pulses through a feedback regulation of laser power by an online measurement of skin temperature at target site, combined with an adaptive staircase algorithm using reaction-time to distinguish between responses triggered by A $\delta$ - and C-fibre input, we show that it is possible to estimate robustly and independently the thermal detection thresholds of A $\delta$ -fibres ( $46.9 \pm 1.7^\circ\text{C}$ ) and C-fibres ( $39.8 \pm 1.7^\circ\text{C}$ ). Furthermore, we show that both thresholds are dependent on the skin temperature preceding and/or surrounding the test stimulus, indicating that the A $\delta$ - and C-fibre afferents triggering the behavioural responses to brief laser pulses behave, at least partially, as detectors of a change in skin temperature rather than as pure level detectors. Taken together, our results show that the difference in threshold between A $\delta$ - and C-fibre afferents activated by brief laser pulses can be exploited to activate C-fibres selectively and reliably, provided that the rise in skin temperature generated by the laser stimulator is well-controlled. Our approach could constitute a tool to explore, in humans, the physiological and pathophysiological mechanisms involved in processing C- and A $\delta$ -fibre input, respectively.

## INTRODUCTION

For the past 30 years, investigators have relied extensively on infrared laser stimulators to study nociception and pain perception in humans (Bromm et al., 1984; Carmon et al., 1976; Plaghki and Mouraux, 2005; Treede, 2003). Indeed, as a source of radiant heat applied onto the skin, infrared lasers can be used to activate heat-sensitive A $\delta$ - and C-fibre nociceptors selectively. Most importantly, because of their very high power output, infrared lasers can generate the very steep heating ramps required to elicit time-locked responses such as reaction-times and event-related brain potentials (Plaghki and Mouraux, 2003), and are thus well suited to study nociception.

However, for reasons that remain a matter of debate, brain responses related to the activation of C-fibres can be identified only if the concomitant activation of A $\delta$ -fibres is avoided (Mouraux et al., 2004; Mouraux and Plaghki, 2007; Plaghki and Mouraux, 2002). Therefore, in humans, most of the current research has focussed exclusively on characterizing the neural processes triggered by A $\delta$ -fibre input, simply because of the lack of reliable methods to study the responses triggered by the selective activation of C-fibres. Hence, to progress in our understanding of the physiology and pathophysiology of nociception, developing means to study the signals ascending through C-fibres is essential (Cruccu et al., 2004).

Based on characteristics differentiating A $\delta$ - and C-fibres, several methods have been proposed to activate C-nociceptors selectively (Plaghki and Mouraux, 2003). A first method exploits the fact that unmyelinated C-fibres are more resistant to pressure than myelinated A-fibres, and consists in applying prolonged force against a peripheral nerve such as to block selectively the nerve conduction of A-fibres

(Bromm et al., 1983; Nahra and Plaghki, 2003). A second method takes advantage of the fact that the distribution density of C-fibres in the epidermis is greater than that of A $\delta$ -fibres, and consists in using a very small stimulation surface area to elicit isolated C-fibre responses (Bragard et al., 1996; Opsommer et al., 2001). A third method uses the difference in the thermal activation thresholds of A $\delta$ - and C-fibre afferents, and consists in heating the skin above the threshold of C-fibres, but below the threshold of A $\delta$ -fibres (Magerl et al., 1999). However, implementing these methods is technically challenging, and the results obtained are often unreliable. Hence, at present, their use as a routine clinical diagnostic tool is not conceivable (Cruccu et al., 2004).

Here, using a new CO<sub>2</sub> laser stimulator able to deliver constant-temperature heat pulses through a feedback regulation of laser power by an online measurement of skin temperature at target site, combined with an adaptive psychophysical algorithm using reaction-times as criterion to distinguish between responses triggered by A $\delta$ - and C-fibre input, we show (1) that it is possible to estimate reliably the thermal detection thresholds of A $\delta$ - and C-fibres and (2) that the difference between C- and A $\delta$ -fibre thresholds can be exploited to activate C-nociceptors selectively and reliably, provided that skin temperature is well controlled. Furthermore, we show (3) that both thresholds are significantly dependent on the skin temperature preceding and/or surrounding the test stimulus.

## **METHODS**

### **Participants**

Nine healthy participants (3 females; 8 right-handed, aged 24-35 years) took part in the study. Before the experiment, they were familiarized with the experimental setup and task. The study was approved by the local Ethics Committee.

### **CO<sub>2</sub> laser stimulation of A $\delta$ - and C-nociceptors**

Thermal stimuli were applied to the dorsum of the non-dominant hand, using a new CO<sub>2</sub> laser stimulator whose power is regulated using a feedback control based on an online measurement of skin temperature at the site of stimulation (Laser Stimulation Device, SIFEC, Belgium). Conception of the laser was inspired by the temperature-controlled laser stimulator proposed by Meyer et al. (1976). Both devices are based on a closed-loop control of laser power by an online monitoring of skin temperature performed using a radiometer collinear with the laser beam. As compared to the device proposed by Meyer et al. (1976), the present device integrates some improvements provided by recent technical progress. The most important difference is the very small lag in the feedback control, which makes it possible to achieve temperature steps with much greater rise rates. For example, in Magerl et al. (1999), heating ramps were approximately 50°C/s and, consequently, the time required to bring the skin temperature from baseline to a temperature supraliminal for A $\delta$ -nociceptors was approximately 150 ms. In contrast, the present stimulator is able to reach similar target temperatures in less than 10 ms, and is thus better suited to record and interpret time-locked responses such as reaction-times and event-related potentials (Baumgärtner et al., 2005). The heat source is a 25 W radio-frequency excited CO<sub>2</sub> laser (Synrad 48-2; Synrad, USA). Power control is achieved by pulse

width modulation (PWM) at 5 KHz clock frequency. Stimuli are delivered through a 10 m optical fibre. By vibrating this fibre at some distance from the source, a quasi-uniform spatial distribution of radiative power within the stimulated area is obtained. At the end of the fibre, optics collimate the beam, resulting in a 6-mm beam diameter at target site. Using this system, thermal stimulation profiles were defined as follows (Figure 1, left panel).

*Baseline skin temperature ( $T_0$ ).* Prior to the actual stimulus, the skin temperature was measured for 100 ms without delivering any laser energy, such as to obtain an estimate of the baseline skin temperature.

*Conditioning skin temperature ( $T_1$ ).* To examine whether A $\delta$ - and C-fibre thermal detection thresholds were dependent on the skin temperature immediately preceding the stimulus, three different conditioning temperatures were used: *unchanged*, 34°C, and 38°C. In the *unchanged* condition, no laser energy was delivered during this 3-second time-interval and the skin temperature of the stimulated area was thus unchanged. In the other two conditions, the skin temperature of the stimulated area was raised to either 34°C or 38°C using a 1-second linear heating-ramp. This conditioning temperature was maintained for 2 seconds before applying the target stimulus.

*Target skin temperature ( $T_2$ ).* The skin temperature of the stimulated area was raised briefly to a defined target temperature using a very steep 10-ms heating ramp, and was maintained at that temperature for 40 ms. As detailed in the next section, possible target skin temperatures ranged from 33°C to 51°C, in steps of 1°C.

Finally, the skin temperature was measured for an additional 2 seconds without delivering any laser energy.

After each trial, the target of the laser was displaced to a random position on the hand dorsum, in order to avoid nociceptor sensitization and/or habituation. The time interval between two consecutive trials varied randomly between 5 and 10 seconds.

## **Procedure**

Stimuli were delivered and detection thresholds were estimated by means of a staircase algorithm (Figure 1, right panel). Participants were asked to respond as quickly as possible by pressing a button held in the dominant hand when perceiving the target stimulus ( $T_2$ ). Because the thermal activation threshold of C-fibres is known to be lower than the thermal activation threshold of A $\delta$ -fibres (Bromm and Treede, 1984; Nahra and Plaghki, 2003), it was expected that the skin temperature required to elicit A $\delta$ -fibre responses would be higher than the skin temperature required to elicit C-fibre responses. Furthermore, because the nerve conduction velocity of unmyelinated C-fibres is markedly lower than that of A $\delta$ -fibres, taking into account the peripheral conduction distance of afferent input originating from the hand, reaction-times to the target stimulus were used to discriminate between C-fibre (reaction-time  $\geq 650$  ms) and A $\delta$ -fibre (reaction-time  $< 650$  ms) responses (Mouraux et al., 2003). As shown in Figure 2, this criterion effectively discriminated the two response categories.

*C-fibre thermal detection threshold.* A staircase using detection vs. no detection as criterion, regardless of reaction-time, was used to determine the absolute detection threshold. This threshold is expected to reflect the C-fibre threshold, provided that the C-fibre threshold is lower than the A $\delta$ -fibre threshold.

*Aδ-fibre thermal detection threshold.* A staircase using detection with a reaction-time <650 ms as criterion was used to determine the Aδ-fibre thermal detection threshold. Indeed, as shown in Figure 2, such reaction-times are compatible only with the greater conduction velocity of myelinated Aδ-fibres.

For both staircase algorithms, the temperature of the first stimulus of the staircase was set randomly to a value ranging between 32 and 41°C. The temperature of each of the following test stimuli was determined by whether the participant's response to the preceding stimulus met the defined detection criterion. If the preceding stimulus was detected, the temperature of the upcoming stimulus was decreased by 1°C; else, it was increased by 1°C. Hence, the staircase converged towards the skin temperature at which the probability of detecting the stimulus with the defined criterion was 50%.

Aδ- and C-fibre thresholds were estimated for each of the three conditions ( $T_1$ =unchanged,  $T_1$ =34°C, and  $T_1$ =38°C). As shown in the right panel of Figure 1, the six staircases required to estimate the two thresholds for each of the three conditions were presented in an interleaved fashion. This procedure avoided any order effect on the estimated thresholds that could have been induced by habituation and/or sensitization if the staircases had been presented in separate successive blocks. Furthermore, the interleaving procedure ensured that participants were unable to predict the temperature of the upcoming stimulus, and unable to understand the relationship between their response and the temperature of the upcoming stimuli, thus preventing any response bias by anticipation.

Each staircase was interrupted after the occurrence of four staircase reversals (i.e. when the current stimulus was detected and the preceding stimulus undetected, or



when the current stimulus was undetected and the preceding stimulus detected). Threshold values were then obtained by averaging the target stimulus temperatures  $T_2$  at which the four staircase reversals had occurred.

## RESULTS

### Baseline skin temperature

Across subjects, the baseline skin temperature of the hand dorsum, averaged across all trials, ranged from 28.5°C to 33.6°C ( $31.2 \pm 1.7^\circ\text{C}$ , group-level mean  $\pm$ SD).

### Reaction times

As shown in Figure 2, the frequency distribution of reaction-times appeared bimodal, and the arbitrarily-defined cut-off to discriminate between A $\delta$ -fibre (reaction-time <650 ms) and C-fibre (reaction-time  $\geq$ 650 ms) responses effectively separated the two response categories.

The bimodal nature of this distribution was confirmed by comparing the fitting of the data to a model describing a unimodal distribution vs. a model describing a bimodal distribution. The distribution of reaction-times was modelled using an asymmetrical function proposed by Zaitsev and Skorik (2002), shown to describe well the right-skewed distribution of sensory-motor responses:

$$y = \frac{\lambda}{b} \exp \left[ -\exp \left( \frac{a-x}{b} \right) + \left( \frac{a-x}{b} \right) \right]$$

Where  $y$  is the distribution function of reaction-times  $x$ , and  $\lambda$  is the width of the class of division. This distribution function is characterized by two parameters:  $a$  defining

the value of  $x$  at which the distribution function reaches its maximum, and  $b$  defining the spread of the distribution function.

The bimodal vs. unimodal nature of the distribution of reaction times was tested by comparing the fitting of the data to this unimodal function (adjusting parameters  $a$  and  $b$ ), with the fitting of the data to a bimodal model consisting of the sum of these two functions (adjusting parameters  $a$  and  $b$  characterizing each of the two functions). The corrected Akaike Information Criterion (AIC) and the F-test were used to select the most likely of the two fitted models, taking into account the different number of parameters in each model. The bimodal model had a lower AIC than the unimodal model (59.2 vs. 89.6). The F-test showed that the data fit significantly better to the bimodal model ( $r^2=0.96$ ) than to the unimodal model ( $r^2=0.76$ ) ( $F=30.6$ ,  $p<.0001$ ), thus indicating that the distribution of reaction-times was indeed bimodal. The first function of the bimodal model characterized the distribution of the short-latency A $\delta$ -fibre responses ( $a=459$  ms;  $b=111$  ms). The second function characterized the distribution of the late-latency C-fibre responses ( $a=961$  ms;  $b=202$  ms).

### **A $\delta$ - and C-fibre thermal detection thresholds**

When the conditioning skin temperature was unchanged ( $T_1$ =unchanged), the C-fibre threshold was  $T_2=39.8\pm1.7^\circ\text{C}$ , whereas the A $\delta$ -fibre threshold was  $T_2=46.9\pm1.7^\circ\text{C}$  ( $\Delta T$  between C-fibre and A $\delta$ -fibre thresholds:  $7.2\pm1.7^\circ\text{C}$ , ranging from  $5.0^\circ\text{C}$  to  $10.0^\circ\text{C}$  across participants) (Figure 3). When the conditioning skin temperature was brought to  $T_1=34^\circ\text{C}$ , the C-threshold was  $T_2=40.3\pm1.3^\circ\text{C}$  and the A $\delta$ -threshold was  $T_2=47.4\pm1.6^\circ\text{C}$  ( $\Delta T=7.1\pm1.4^\circ\text{C}$ , ranging from  $5.3^\circ\text{C}$  to  $9.3^\circ\text{C}$ ). When  $T_1$  was brought

to 38°C, the C-threshold was  $T_2=42.4\pm1.0^\circ\text{C}$  and the A $\delta$ -threshold was  $T_2=46.7\pm1.8^\circ\text{C}$  ( $\Delta T=4.3\pm1.1^\circ\text{C}$ , ranging from 3.0°C to 5.5°C) (Figures 3 and 4).

To assess the difference between A $\delta$ - and C-thresholds, and to examine the effect of the conditioning skin temperature ( $T_1$ ), a repeated-measures ANOVA was performed with the following two factors: 'nociceptor type' (A $\delta$  or C) and 'conditioning skin temperature' (unchanged, 34°C or 38°C). The analysis showed a very significant main effect of the factor 'nociceptor type' ( $F=481.1$ ,  $p<.001$ ), a significant main effect of the factor 'conditioning skin temperature' ( $F=7.5$ ,  $p=.006$ ), as well as a significant interaction between the two factors ( $F=10.8$ ,  $p=.001$ ). Post-hoc comparisons, performed using the Tukey test, showed that the A $\delta$ -threshold was markedly higher than the C-threshold ( $\Delta T=+6.2^\circ\text{C}$ ,  $p<.001$ ). Furthermore, they showed that the 'conditioning skin temperature' significantly affected the C-threshold, which was significantly higher when the conditioning skin temperature was brought to 38°C, as compared to when the conditioning skin temperature was brought to 34°C ( $\Delta T=+2.1^\circ\text{C}$ ,  $p<.001$ ), or when it was unchanged ( $\Delta T=+2.6^\circ\text{C}$ ,  $p<.001$ ). In contrast, the 'conditioning skin temperature' had no significant effect on the A $\delta$ -threshold.

Finally, it was examined whether A $\delta$ - and C-thresholds were modulated by the baseline skin temperature ( $T_0$ ), i.e. the temperature of the skin before bringing the stimulated area to the conditioning skin temperature ( $T_1$ ), also corresponding to the temperature of the skin surrounding the stimulated area during the entire trial duration. As shown in the left panels of Figure 5, a significant positive correlation was found between the baseline skin temperature ( $T_0$ ) and the C-threshold when the conditioning skin temperature was unchanged ( $T_1$  unchanged: slope  $0.78\pm0.20$ ;  $r^2=0.71$ ;  $p=.008$ ), but not when the conditioning skin temperature was raised to 34°C

(slope  $0.41 \pm 0.23$ ;  $r^2=0.34$ ;  $p=.129$ ) or  $38^\circ\text{C}$  (slope  $0.33 \pm 0.18$ ;  $r^2=0.37$ ;  $p=.110$ ). As shown in the right panels of Figure 5, a significant positive correlation was also found between  $T_0$  and the A $\delta$ -threshold when  $T_1$  was unchanged (slope  $0.56 \pm 0.24$ ;  $r^2=0.44$ ;  $p=.050$ ), and even more so when  $T_1$  was brought to  $34^\circ\text{C}$  (slope  $0.73 \pm 0.14$ ;  $r^2=0.80$ ;  $p=.001$ ) and  $38^\circ\text{C}$  (slope  $0.75 \pm 0.19$ ;  $r^2=0.68$ ;  $p=.006$ ).

## DISCUSSION

In the present study, we show that when brief (50 ms duration) and focal (6-mm beam diameter) CO<sub>2</sub> laser stimuli are applied to the distal end of a limb, such as to maximize the difference between the peripheral conduction times of unmyelinated C-fibres and myelinated A $\delta$ -fibres, reaction-time latencies can be used to effectively discriminate between behavioural responses to C-fibre input and behavioural responses to A $\delta$ -fibre input. Most importantly, using a feedback-controlled CO<sub>2</sub> laser stimulator to deliver constant-temperature thermal stimuli combined with a staircase algorithm relying on reaction-times to distinguish between A $\delta$ -fibre and C-fibre responses, we show that it is possible to obtain, at individual level, a reliable estimate of the thermal detection threshold of each of the two classes of nociceptive fibres.

Thereby, we confirm that the threshold to obtain behavioural responses related to the thermal activation of A $\delta$ - and C-fibres by brief and focal infrared laser pulses applied to the hairy skin are significantly different. The C-fibre threshold was  $39.8 \pm 1.7^\circ\text{C}$ , whereas the A $\delta$ -fibre threshold was  $46.9 \pm 1.7^\circ\text{C}$  ( $\Delta T = 7.2 \pm 1.7^\circ\text{C}$ ). Hence, provided that the rise in skin temperature generated by the stimulator is well-

controlled, brief infrared laser pulses below the threshold of A $\delta$ -fibres but above the threshold of C-fibres can be used to activate C-fibres selectively and reliably.

### **C-fibre thermal detection threshold**

When the baseline skin temperature was unchanged, the C-fibre threshold was  $39.8 \pm 1.7^\circ\text{C}$ . Increasing the temperature of the stimulated area to  $34^\circ\text{C}$  or  $38^\circ\text{C}$  for a short duration before applying the test stimulus led to a significant increase of the C-fibre threshold (Figure 4). One likely explanation to this observation is that increasing the skin temperature before applying the stimulus activates a number of low-threshold heat-sensitive C-fibre afferents, such as C-warm receptors that have been shown to respond to small increases in skin temperature within a range of  $30\text{--}50^\circ\text{C}$  (Hallin et al., 1982). This background C-fibre activity, by reducing the contrast of the C-fibre input generated by the test stimulus, could explain why higher stimulus temperatures were required for participants to reliably detect the test stimulus in these two conditions. In fact, it is likely that the behavioural responses triggered by C-fibre input observed in the present study were themselves related mainly to the activation of C-warm receptors, rather than to the activation of heat-sensitive C-fibre *nociceptors*. Indeed, such as what was observed in the present study, C-warm receptors are considered to respond preferentially to sudden increases in skin temperature relative to baseline and, hence, their thresholds are dependent on the background skin temperature (Darian-Smith et al., 1979a; Darian-Smith et al., 1979b; Johnson et al., 1979; LaMotte and Campbell, 1978). Furthermore, these studies showed that C-warm receptors respond to such increases in skin temperature in a very phasic manner, compatible with the triggering of time-locked responses such as reaction times. Finally, the C-fibre related detection thresholds

observed in the present study were lower than the usually-reported thresholds of C-fibre nociceptors, which are considered to respond only to skin temperatures around and above 45°C (Meyer and Campbell, 1981a).

### **A $\delta$ -fibre thermal detection threshold**

When the baseline skin temperature was unchanged, the A $\delta$ -fibre threshold was  $46.9 \pm 1.7^\circ\text{C}$ . In contrast to what was observed for C-fibres, increasing the temperature of the stimulated area to 34°C or 38°C immediately before applying the test stimulus did not modulate the A $\delta$ -threshold (Figure 4, right panel). This suggests that the increase in background C-fibre input engendered by the increase in baseline skin temperature did not interfere significantly with the detection of A $\delta$ -fibre input. This could be due to the fact that the pricking sensation generated by A $\delta$ -fibre input is qualitatively very distinct from the sensation elicited by C-fibre input (Nahra and Plaghki, 2003) and, hence, easier to discriminate qualitatively from background C-fibre input.

In contrast to the absence of effect of briefly increasing the skin temperature prior to applying the test stimulus ( $T_1$ ), we did observe a very significant positive relationship between the A $\delta$ -threshold and  $T_0$ , i.e. the skin temperature before the onset of the trial, also reflecting the temperature of the skin surrounding the stimulus during the entire trial duration (Figure 5, right panels). This indicates that the A $\delta$ -nociceptors triggering the behavioural responses observed in the present study cannot be considered as pure level detectors, i.e. as receptors responding when a given skin temperature is reached regardless of the preceding and/or surrounding skin temperature but, instead, at least partially act as detectors of a change in skin temperature (change relative to the preceding skin temperature, change relative to

the surrounding skin temperature). This observation is clearly at variance with the observations of previous studies (Hardy et al., 1951; Plaghki et al., 2010) showing that the threshold for pricking pain is largely invariant with regard to the baseline skin temperature ( $T_0$ ). This discrepancy may result from a difference in stimulus parameters. For example, as compared to the 10-ms rise time used in the present study, the rise time of the temperature steps used in these previous studies was much lower (e.g., 3-s in Hardy et al., 1951). Interestingly, the correlation between  $T_0$  and the A $\delta$ -fibre threshold was present even when the temperature of the stimulated area was briefly brought to  $T_1=34^\circ\text{C}$  or  $T_1=38^\circ\text{C}$  before applying the stimulus, thus suggesting that the A $\delta$ -threshold was dependent on the skin temperature surrounding the stimulated area rather than the skin temperature of the stimulated area immediately preceding the stimulus. Assuming that A $\delta$ -nociceptors are entirely unresponsive at baseline skin temperatures ( $T_0$ ), one must postulate that the threshold for A $\delta$ -fibre input to trigger a behavioural response is not only dependent on the activity conveyed by A $\delta$ -fibres but, instead, that it also integrates other sources of input such as the background C-fibre input whose firing rate is dependent on the baseline skin temperature. In fact, previous studies have also suggested that the perception induced by heating the skin results from a central integration, at spinal and/or cortical level, of the activity of the different classes of heat-sensitive free nerve endings (Meyer and Campbell, 1981b).

Using single-fibre recordings in monkeys, Treede et al. (1995) described two distinct classes of heat-sensitive A $\delta$ -fibre polymodal nociceptors, referred to as slowly adapting (type I) and rapidly adapting (type II). Because the response profiles of type I A $\delta$ -nociceptors is not sufficiently phasic, it is usually considered that heat-evoked time-locked responses related to the activation of A $\delta$ -fibres, such as reaction times

or event-related potentials triggered by brief laser pulses, are exclusively related to the activation of rapidly-adapting type II A $\delta$ -nociceptors. Compatible with this view, the A $\delta$ -thresholds estimated in the present study matched more closely the thermal activation threshold of type II A $\delta$ -nociceptors (46°C), as compared to type I A $\delta$ -nociceptors (>53°C) (Treede et al., 1995).

## **Conclusion**

Using a CO<sub>2</sub> laser stimulator able to deliver constant-temperature heat pulses using a regulated feedback-control of laser power, combined with an adaptive staircase algorithm using reaction-times to distinguish between responses related to A $\delta$ - and C-fibres, we show that it is possible to estimate reliably the thermal detection thresholds of A $\delta$ - and C-fibres, respectively. Furthermore, we show that it is possible to activate C-fibres selectively and reliably, using a stimulus temperature above the C-fibre threshold, but below the A $\delta$ -fibre threshold, provided that skin temperature is well controlled.

The low threshold of the responses triggered by C-fibre input, as well as the dependency of this threshold on the skin temperature immediately preceding stimulus onset, suggests that these responses were related mainly to the activation of C-warm receptors. In contrast, the higher threshold of the responses triggered by A $\delta$ -fibre input suggests that these responses were related mainly to the activation of rapidly-adapting type II A $\delta$ -nociceptors.

Taken together, our approaches could constitute a useful tool for assessing A $\delta$ - and C-fibre function in patients.



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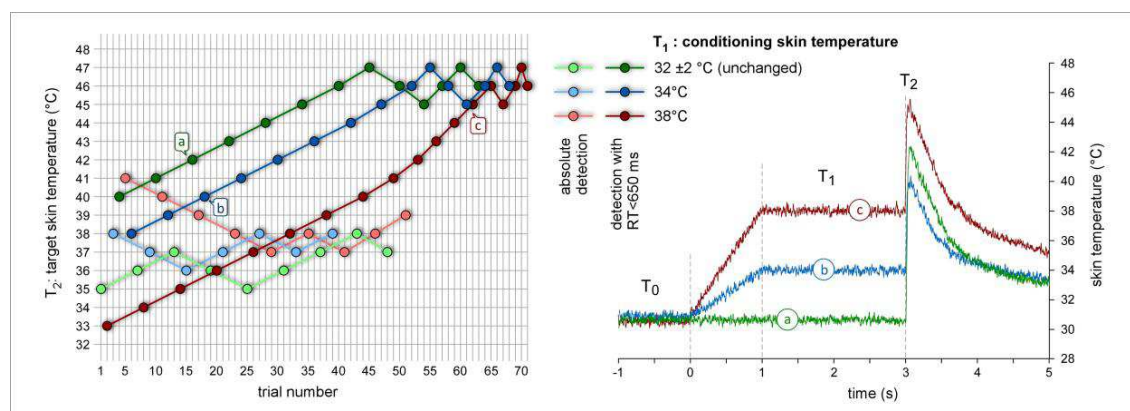
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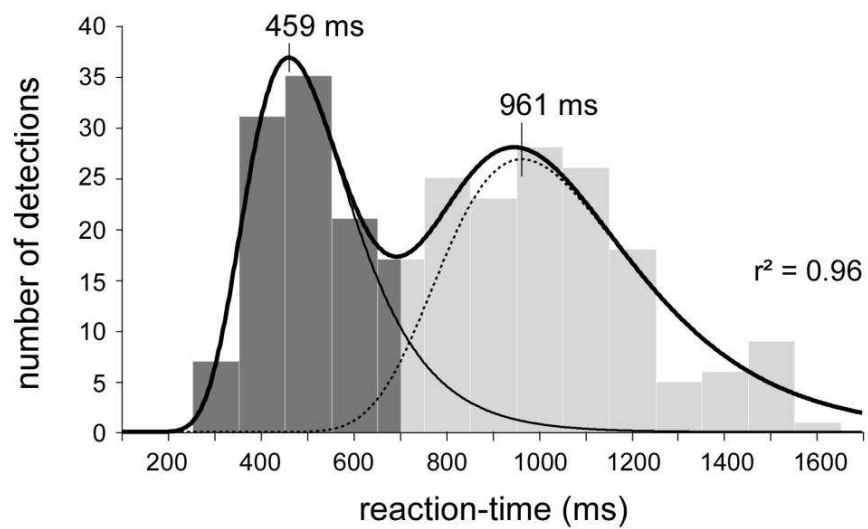
## FIGURE LEGENDS



**Figure 1.** Experimental paradigm. Thermal stimuli were applied to the dorsum of the non-dominant hand using a CO<sub>2</sub> laser (beam diameter: 6 mm). After measuring the baseline skin temperature for one second ( $T_0$ ), the stimulated area was raised to the conditioning skin temperature using a 1-s heating ramp and was maintained at that temperature for 2 seconds ( $T_1$ ). Three different conditioning skin temperatures were used: (a)  $T_1$  = unchanged, (b)  $T_1$  = 34°C and (c)  $T_1$  = 38°C. Following this conditioning of the stimulated area, the test stimulus ( $T_2$ ) was applied, consisting of a 10-ms heating ramp followed by a 40-ms plateau. The data shown in the left panel corresponds to an actual measurement of skin temperature performed by the radiometer in three trials randomly selected in each of the three conditions. The right panel shows the staircase procedure used to estimate A $\delta$ - and C-fibre thresholds (one subject shown as example). Participants were asked to respond as quickly as possible by pressing a button held in the dominant hand when perceiving the test stimulus ( $T_2$ ). Reaction times were used to discriminate between responses triggered by C-fibre input (reaction-time  $\geq 650$  ms) and responses triggered by A $\delta$ -fibre input (reaction-time  $< 650$  ms). For all three conditions ( $T_1$  = unchanged,  $T_1$  = 34°C and  $T_1$

= 38°C), an adaptive staircase algorithm was used to estimate (1) the absolute detection threshold assumed to reflect the detection threshold of C-fibre input and (2) the detection threshold of responses with a reaction-time <650 ms assumed to reflect the detection threshold of A $\delta$ -fibre input. The six staircases required for this procedure were presented in an interleaved fashion, such as to avoid order effects related to habituation and/or sensitization as well as a possible response bias due to anticipation.

A.



B.

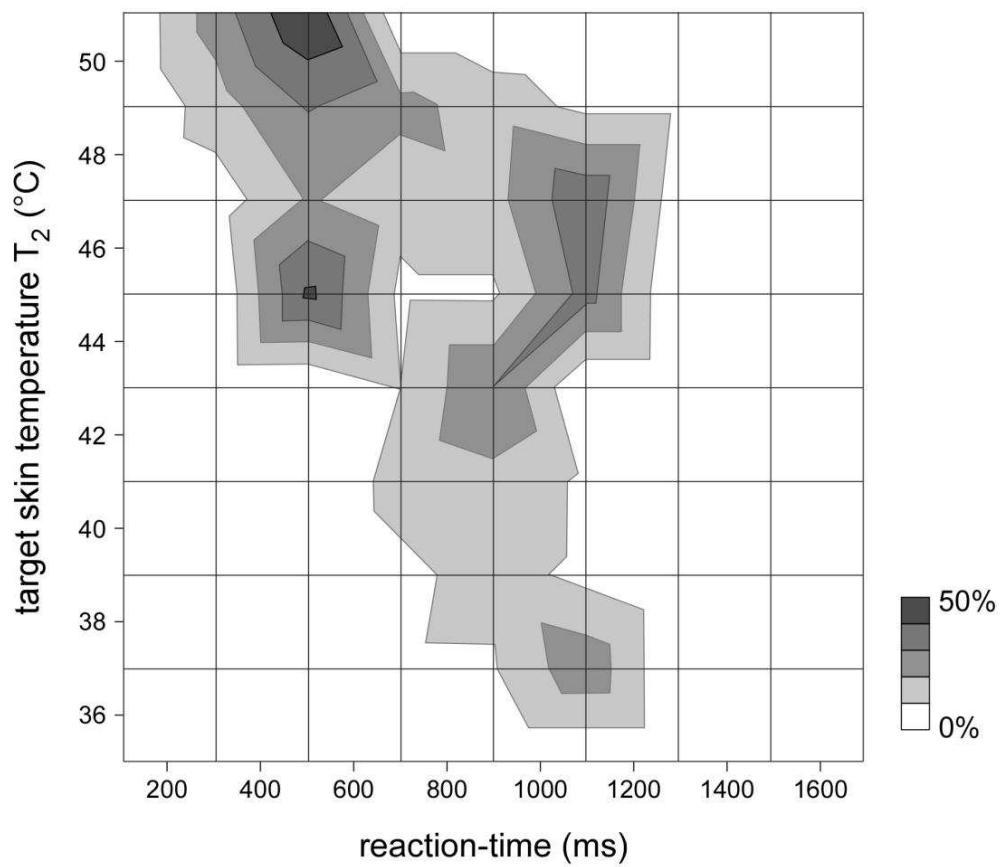
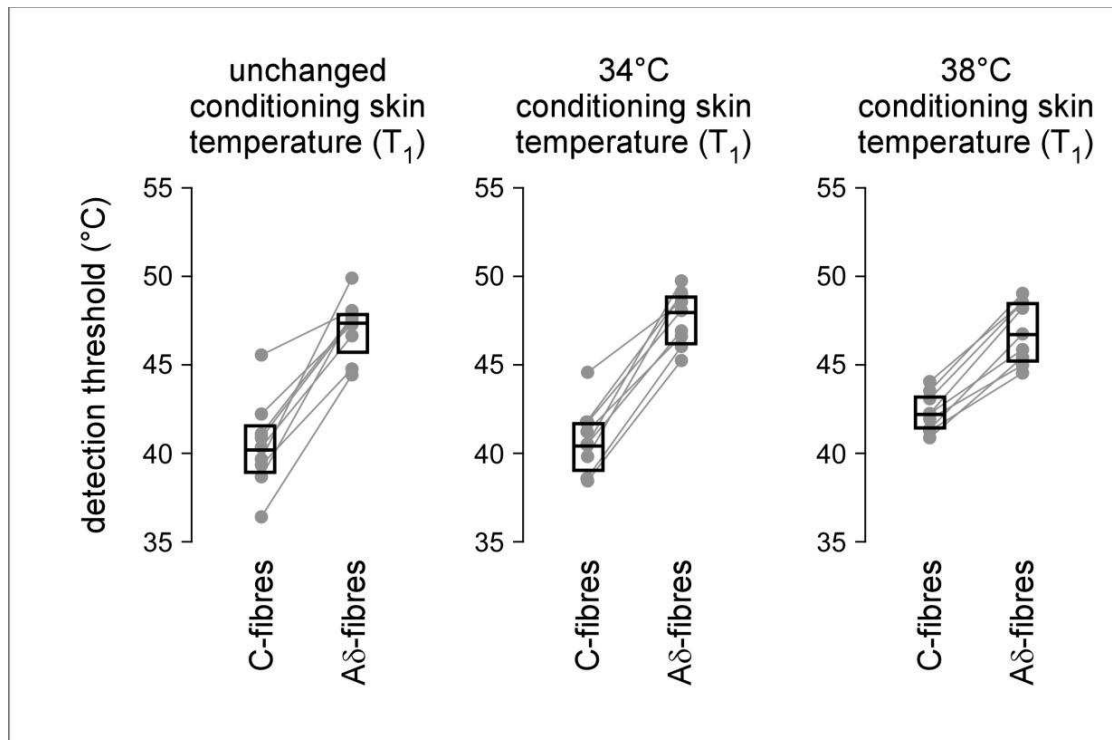
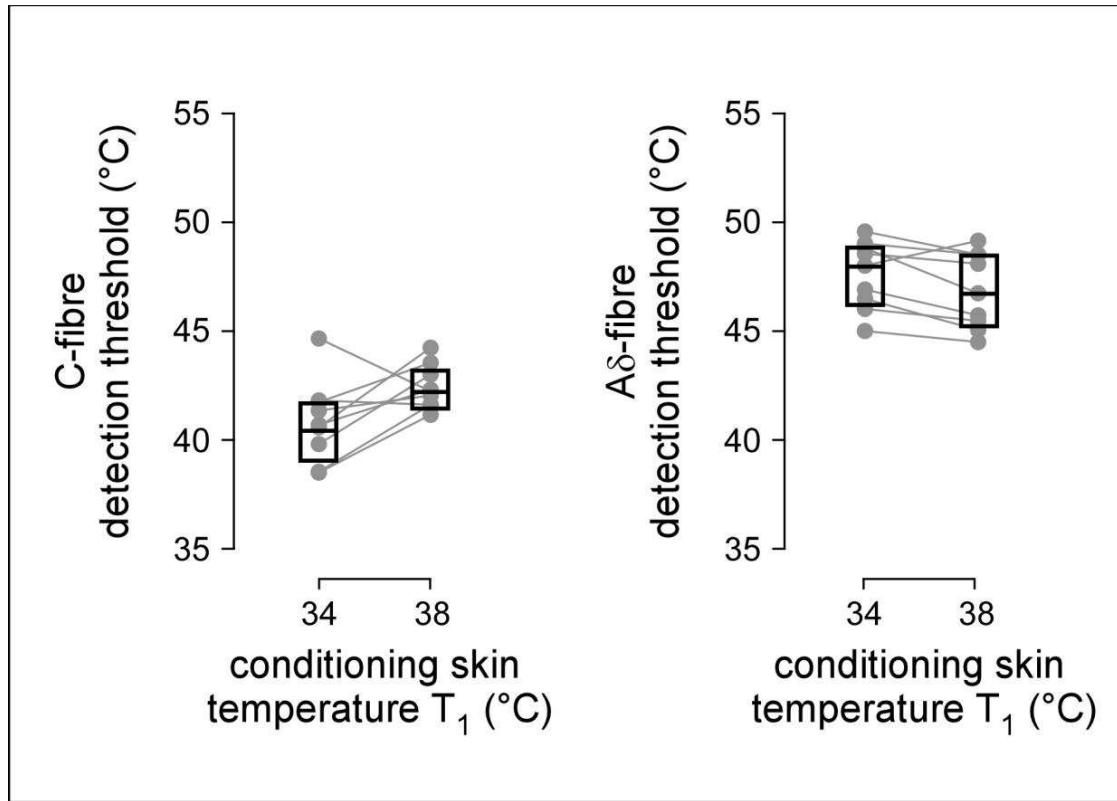


Figure 2. Frequency distribution of the reaction-times to brief CO<sub>2</sub> laser pulses applied onto the dorsum of the non-dominant hand using target skin temperatures ranging from 35 to 51°C. The upper panel shows the overall distribution of reaction-times regardless of the target skin temperature ( $T_2$ ). The lower panel shows the distribution of reaction-times as a function of  $T_2$ . Note the bimodal distribution of reaction-times, related to the fact that lower target skin temperatures triggered late-latency responses compatible with the conduction velocity of unmyelinated C-fibres, whereas higher target skin temperatures triggered early-latency responses compatible with the conduction velocity of myelinated A $\delta$ -fibres. The bimodal nature of this distribution was confirmed by comparing the fitting of the data to a model describing a unimodal vs. a bimodal distribution of sensory-motor responses (see Results). The first function of the bimodal model characterized the distribution of the short-latency A $\delta$ -fibre responses ( $a=459$  ms;  $b=111$  ms). The second function characterized the distribution of the late-latency C-fibre responses ( $a=961$  ms;  $b=202$  ms).



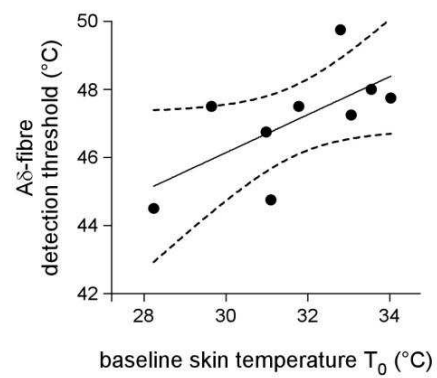
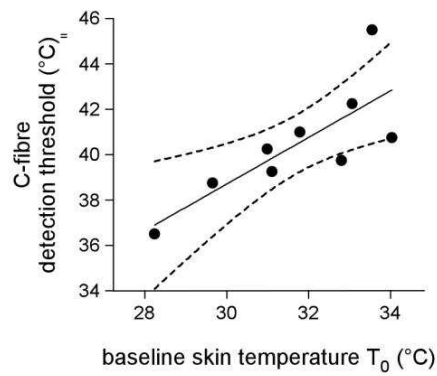


**Figure 3.** Thermal detection threshold of the behavioural responses triggered by Aδ- and C-fibre input obtained in the three experimental conditions ( $T_1$ =unchanged,  $T_1$ =34°C and  $T_1$ =38°C). The thin grey lines represent the individual thresholds obtained in each participant. The box plots represent the group-level median and interquartile range. Note that in all participants, the Aδ-fibre threshold is markedly greater than the C-fibre threshold. Also note that this difference is less marked in the condition  $T_1 = 38^\circ\text{C}$ .

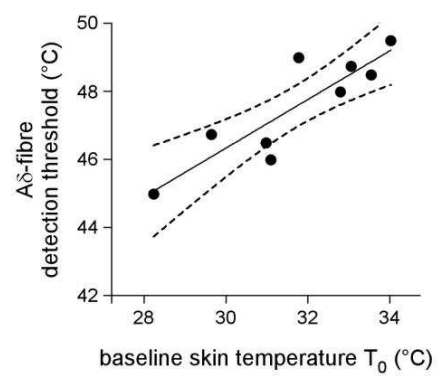
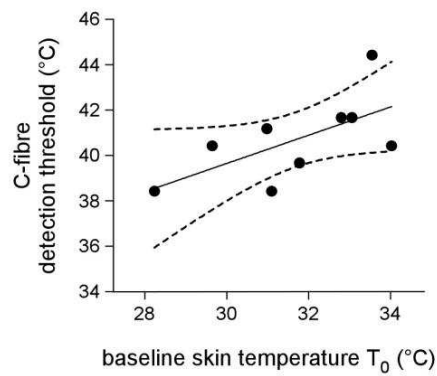


**Figure 4.** Thermal detection threshold of the behavioural responses triggered by Aδ- and C-fibre input obtained when the stimulated area is first heated to  $T_1=34^\circ\text{C}$  or  $T_1=38^\circ\text{C}$  during the two seconds preceding the test stimulus ( $T_2$ ). The thin grey lines represent the individual thresholds obtained in each participant. The box plots represent the group-level median and interquartile range. Note that the C-fibre threshold is increased for  $T_1=38^\circ\text{C}$  vs.  $T_1=34^\circ\text{C}$ , whereas the Aδ-fibre threshold appears unaffected by the conditioning skin temperature.

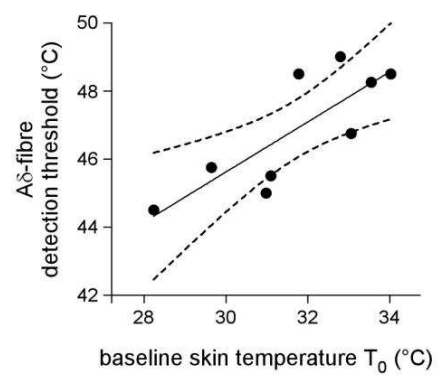
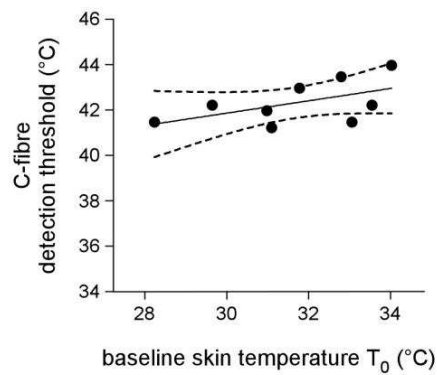
### unchanged conditioning skin temperature ( $T_1$ )



### 34°C conditioning skin temperature ( $T_1$ )



### 38°C conditioning skin temperature ( $T_1$ )



**Figure 5.** Thermal detection threshold of the behavioural responses triggered by A $\delta$ - and C-fibre input obtained in the three experimental conditions ( $T_1$ =unchanged,  $T_1=34^\circ\text{C}$  and  $T_1=38^\circ\text{C}$ ), as a function of the baseline skin temperature ( $T_0$ ), measured immediately before trial onset. Note the positive relationship between the two variables. Most importantly, note that the positive relationship between baseline skin temperature ( $T_0$ ) and the A $\delta$ -fibre threshold persists even when the stimulated area is brought for two seconds to  $34^\circ\text{C}$  or  $38^\circ\text{C}$  prior to applying the test stimulus.