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Evidence of a Specific Spinal Pathway for the Sense of Warmth in Humans

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Iannetti, G.D., A. Truini, A. Romaniello, F. Galeotti, C. Rizzo, M. Manfredi, and G. Cruccu. Evidence of a specific spinal pathway for the sense of warmth in humans. *J Neurophysiol* 89: 562–570, 2003; 10.1152/jn.00393.2002. While research on human sensory processing shows that warm input is conveyed from the periphery by specific, unmyelinated primary sensory neurons, its pathways in the central nervous system (CNS) remain unclear. To gain physiological information on the spinal pathways that convey warmth or nociceptive sensations, in 15 healthy subjects, we studied the cerebral evoked responses and reaction times in response to laser stimuli selectively exciting A δ nociceptors or C warmth receptors at different levels along the spine. To minimize the conduction distance along the primary sensory neuron, we directed CO₂-laser pulses to the skin overlying the vertebral spinous processes. Using brain source analysis of the evoked responses with high-resolution electroencephalography and a realistic model of the head based on individual magnetic resonance imaging scans, we also studied the cortical areas involved in the cerebral processing of warm and nociceptive inputs. The activation of C warmth receptors evoked cerebral potentials with a main positive component peaking at 470–540 ms, i.e., a latency clearly longer than that of the corresponding wave yielded by A δ nociceptive input (290–320 ms). Spinal neurons activated by the warm input had a slower conduction velocity (2.5 m/s) than the nociceptive spinal neurons (11.9 m/s). Brain source analysis of the cerebral responses evoked by the A δ input yielded a very strong fit for one single generator in the mid portion of the cingulate gyrus; the warmth-related responses were best explained by three generators, one within the cingulate and two in the right and left opercular-insular cortices. Our results support the existence of slow-conducting second-order neurons specific for the sense of warmth.

INTRODUCTION

The anatomical-physiological characteristics of the spinal sensory pathways have been more precisely defined for the painful than the thermal input (Craig and Dostrovsky 1999, 2001; Darian-Smith 1984; Dostrovsky and Craig 1996; Han et al. 1998; Perl 1984). Small-myelinated (A δ) and unmyelinated (C) primary afferents from nociceptors and thermoreceptors connect with lamina I/II/V second-order neurons (Darian-Smith 1984; Doubell et al. 1999; Perl 1984). Most axons from lamina I/II/V cells decussate at segmental level and ascend within the anterolateral quadrant to the thalamus, constituting the spinothalamic tract (STT) (Craig and Dostrovsky 1999).

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The STT and trigeminothalamic tract are commonly considered the most important pathways for signaling painful stimuli. In contrast, in animal studies, based on antidromic activation of trigeminothalamic (Craig and Dostrovsky 1991; Price et al. 1978) or spinothalamic cells (Christensen and Perl 1970; Craig and Dostrovsky 2001; Dostrovsky and Craig 1996; Kumazawa et al. 1975), the finding of second-order warmth-specific neurons within these pathways was rare. Most spinothalamic cells coding thermal stimulation were excited by graded cooling and inhibited by warming (Craig et al. 2001; Dostrovsky and Craig 1996). Whereas cold and warmth sensations are undoubtedly mediated by independent receptors and mainly conveyed by physiologically distinct peripheral fiber groups (classically myelinated for cold and unmyelinated for warmth) (Darian-Smith 1973, 1979), whether a class of warmth-specific second-order neurons exists in the human spinal cord is still unknown.

Brief radiant heat pulses, generated by a CO₂ laser stimulator, excite free nerve endings in superficial skin layers and can activate A δ and C fibers (Bromm and Treede 1984). The brain responses evoked by a painful laser stimulus (*late* laser-evoked potentials, LEPs) are related to the activation of type II A δ mechano-heat nociceptors (AMH II), small-myelinated primary neurons, and STT neurons. Late LEPs are widely used in physiological and clinical studies in patients with peripheral or central lesions (Bromm and Treede 1991; Iannetti et al. 2001a; Kakigi et al. 1991).

In contrast, the LEPs related to activation of C fibers (*ultralate* LEPs) are difficult to record, probably because the A δ volley inhibits transmission along the C-fiber central pathways or the preceding late LEP interferes with the generators of the ultralate LEP in the brain (Bromm et al. 1984). Since the first reported recording of an ultralate LEP, obtained with the experimental block of the group A fibers (Bromm et al. 1983), several techniques have been proposed: stimulation of tiny skin areas (Bragard et al. 1996), spectral analysis of the expected time window (Arendt-Nielsen 1990; Bragard et al. 1996), and selection of single trials devoid of late LEPs (Towell et al. 1996). Because the heat threshold is slightly lower in C than in A δ receptors (Lynn and Baranowski 1987; Treede et al. 1995), the use of stimulus intensities below the A δ activation threshold also is a useful tool for the selective activation of C fibers

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(Magerl et al. 1999). The threshold of warmth receptors is even lower than that of C nociceptors (Campbell et al. 1989; LaMotte and Campbell 1978). Because warmth receptors have small receptive fields and a very low density in the skin (Campbell et al. 1989; Green and Cruz 1998; LaMotte and Campbell 1978), purely warm sensations are evoked only by laser pulse that irradiate a large skin area (Agostino et al. 2000; Towell et al. 1996).

To investigate the physiological properties of spinal cord pathways and cerebral structures involved in the processing of thermal-pain afferent inputs in humans, we studied the cerebral evoked responses to CO₂ laser pulses, with stimulus variables optimal for exciting selectively either A δ nociceptors or C warmth receptors, in 15 healthy subjects. To minimize the conduction distance along the primary sensory neuron, we directed the laser pulses to the skin overlying the vertebral spinous processes. Preliminary results have been presented elsewhere (Iannetti et al. 2001b).

METHODS

Fifteen healthy volunteers (10 women, 5 men) aged between 24 and 64 yr (mean: 31 yr) participated in the study. All participants gave their informed consent and the research was approved by the local ethical committee.

Stimulation procedure

Using a CO₂ laser stimulator (Neurolas, Electronic Engineering, Florence, Italy) we delivered brief pulses (wavelength: 10.6 μ m) to the skin overlying C₅, T₂, T₆, and T₁₀ vertebral spinous processes (four subjects) or C₅ and T₁₀ (15 subjects). To avoid habituation, sensitization, and tissue damage, interstimulus intervals were randomly varied between 15 and 30 s, and the stimulation site was slightly changed within a transverse area of 3 \times 2 cm centered over the spinous process. Before the recording, individual thresholds to pinprick and warmth sensation were determined by delivering a series of stimuli at increasing and decreasing intensities; threshold was defined as the lowest intensity at which the subject perceived at least 50% of stimuli (Agostino et al. 2000). The skin temperature was measured with a thin thermocouple (0.7-mm diam) connected to an oscilloscope and laid on the skin surface, with the tip at the center of the laser spot. In preliminary experiments, we observed that laser pulses of low intensity (4.5–7.5 mJ/mm²), relatively long duration (30–50 ms), and a large irradiated area (spot diameter: 5 mm) were optimal to elicit purely warmth sensations (C warmth input); the mean temperature was 39°C. In contrast, laser pulses of higher intensity (9–18 mJ/mm²), short duration (5–5 ms), and a small irradiated area (spot diameter: 2.5 mm) were optimal to elicit pinprick sensation (A δ input) (Romaniello et al. 2002); the mean temperature was 48°C. Stimulation sites were marked on the skin and cutaneous temperature was regulated at 30–32°C.

LEP recordings

Subjects lay prone and were asked to stay awake and relax their muscles. Brain electrical activity was recorded with disc electrodes from the vertex (Cz) referenced to the linked earlobes (A₁–A₂) with a bandwidth of 0.3–100 Hz, a sampling rate of 256 Hz, and a conversion on 12 bit giving a resolution of 0.195 μ V/digit (Brain Quick System98, Micromed, Treviso, Italy). Analyzed signals were band-pass digitally filtered between 0.3 and 30 Hz. The electrode impedance was always kept below 5 k Ω . White noise through earphones ensured acoustic isolation. The simultaneous recording of the electrooculogram (EOG) was used to discard trials contaminated by eye blinks or ocular

movements. For each site of stimulation, two series of 15 artifact-free trials were selected and averaged off-line. The window analysis time was 1 s.

Each subject underwent two separate sessions for recording late (A δ -fiber related) and ultralate (C-fiber related) LEPs. For the late responses, we measured the latency of the main negative (N) and positive (P) components and the peak-to-peak amplitude. For the ultralate responses we measured the latency of the main positive (P) component and its amplitude from baseline.

Reaction times

The reaction times for both A δ and C fiber activation were measured in two dedicated sessions separate from LEP recordings. The subjects lay prone as for the LEP recording and were instructed to respond as fast as possible to any kind of perceived sensation by pressing a switch with the index finger of the dominant hand. The data were digitally recorded and analyzed off-line. For each site of stimulation, the mean reaction time was measured over 30 trials. Participants were asked to describe the evoked sensation by choosing one of the following qualities: warmth, pinprick, burning, touch, or an open category to be specified by the subject.

Conduction velocity along the spinal cord

We measured the conduction distance between the vertebral spinous processes and corrected it for the spine convexity (the real length of the spinal cord is on average 13% shorter than the length measured from the skin) (Desmedt and Cheron 1983; Kakigi and Shibasaki 1991). Spinal conduction time was taken from the latency of the main positive LEP component and the reaction times. The conduction velocity (CV) of the afferent volleys along the spinal cord was obtained by calculating, for reaction times and LEPs, the reciprocal of the slope of the regression line for all the latency values obtained at all sites of stimulation along the spine.

Electrical source analysis

To calculate a hypothesis-based model of dipolar generator of the scalp LEPs, three subjects were studied with multi-electrode recordings. We used a 31-channel cap plus 1 EOG channel, recording with the same variables previously described. For source analysis we used Advanced Source Analysis (ASA, courtesy of ANT Software, www.ant-software.nl), which permits dipole localization using a realistic model obtained from the magnetic resonance imaging (MRI) scan of the subject. Anatomical MRI scans were acquired with a 1.5 T scanner (Gyroscan, Philips) using a three-dimensional (3D) T1-weighted sequence (191 axial slices; slice thickness: 0.8 mm, gap: 0 mm, matrix: 256 \times 256). Immediately before MRI acquisition, three anatomical landmarks were placed in standard positions: left preauricular point, right preauricular point, and nasion; they were visualized on the images using vitamin E capsules. After the MRI acquisition and before the LEP recording session, once the recording cap was placed on the scalp, all electrodes and vitamin marker positions were measured using a 3D digital localizer (Polhemus, Colchester, VT). At this point the LEPs were recorded, taking care to keep the electrode position fixed.

The realistic volume-conductor model of the subject's head was obtained from the MRI data using the boundary element method (BEM) (Fuchs et al. 1998). We assumed that the localization of the dipole remained unchanged during the tested interval, and a model with rotating dipoles was chosen for each examined component.

The goodness of fit of the dipolar configuration was assessed with the residual variance and correlation. The residual variance (expressed as a percentage) is a function of the total amount of signal that cannot be explained by the dipole characteristics; the correlation between the

TABLE 1. Latencies and amplitudes of brain-evoked responses and reaction times to laser stimulation of the skin overlying the vertebral spinous processes in healthy subjects

	C5				T10			
	RT, ms	N-Wave Latency, ms	P-Wave Latency, ms	Amplitude, μ V	RT, ms	N-Wave Latency, ms	P-Wave Latency, ms	Amplitude, μ V
A δ input	287 \pm 30*	195 \pm 14†	292 \pm 38†	10.7 \pm 2.9†	310 \pm 36*	210 \pm 15†	320 \pm 33†	10.4 \pm 2.8†
C input	537 \pm 118*	—	471 \pm 43‡	5.7 \pm 4.2‡	641 \pm 142*	—	584 \pm 42‡	5.1 \pm 2.5‡

Values are means \pm SD. RT, reaction time. * $n = 10$. † $n = 15$. ‡ $n = 14$.

originally recorded signal and the signal generated by the estimated dipoles was assessed with the linear correlation index (r).

Statistical analysis

All results are given as means \pm SD. Differences between LEP latencies and LEP amplitudes for the different stimulated districts (having a Gaussian distribution) were evaluated by paired Student's t -test, and those in threshold and reaction times (not having a Gaussian distribution) by the Mann-Whitney U test. Goodness of fit of the linear regression was tested with the coefficient of correlation (r^2) and its deviation from zero with the F test. For statistical analysis and graphs we used Prism 3.0 (GraphPad, Sorrento Valley, CA).

RESULTS

Perceptive thresholds and sensations

Laser pulses with optimal characteristics for exciting A δ receptors elicited a clear pinprick sensation in all irradiated sites in all subjects. Most subjects had the same perceptive threshold in the different sites of stimulation, and the mean perceptive threshold was similar at C₅ and T₁₀ (4.4 \pm 2.9 vs. 5.5 \pm 3.6 mJ/mm²; Mann-Whitney: $P > 0.20$).

Laser pulses with optimal characteristics for exciting C receptors elicited a warm sensation in all irradiated sites in 15 of 17 subjects (2 subjects were excluded from those preliminarily examined because laser stimuli failed to evoke a warm sensation; they described threshold sensations as slight burning and pinprick). Most subjects had the same perceptive threshold in the different sites of stimulation, and the mean perceptive threshold was similar at C₅ and T₁₀ (4.1 \pm 0.9 vs. 4.4 \pm 1.3 mJ/mm²; Mann-Whitney: $P > 0.70$).

Reaction times

The reaction times were measured in 10 subjects. After stimulation of A δ afferents, no significant difference was found between reaction times for stimuli applied to C₅ and T₁₀ (287 \pm 30 vs. 310 \pm 36 ms; Mann-Whitney: $P > 0.20$). In contrast, the reaction times related to C-fiber activation were significantly shorter after C₅ than after T₁₀ stimulations (537 \pm 118 vs. 641 \pm 142 ms; Mann-Whitney: $P < 0.0001$; Table 1).

LEPs

In all 15 subjects, laser stimulation of A δ receptors easily evoked clear and reproducible late LEPs. The earliest identifiable LEP was a negative wave peaking at about 200 ms, followed by a positive wave at about 300 ms, with a peak-to-peak amplitude of about 10 μ V (Fig. 1). Even single trials often yielded a clear LEP, and after few averaged trials, its

peak latency and shape became stable. The latency was significantly shorter after C₅ than after T₁₀ stimulation (paired t -test, $P < 0.001$; Table 1); conversely the peak-to-peak amplitude was similar (paired t -test, $P > 0.5$; Table 1).

The stimulation of C afferents yielded clear and reproducible ultralate LEPs in 14 of the 15 subjects who reported unmistakable sensations of warmth (see preceding text). The main signal was a positive wave peaking at about 500 ms, with an amplitude of about 5 μ V, only occasionally preceded by a negative wave (Fig. 1). No earlier potentials were identified. P-wave latencies were significantly shorter after C₅ than after T₁₀ stimulation (471 \pm 43 vs. 584 \pm 42 ms; t -test: $P < 0.0001$); the amplitudes did not differ (t -test: $P > 0.2$; Table 1).

Conduction velocity of the central pathway activated by A δ input

The regression line calculated from P-wave latencies of late LEPs from all the stimulated sites along the dorsal skin ($n = 38$) indicated a significant linear relationship between distance and time ($r^2 = 0.1565$, $F = 7.422$, $P < 0.01$; Fig. 2A); the resulting conduction velocity (reciprocal of the slope) was 11.9 m/s.

Probably owing to the wide range of reaction times and their relatively small latency difference after A δ stimulation, the regression line for the reaction times was not statistically significant, thus precluding a reliable measurement of conduction velocity.

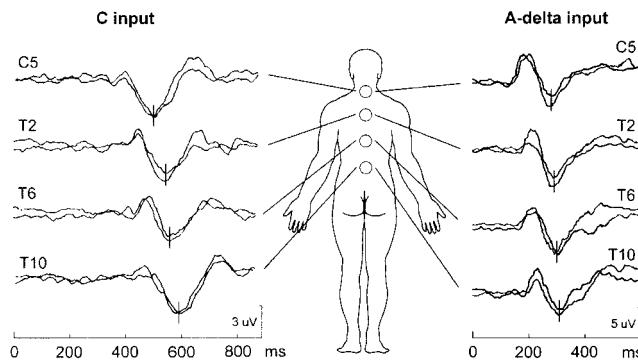


FIG. 1. Late and ultralate laser-evoked brain potentials (LEPs) after stimulation of the skin overlying the C₅, T₂, T₆, and T₁₀ vertebral spinous processes in a representative subject. Negativity upward. Two averages (15 trials each) per site of stimulation. The vertical bars indicate the peak latency of the positive components. Right: stimulation of A δ afferents. Left: stimulation of C warmth afferents.

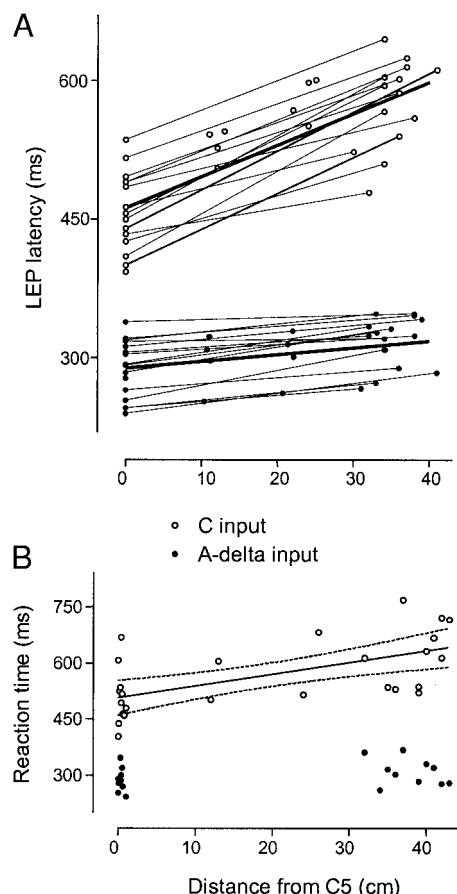


FIG. 2. A: scatterplot of individual latencies of the main positive component of late (15 subjects) and ultralate (14 subjects) LEPs. *y* axis: peak latency of the considered component. *x* axis: each stimulated site is ranked according to its distance from C₅. Each symbol indicates the mean peak latency obtained from 1 site of stimulation (open circles: C input; closed circles: A_δ input). Thin lines show intraindividual latency changes: they either represent the individual regressions in the 4 subjects who had multiple-site stimulations or connect the latencies of the subjects who had C₅ and T₁₀ stimulated. The 2 thick lines are the mean regressions calculated on all stimulated districts (late LEPs: 38 stimulated sites; ultralate LEPs: 36 stimulated sites). The reciprocals of the slopes of each regression line indicate the mean conduction velocities (late LEP: 11.9 m/s; ultralate LEP: 2.55 m/s). Note, in both late and ultralate LEPs, the widely scattered absolute latencies at C₅ (*x* axis = 0), whereas the thin lines showing intraindividual latency changes have remarkably parallel slopes. B: scatterplot of individual mean latencies of reaction times after A_δ fiber and C fiber laser stimulation of the skin overlying vertebral spinous processes (10 subjects). *y* axis: mean reaction time. *x*-axis: each stimulated site is ranked according to its distance from C₅. Each symbol indicates the mean peak latency obtained from 1 site of stimulation (open circles: C input; closed circles: A_δ input). The line is the mean regression calculated on all 24 districts after C fiber stimulation; dashed line indicates the 95% confidence limits. The reciprocal of the slope of the regression line (2.77 m/s) indicates mean conduction velocity. Note that latencies of reaction times after A_δ fiber stimulation were not significantly different at C₅ and T₁₀ (Mann-Whitney: $P > 0.20$).

Conduction velocity of the central pathway activated by C input

The regression line calculated from P-wave latencies of ultralate LEPs from all the stimulated sites along the dorsal skin ($n = 36$) indicated a significant linear relationship between distance and time ($r^2 = 0.5823$, $F = 46.74$, $P < 0.0001$; Fig. 2A). The resulting conduction velocity was 2.55 m/s.

Considering the reaction times to the C-warmth input, the

regression line calculated from all the stimulated sites ($n = 24$) indicated a significant linear relationship between distance and time ($r^2 = 0.3585$, $F = 13.41$, $P < 0.005$; Fig. 2B). The resulting conduction velocity was 2.77 m/s.

Source analysis

In all subjects, for the main positive component of the late (A_δ) LEPs, source analysis yielded a very strong fit for one generator in the posterior portion of the anterior cingulate cortex (pACC; subject 1: residual variance = 9%; correlation = 0.9553; subject 2: residual variance = 8.7%; correlation = 0.9586; subject 3: residual variance = 10.2%; correlation = 0.9507).

In contrast, even though source analysis placed a generator in the cingulate cortex also for the positive component of the ultralate (C) LEPs, it fitted less well than that for late LEPs (mean residual variance = 14.4%). This relatively high residual variance was minimized by a three-dipole model: the best fit was yielded by one generator in the pACC and two symmetrical generators in the right and left opercular-insular cortices (subject 1: residual variance = 10.2%; correlation = 0.9502; subject 2: residual variance = 9.8%; correlation = 0.9521; subject 3: residual variance = 11.5%; correlation = 0.9449). Any other possible location had far lower probabilities. Figure 3 shows the dipoles of the ultralate LEPs superimposed on the MRI scans in one subject.

DISCUSSION

By applying CO₂-laser pulses, with stimulus variables optimal for exciting selectively A_δ nociceptors or C warmth receptors, to the dorsal skin in healthy subjects, we obtained useful physiological information on the spinal cord pathways and brain structures involved in processing of thermal-pain inputs. In the spinal cord, warmth sensations are conveyed by slowly conducting fibers, and in the brain are probably processed by the anterior cingulate and the opercular-insular cortices.

Afferent input, subjective sensation, and brain signals

Laser stimuli at a relatively high intensity, exciting both A_δ and C fibers, can simultaneously activate the sensory systems that mediate warmth, burning, and pinprick sensations (Bromm and Treede 1984). But the brain-evoked responses that are recorded after high-intensity laser stimuli, and are used as a diagnostic test (late LEPs), are related only to the activation of small-myelinated primary sensory neurons and STT neurons (Arendt-Nielsen 1994). Although laser stimulation coactivates both A_δ and C fibers, the recording of brain responses related to the activation of unmyelinated afferents (ultralate LEPs) poses a series of problems. First, the slow conduction velocity of C afferents (calculated in humans with different techniques, it ranges between 0.5 and 2.5 m/s) (Magerl et al. 1999; Nordin 1990; Opsommer et al. 1999; Torebjörk 1974; Torebjörk and Hallin 1974; Tran et al. 2001) induces a dispersion of the ascending volley and probably entails long and variable synaptic times, thus decreasing the amplitude of the brain signals. Second, larger fibers inhibit smaller fibers in the spinal dorsal horn, and, in particular in primates, the A_δ afferent input strongly inhibits the activity of STT cells innervated by C

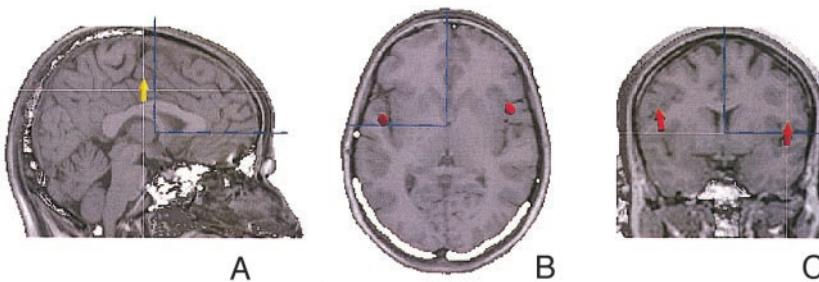


FIG. 3. Source analysis of the cerebral responses evoked by laser thermal stimulation (ultralate LEP) in 1 representative subject. A 3-dipole model gave the best source explanation for the scalp topography of the ultralate LEP. One dipole (*A*, in yellow) was located in the posterior part of the anterior cingulate cortex (pACC) and 2 dipoles (*B* and *C*, in red) were located in the opercular-insular cortex, bilaterally. Dipoles are overlaid on individual high-resolution magnetic resonance images. *A*: sagittal section through the cingulate dipole; *B* and *C*: axial and coronal sections through 1 of the opercular-insular dipoles; the symmetrical dipole is visible too.

afferents (Chung et al. 1984). Third, the activation of cerebral areas generating late LEPs may induce a refractory state so that brain structures become less sensitive to the subsequent C-fiber input (Bromm and Treede 1987). Indeed, the first recordings of ultralate LEPs were obtained after experimental block of the A fibers (Bromm et al. 1983) or in patients with A-fiber neuropathy (Lankers et al. 1991).

Hence, to detect C-related brain activity, we need selective stimuli that avoid A δ fiber activation. The technique proposed by Bragard et al. (1996) takes advantage of the different spatial density of the epidermal free nerve endings that is far higher for C mechano-heat nociceptors (CMH) than for A δ mechano-heat nociceptors (AMH) (Campbell et al. 1989; Ochoa and Mair 1969). Laser stimuli delivered to a very tiny skin area (0.15 mm^2) are unlikely to excite A δ nociceptors and elicit brain responses at a latency compatible with a peripheral C-fiber conduction. These ultralate LEPs seem to be related to CMH units (Opsommer et al. 1999). The technique proposed by Magerl et al. (1999) exploits the different physiological characteristics in the heat threshold of epidermal-free nerve endings. Because experiments in monkeys show that A δ receptors have a higher heat threshold than C receptors (Treede et al. 1995), the use of a stimulus intensity slightly lower than the threshold for A δ nociceptors will selectively activate C fibers and elicit ultralate LEPs in humans.

In an earlier study, we found that laser stimulation of the dorsal skin overlying the vertebral spinous processes, because the receptors are more dense and superficial and because the conduction distance along the primary neuron is very short, yields lower sensory thresholds and larger-amplitude brain potentials than hand stimulation (Cruccu et al. 2000). Furthermore, laser stimulation applied at adequate intensities, duration, and irradiated areas to the dorsal skin allows selective excitation of A δ or C receptors (Agostino et al. 2000). In this study, A δ fibers (type II AMH receptors) were activated with pulses of comparatively high-intensity (mean $9\text{--}18 \text{ mJ/mm}^2$), brief duration (15 ms), and a small irradiated area (about 5 mm^2). Laser pulses of this kind increased the skin temperature from baseline to 48°C , i.e., above the heat threshold of the type II A δ nociceptors (Treede et al. 1995) and were perceived as a sharp pinprick, a sensation conveyed by A δ nociceptive fibers (Arendt-Nielsen and Bjerring 1988; Romaniello et al. 2002). The peak latency of the N-wave (195–210 ms) came between the latencies of the corresponding components after stimuli delivered to the face (170 ms) and hand (240 ms) with the same laser and recording apparatus (Cruccu et al. 1999). This kind of stimulation has proved reliable in physiological and clinical studies (Cruccu et al. 2000; Iannetti et al. 2001a).

To activate C fibers (C warmth receptors), we reduced the stimulus intensity (mean $4.5\text{--}7.5 \text{ mJ/mm}^2$), increased the du-

ration (30–50 ms), and irradiated a larger skin area (about 20 mm^2). The large irradiated area compensated for the low-density and punctate receptive fields of warmth receptors (Campbell et al. 1989; Green and Cruz 1998); the skin temperature increased from baseline to 39°C , i.e., above the heat threshold of C warm receptors (1°C above skin temperature) (Hallin et al. 1981; LaMotte and Campbell 1978) and below that of C nociceptors ($41\text{--}46^\circ\text{C}$) (Hallin et al. 1981; LaMotte and Campbell 1978; Treede et al. 1995). All subjects perceived these stimuli as a warmth sensation; the peak latency of the main positive component of the evoked response (470–580 ms) matched that reported for ultralate LEPs evoked by activation of CMH nociceptors after stimulation of tiny skin areas (460–630 ms) (Qiu et al. 2001; Tran et al. 2002) and was consistently shorter than those found after activation of CMH units of the hand (840–1,000 ms) (Bragard et al. 1996; Magerl et al. 1999; Towell et al. 1996; Tran et al. 2001) and foot (1500 ms) (Opsommer et al. 1999). From the skin temperature induced by the stimulus, the reported sensations, and the latency of the brain signals, we conclude that our stimulation selectively activates unmyelinated afferents and that, although we cannot exclude the co-activation of some CMH units, the evoked responses arise predominantly from the excitation of warmth receptors.

Spinal pathways

The estimated conduction velocity along the spinal cord of the afferent volley generated by the warmth input was slower than that related to the A δ input (2.5 vs. 11.9 m/s).

Estimating a conduction velocity using LEP latencies or reaction times raises two main problems. First, because the number of receptors activated probably differs according to the dorsal site stimulated, spatial summation at central synapses may also differ. To minimize this problem, we used the same multiples of perceptive threshold and delivered similar energy, elicited similar sensations, and thus matching amplitude LEPs. Second, the LEPs, like reaction times, are highly integrated responses; their inherent variability, and possibly cognitive factors, may influence the latency. We used the same mean frequency of arrhythmic stimulation and alternated the sites of stimulation, trying to keep the subjects' attentiveness unchanged. The wide interindividual variability in latency probably depended on individual characteristics, including peripheral (receptor times) and central factors (signal processing within the brain). But neither of these would affect the measurement of conduction velocity. Indeed, as the diagram in Fig. 2A shows, although the absolute latencies were widely scattered, the regression lines for velocity had remarkably parallel slopes.

For similar reasons, underestimating the conduction velocity because of an additional synapse along the pathway between caudal and rostral sites of stimulation seems improbable. LEP latencies and reaction times invariably increased with distance along the spine and the linear fit was highly significant for all measures in particular for the latency of LEPs related to C-fiber activation ($r^2 = 0.604$, $P < 0.0001$). Hence, either the pathways ascending from the caudal spinal cord have no additional synapses or these are homogeneously distributed between T₁₀ and C₅ and do not affect the estimated velocity.

Our estimated conduction velocity for spinal neurons that mediate the pinprick sensation (A δ input) was relatively similar to velocities previously found with laser-stimulation methods measuring P latencies (about 10 m/s) (Kakigi and Shibasaki 1991; Rossi et al. 2000) or N latencies (about 20 m/s) (Crucu et al. 2000) in humans and with direct recording of spinal and thalamic cells in primates (17–22 m/s) (Ferrington et al. 1987; Willis et al. 1974). The A δ -related volley ascends along the STT, the main pathway that conveys nociceptive information from the spinal cord to the forebrain (Craig and Dostrovsky 1999). In primates, the cell bodies of STT neurons lie predominantly in lamina I and V of the superficial dorsal horn (Craig and Dostrovsky 1999; Doubell et al. 1999; Perl 1984); axons of thermoreceptive lamina I cells ascend in the lateral spinothalamic tract (Bowsher 1961; Craig et al. 2002; Zhang et al. 2000a,b). Delayed or absent brain responses after laser stimulation of A δ fibers (late LEPs) have been reported in patients with spinal lesions involving this pathway (Iannetti et al. 2001a; Kakigi et al. 1991; Treede et al. 1991).

Innocuous thermoreceptive primary afferents (A δ “cold” and C “warm” neurons) mainly project to lamina I of the spinal dorsal horn and its trigeminal equivalent, the medullary subnucleus caudalis (Darian-Smith 1984; Kumazawa et al. 1975), where the cell bodies of thermoreceptive-specific second-order neurons lie anatomically distinct from the nociceptive-specific neurons (Han et al. 1998). The majority of these thermoreceptive neurons are cold-responding cells, while neurons selectively excited by warmth are comparatively rare (Andrew and Craig 2001; Dostrovsky and Craig 1996) and have never been described in humans. In cats, the ascending pathways for thermal sensations are probably multiple and scattered in the lateral funiculus (Norrsell 1979); those arising from lamina I project to three thalamic nuclei, nucleus submedius, ventral posterior medial nucleus, and the ventral aspect of the ventral medial basal nucleus (vVMB), with vVMB being probably the most important for thermosensory behavior (Norrsell and Craig 1999); its homologue in humans and nonhuman primates, the posterior ventral medial nucleus (VMpo), is critical for pain and temperature sensation (Blomqvist et al. 2000).

Our study is, to our knowledge, the first to describe a specific spinal pathway for the sense of warmth in humans. From measurements of LEP latencies and reaction times, we estimated a mean conduction velocity of about 2.5 m/s (Fig. 2), indicating that these second-order neurons probably have unmyelinated ascending fibers. Studies of structure-function correlation of lamina I neurons indicate that only cells with a conduction velocity less than 3 m/s have unmyelinated axons (Craig et al. 2001; Han et al. 1998). Our results for the

spinal pathways conveying warmth input are similar to those, obtained by stimulating tiny skin areas, for the spinal pathways related to CMH nociceptors: 1.4–4 m/s (Qiu et al. 2001; Tran et al. 2002). In animal studies investigating thermoreceptive neurons within the dorsal horn, only few data have been reported regarding the activity of central neurons responding to innocuous warming, possibly because of their small number or small size (Darian-Smith 1984). Several studies investigated medullary (Craig and Dostrovsky 1991; Craig et al. 2001; Dickenson et al. 1979) or spinal dorsal horn (Andrew and Craig 2001; Christensen and Perl 1970; Kumazawa et al. 1975) neurons excited orthodromically by innocuous warming of the skin and antidromically by supraspinal electrical stimulation. In all these studies, only few second-order warming neurons were identified. One warm-sensitive cell of the spinal lamina I was excited by stimulation of the contralateral ventral funiculus at mid-cervical level in a monkey; its antidromic response indicated a conduction velocity of about 5 m/s (Kumazawa et al. 1975). One warm-sensitive lamina I cell of the trigeminal nucleus caudalis was excited by stimulation of the thalamic nucleus submedius in a cat; the antidromic response had a 3.6-ms latency (Craig and Dostrovsky 1991). In a recent study, Andrew and Craig (2001) described a small subset (10/474 total cells) of superficial dorsal horn cells that were selectively excited by cutaneous warming in cats. These cells, responding with increasing excitation to innocuous warm stimulation of the hindpaw, had slow conduction velocities (mean: 2.3 m/s), similar to those found in our study.

Brain structures

Using a high number of trials and grand averages, some studies aimed at identifying the cerebral generators of the late LEPs (Bromm and Chen 1995; Valeriani et al. 1996, 2000), found early components (termed N1-P1) that probably originate in the somatosensory cortex, in agreement with functional imaging studies (Brooks et al. 2002; Hofbauer et al. 2001; Talbot et al. 1991). Because of the relatively small number of trials at each stimulated site, we could not identify any components earlier than the main negative-positive complex (termed N2-P2) described in RESULTS. This by no means excludes the possibility that the thermal-pain input yielded by laser stimulation of the dorsal skin also reaches the somatosensory cortex.

The realistic head model based on high-resolution MRI we used for electrical source analysis of the main positive component of late dorsal LEPs yielded the best fit assuming a single-dipole model; the resulting generator had a deep midline location, corresponding to the posterior part of the anterior cingulate cortex (pACC). Hence, also with laser stimulation of a proximal territory such as the skin of the back, the source analysis located the generator in the same region found with hand, foot, or face stimulation (Bromm and Chen 1995; Valeriani et al. 1996, 2000).

Although the ultralate dorsal LEP also had a central generator in the pACC, it was better explained by a three-dipole model, with one generator in the pACC and two symmetrical generators in the right and left opercular-insular cortices (Fig. 3).

The only other study that investigated the neural generators of the ultralate LEPs with source analysis (Opsommer et al. 2001) suggested two possible models: one consisted of two dipoles in the bilateral SII areas and the other consisted of these two dipoles plus a third dipole in the ACC region. This second result almost matched ours. Some differences may arise from the different body district of stimulation (hand vs. back) or may depend on the stimulation techniques: Opsommer et al. used a technique that activates CMH nociceptors, whereas our stimulation technique activates C warmth receptors. Nevertheless the inherent imprecision of the dipolar analysis techniques limits the spatial accuracy of these results. Functional neuroimaging studies may yield more precise information on the brain areas specifically involved in thermal and pain processing.

Several human and animal studies have shown that the cingulate gyrus is involved in the representation of the affective dimension of pain as well as in arousal, attention, response selection, and autonomic activity (Devinsky et al. 1995). Some fMRI studies, using contact thermode (Becerra et al. 1999; Kwan et al. 2000) or laser stimulations (Büchel et al. 2002; Sawamoto et al. 2000) to evoke warmth, innocuous sensations, found an involvement of the anterior cingulate cortex. The ACC activation in response to thermal stimuli probably reflects its broad role in modulating behavioral reactions to external stimuli (Vogt and Sikes 2000).

The other two sources of our warmth-related LEPs were roughly symmetrical and located in the opercular-insular regions. Patients with lesions in the parietal operculum and posterior insula had an increase of pain and thermal threshold on the contralateral body (Greenspan et al. 1999). The posterior insular cortex, in particular, seems to play a crucial role in the processing of thermal sensations (Augustine 1996; Mesulam and Mufson 1982). Using PET in humans, Craig et al. (2000) have recently observed that the activation of the dorsal margin of the middle/posterior insula is linearly correlated with both the intensity of cold stimuli and the subjective rating of the perceived temperature, indicating an involvement of this area in the discriminative representation of thermal perception. In contrast, the anterior insula is more effectively activated during noxious stimulation (Ingvar and Hsieh 1999).

In conclusion, our findings provide new information on the CNS pathways conveying and processing warmth input. Our results support the existence of second-order neurons specific for the sense of warmth. These neurons convey warmth input to the brain through a slow-conducting pathway; in the brain, the warmth input is probably processed by the anterior cingulate and the opercular-insular cortices.

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