Unmyelinated trigeminal pathways as assessed by laser stimuli in humans

G. Cruccu,1,6 E. Pennisi,2 A. Truini,1 G. D. Iannetti,1 A. Romaniello,1 D. Le Pera,3 L. De Armas,3 M. Leandri,5 M. Manfredi6 and M. Valeriani3,4

1Department of Neurological Sciences, Università La Sapienza, 2Ospedale S.Filippo Neri, 3Institute of Neurology, Università Cattolica del Sacro Cuore, and 4Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, 5Inter-University Centre Neurophysiology of Pain (CIND), Genova, and 6Neuromed Institute, Pozzilli, Italy

Summary
Laser pulses excite superficial free nerve endings innervated by small-myelinated (Aδ) and unmyelinated (C) fibres. Whereas laser-evoked scalp potentials (LEPs) are now reliably used to assess function of the Aδ-fibre nociceptive pathways in patients with peripheral or central lesions, the selective activation of C-fibre receptors and recording of the related brain potentials remain difficult. To investigate trigeminal C-fibre function, we directed laser pulses to the facial skin and studied sensory perception and scalp evoked potentials related to Aδ- or C-fibre activation in healthy humans and patients—one having a bilateral facial palsy, two a trigeminal neuropathy, and two a Wallenberg syndrome. We also measured afferent conduction velocity and, with source analysis, studied the brain generators. Whereas laser pulses of low intensity and small irradiated area elicited pinprick sensations and standard Aδ-LEPs, laser pulses of very-low intensity and large irradiated area elicited warmth sensations and scalp potentials with a latency compatible with C-fibre conduction (negative wave 280 ms, positive wave 380 ms); the estimated conduction velocity was 1.2 m/s. The main waves of the scalp potentials originated from the anterior cingulate gyrus; they were preceded by activity in the opercular region and followed by activity in the insular region. The patient with bilateral facial palsy, who had absent trigeminal-facial reflexes, had normal Aδ- and C-related scalp potentials; the patients with trigeminal neuropathy, characterized by loss of myelinated and sparing of unmyelinated fibres, had absent Aδ- but normal C-related potentials; and the patients with Wallenberg syndrome had absent Aδ- and C-related potentials. We conclude that laser pulses with appropriate characteristics evoke brain potentials related to the selective activation of trigeminal nociceptive Aδ or thermal C fibres. The trigeminal territory yields rewarding LEP findings owing to the high density of thermal receptors and, because the short conduction distance, minimizes the problem of signal dispersion along slow-conducting unmyelinated afferents. The opercular-insular region and the cingulate gyrus are involved in the processing of C-fibre trigeminal inputs. The method we describe may prove useful in patients with lesions affecting the trigeminal thermal pain pathways.

Keywords: trigeminal nerve; unmyelinated fibres; laser evoked potentials; thermal-pain perception; trigeminal nerve biopsy

Abbreviations: Aδ-LEP and C-LEP = laser evoked potentials after selective activation of small-myelinated (Aδ) and unmyelinated (C) afferents; LEPs = laser-evoked scalp potentials; Nd:YAP = neodymium-doped yttrium aluminium perovskite; SI and SII = primary and secondary somatosensory cortex

Introduction
Radiant heat pulses delivered by high-power laser stimulators and directed to the hairy skin excite superficial free nerve endings innervated by small-myelinated (Aδ) and unmyelinated (C) fibres. Laser-evoked scalp potentials (LEPs) are now reliably used to assess function of the Aδ-fibre nociceptive pathways in patients with peripheral neuropathy or central lesions (Bromm and Treede, 1991; Kakigi et al., 1991, 1992; Treede et al., 1991), including mandibular neuropathy, trigeminal neuralgia, and Wallenberg syndrome, which impair the trigeminal system (Cruccu et al., 1999, 2001). Although the selective activation of C-fibre receptors and recording of the related brain potentials are more
difficult, C-fibre evoked potentials after hand and foot stimulation have been described in healthy humans (Bromm et al., 1983; Bragard et al., 1996; Towell et al., 1996; Magerl et al., 1999) and patients (Treede et al., 1988, 1991; Lankers et al., 1991; Granot et al., 2001). These studies used various techniques: experimental block of group A fibres (Bromm et al., 1983); spectral analysis of the expected time window (Arendt-Nielsen, 1990; Bragard et al., 1996); selection of single trials devoid of Aδ-LEPs (Towell et al., 1996); ‘microspot’ stimulation (Bragard et al., 1996; Opsommer et al., 1999); or stimulus intensities below the Aδ activation threshold (Treede et al., 1995; Magerl et al., 1999; Iannetti et al., 2003).

To select further between C nociceptors and C warmth receptors, the investigators exploited differences in their threshold and density. C warmth receptors have a slightly lower threshold than C nociceptors and a far lower density in the skin (LaMotte and Campbell, 1978; Tillman et al., 1995; Green and Cruz, 1998). Hence, the highest probabilities of selective activation for warmth receptors are yielded by low-intensity laser pulses irradiating a large skin area (Towell et al., 1996; Agostino et al., 2000; Iannetti et al., 2003) and those for C nociceptors by stimulation of very small areas (~0.2 mm) (Bragard et al., 1996; Opsommer et al., 1999; Qiu et al., 2001; Tran et al., 2002).

To assess trigeminal C-fibre function, we used laser pulses directed to the facial skin and recorded the related brain potentials in healthy humans. To verify that these brain potentials were generated by the thermal-pain sensory system, we also studied five patients: one with bilateral facial palsy; two with trigeminal neuropathy; and two with Wallenberg syndrome.

**Subjects and methods**

Sixteen healthy volunteers (authors, research staff, PhD students and residents of the School of Neurology, La Sapienza University, Rome), aged 22–52 years, and five patients participated in the study. Patient 1 was a 27-year-old man, who had a head injury 2 weeks before examination; bilateral fractures at the base of the petrous bone had caused a bilateral facial palsy. Patients 2 and 3 were 63- and 64-year-old men, both with trigeminal neuropathy; Patient 2 had undergone a supraorbital nerve biopsy. Patient 4 was a 61-year-old man and Patient 5 a 44-year-old woman; both had a Wallenberg syndrome. MRI scans showed an ischaemic infarction in the dorsal-lateral medulla, on the left side in Patient 4 and the right in Patient 5. In these patients, we also assessed trigeminal function by recording the blink reflex, masseter inhibitory reflex, jaw jerk and motor evoked potentials after transcranial magnetic stimulation (Cruccu et al., 1989; Ongerboer de Visser and Cruccu, 1993).

All subjects and patients gave informed consent according to the Declaration of Helsinki and the Comitato Etico Ricerche Neurofisiologiche Dipartimento Scienze Neurologiche approved the procedures.
Laser stimulation
We used two kinds of stimulator: a CO₂-laser (wavelength 10.6 μm, pulse duration 5–50 ms, maximum energy 1.5 J) commonly used in clinical practice and a neodymium:yttrium-aluminium-perovskite laser (Nd:YAP) (wavelength 1.34 μm, duration 1–10 ms, maximum energy 7 J) with fibre-optic guidance. Both were produced by Electronic Engineering, Florence, Italy. In preliminary experiments, we found that CO₂-laser pulses (pulse duration 30–50 ms, irradiated area ~20 mm², intensity 6–11 mJ/mm²), elicited warmth sensations and brain potentials (C warmth input) if directed to the region of the upper-lip, in particular close to the sulcus nasus-genius and ala nasi. Stimuli directed elsewhere failed to evoke a purely warmth sensation or brain potentials. We then tried other laser stimulators and found that the Nd:YAP laser proved optimal to elicit warmth sensations in all facial territories. Nd:YAP laser pulses also had the advantage, as reported for the thulium laser (Spiegel et al., 2000), of inducing no damage to the facial skin—not even the transient dyschromic spots sometimes produced by CO₂-lasers (Cruccu et al., 1999, 2001; Romaniello et al., 2002).

Nd:YAP laser pulses of low intensity (16–37 mJ/mm²), relatively-long duration (10 ms) and large irradiated area (~180 mm²), raising the temperature of the facial skin to 39°C [as measured with a thermocouple (Iannetti et al., 2003)], were optimal to elicit purely warmth sensations (C-input). Pulses of higher intensity (49–76 mJ/mm²), short duration (1 ms), and small irradiated area (~5 mm²), raising the temperature to 48°C, were optimal to elicit pinprick sensations (Aδ-input).

Laser pulses were directed to the perioral or supraorbital skin. The irradiated spot was slightly shifted after each stimulus to avoid receptor sensitisation, and stimuli were delivered arrhythmically with 10–30 s intervals to minimize central habituation. Sensory thresholds were determined with series of increasing and decreasing stimulus-intensities (Cruccu et al., 1999; Agostino et al., 2000). Subjects were asked to describe the evoked sensation by choosing one of the following descriptors: ‘touch’; ‘pinprick’; ‘warmth’; ‘burning’; or ‘it’s difficult to describe’.

To estimate the afferent conduction, we stimulated the supraorbital skin at three sites along the course of the supraorbital nerve branches in eight subjects (Fig. 1). We drew three points on the forehead skin: the most proximal above the eyebrow; the most distal on the scalp; and the third midway between the two. For each point, stimuli were delivered to an area of ~4 × 2 cm. To ensure a reproducible input and thus a similar level of spatial summation at central synapses, we set the stimulus intensity at the same multiples of the perceptive threshold at each area of stimulation. To avoid receptor sensitization or fatigue or a different level of habituation, we alternated the proximal, intermediate and distal areas of stimulation. Finally, we measured the LEP latencies (at the peak of the N2 wave) and distances, and calculated the slope of the total regression line obtained from the 24 couples of samples (Cruccu et al., 2000; Iannetti et al., 2003).

Electrical source analysis
Ten subjects also underwent multi-electrode recordings (31-channel cap plus 1 EOG channel) to calculate a hypothesis-based model of a dipolar generator of the scalp signal. Using a Quick Brain System 98 (Micromed, Treviso, Italy), we averaged three blocks of 20 artifact-free trials after stimulation of the right perioral region for each subject and examined the grand-average (60 × 10). We performed dipolar source modelling using Brain Electrical Source Analysis (BESA) with a ‘sequential strategy’ as described in detail elsewhere (Valeriani et al., 2001). The model was built initially from the grand-average traces calculated across all 10 subjects; it was then applied to the individual C-related LEPs. BESA is a program that uses the surface-recorded EEG to estimate the source activities generating the scalp EP topography and then verifies whether the hypothesized dipolar model accounts for the recorded traces. The percentage of the recorded signal that cannot be explained by the dipolar model is indicated as residual variance (RV) (Scherg, 1990; Bromm and Chen, 1995). The dipole locations were expressed by Talairach’s coordinates and converted into the Montreal Neurological Institute’s MRI template (Evans et al., 1993).

Scalp recordings
Participants were seated in a dentist’s chair and wore protective goggles. White noise was given through earphones. Subjects were instructed to keep their eyes open and gaze slightly downwards. In all subjects, the signals were recorded with disk electrodes from the vertex and referenced to linked earlobes (bandwidth 0.3–30 Hz). Simultaneous electrooculography monitored ocular movements or eye-blanks. Two series of 20 artifact-free trials were collected and averaged off-line. We measured the peak latencies of the main negative (N2 wave) and positive (P2 wave) components and their peak-to-peak amplitude (Fig. 1).

Statistics
We analysed intra-individual differences using the Wilcoxon matched-pairs test. Because the variance of latency values differed significantly between some groups, we assessed mean differences between groups with Welch’s corrected test. We assessed the significance of the regression line used for estimating conduction velocity with the r correlation index of linear regression and the deviation from zero of the slope with F test. For all statistics and graphs, we used Prism 3.0 (GraphPad, Sorrento Valley, CA, USA). Throughout the text and tables, data are given as means ± 1SD.
Results

Sensory perception

When determining the sensory threshold with laser pulses optimal for Aδ-fibre activation, one subject reported a ‘slight feeling of warmth’, two said that they could not describe the sensation, and 13 reported a ‘slight pinprick’.

At the intensity used for evoked potential recording (1.5–2 × sensory threshold), all subjects reported a clear ‘pinprick’ sensation.

When the sensory threshold was determined with laser pulses optimal for C-fibre activation, all subjects reported a ‘slight warmth’. At the intensity used for evoked potential recording (1.5 × sensory threshold), all subjects reported a clear ‘warmth’ and, in one subject, the N2 wave was poorly reproducible.

LEPs

In all subjects, stimuli set for Aδ-fibre activation evoked vertex potentials consisting of a negative wave at 160 ms and a positive wave at 240 ms, similar to those commonly elicited by CO2 lasers (Cruccu et al., 1999, 2001). Perioral and supraorbital stimulations gave similar results (Table 1).

In all subjects, stimuli set for C-fibre activation and directed to the perioral region elicited clear scalp potentials, consisting of a widespread negative–positive complex (N2 wave 280 ms; P2 wave 380 ms), with maximum amplitude at the vertex. In two subjects, however, the N2 wave was small and poorly reproducible (Table 1). In 10 subjects, we delivered stimuli set for C-fibre activation to the supraorbital skin. Again all subjects had a clear and reproducible P2 wave with maximum amplitude at the vertex and, in one subject, the N2 wave was poorly reproducible. LEPs after perioral and supraorbital stimulations did not differ significantly (Wilcoxon, P > 0.10). The intra-individual latency difference between C-related and Aδ-related LEPs always exceeded 100 ms (Wilcoxon, P < 0.0001).

After CO2 laser stimulation of the upper lip, LEPs had a slightly longer latency and lower amplitude than those after Nd:YAP stimulations; the differences were not significant (Table 1).

Conduction velocity

Probably because the available conduction distance was relatively short (maximum 150 mm) and the Aδ afferents have a comparatively high conduction velocity, no significant correlation was found between their latency and distance.

In contrast, the low conduction velocity of unmyelinated afferents yielded large differences in the latency of C-related LEPs elicited along the course of the supraorbital nerve. Stimulation at proximal and distal sites yielded LEPs of similar amplitude (Welch’s test, P > 0.10). The regression line between latency and distance for all the 24 stimulated sites was highly significant (r = 0.6269, P < 0.005), as was the deviation of the slope from zero (F = 10.36, P < 0.005). The conduction velocity, indicated by the reciprocal of the slope, was 1.2 m/s (Fig. 1). Individual velocities ranged from 0.7 to 1.6 m/s.

Table 1

<table>
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<tr>
<th>Input and laser</th>
<th>Supraorbital</th>
<th>Perioral</th>
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<tr>
<td></td>
<td>N2 wave latency</td>
<td>P2 wave latency</td>
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<tr>
<td>Aδ-fibre Nd:YAP</td>
<td>16 ± 15</td>
<td>16 ± 24</td>
</tr>
<tr>
<td>C-fibre Nd:YAP</td>
<td>14 ± 276</td>
<td>16 ± 377</td>
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<tr>
<td>C-fibre CO2</td>
<td>10 ± 297 ± 30</td>
<td>10 ± 404 ± 38</td>
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<tr>
<td>C-fibre CO2</td>
<td>10 ± NS</td>
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1Stimulation confined within the region between upper lip and sulcus nasus-genius; 2Statistical significance of differences between C-fibre LEPs after Nd:YAP and CO2 stimulations (Welch’s test). NS = not significant.

Fig. 2 Findings in a patient with bilateral facial palsy (Patient 1). Right- and left-side responses. Supramaximal electrical stimulation failed to evoke reflex responses (S1). Laser pulses set for Aδ-fibre activation (S2, 50 mJ/mm2, 4.9 mm2) or C-fibre activation (S3, 20 mJ/mm2, 177 mm2), evoked the corresponding late and ultralate evoked potentials (Aδ-LEP and C-LEP). Stimulation of left (L) and right (R) sides. Note that calibration changes in S1: sweep 100 ms; sensitivity 50 mV.
Patients

In Patient 1, who had a bilateral facial palsy, EMG examination showed complete absence of voluntary activity and absence of all trigeminal facial reflexes bilaterally. But he had normal pinprick and warmth thresholds, as well as normal Aδ-related and C-related LEPs (Fig. 2).

In Patient 2, who had trigeminal neuropathy, a sensory deficit in the intra- and perioral region had begun at 46 years and had progressed slowly to involve all the face bilaterally. At the age of 57 years, all the trigeminal reflexes including the blink reflex, the maserter inhibitory reflex and the jaw jerk were absent, whereas trigeminal motor function was normal; a supraorbital-nerve biopsy, compared with those of other patients (Pennisi et al., 1997), showed severe degeneration of myelinated axons, whereas unmyelinated fibres had a normal density (35 000/mm²) and showed no collagen pockets or other histological abnormality on electron microscopy (Fig. 3). When we performed the laser study, the patient was 63 years old; he had probably lost further myelinated fibres because he had completely lost sharp–dull discrimination in all trigeminal territories. The laser-evoked blink reflex, a response mediated by Aδ-afferents (Romaniello et al., 2002) was absent bilaterally. Laser pulses set for eliciting Aδ-related LEPs, even at high stimulus intensities, failed to elicit pinprick sensations; the patient felt burning sensations and the scalp signals had a latency compatible with unmyelinated afferent activation. Laser pulses irradiating a large spot revealed a normal warmth threshold and elicited normal C-related LEPs (Fig. 3). Patient 3 reported pain and paraesthesias in the maxillary and mandibular trigeminal divisions 2 years ago. Now, he has a severe sensory loss in the intraoral and perioral territories; all the trigeminal reflexes from the maxillary and mandibular divisions are absent bilaterally, and the early and late blink reflexes are markedly abnormal bilaterally. Laser pulses directed to the perioral territory, whether set for eliciting Aδ- or C-related LEPs, failed to elicit pinprick sensations; the patient felt only burning sensations and the scalp signals had a latency compatible with unmyelinated afferent activation (N 330 ms, P 416 ms, 7 μV; N 338 ms, P 432 ms, 5 μV).

Patient 4 underwent the laser study 2 years after the onset of his Wallenberg syndrome. He still had a slight anisocoria, a slight thermal-pain hypesthesia on the left face and right limbs, and mild allodynic pain to light touch or stroking the skin of the left eyebrow and cheek. On the affected side, both pinprick and warmth thresholds to laser stimuli were increased, and both Aδ-related and C-related LEPs were absent (Fig. 4). Patient 5 had an acute Wallenberg syndrome, with thermal-pain hypesthesia on her right face and left limbs, right Bernard-Horner syndrome, dysphagia and mild cerebellar signs. As for Patient 4, on the affected side the laser thresholds were increased and both Aδ-related and C-related LEPs were absent.

Brain generators

In the grand-average from the 10 subjects who underwent the multi-electrode recordings of the C-related LEPs, scalp topography was best explained by a five-dipole model (Fig. 5). Two first dipoles were localized bilaterally in the parietal opercular region, commonly identified as the secondary somatosensory area (SII). The latency of their peak activity (~200 ms) corresponded to an early negative wave disclosed by the grand-average in the temporal region (N1). A generator in the posterior portion of the anterior cingulate...
cortex was activated at the latency of the main negative-positive complex (N2±P2, 280±380 ms), with the P2 wave also supported by bilateral generators in the insular region. Although the SII and insular dipoles were closely located, their time courses clearly differed (Fig. 5E). This five-dipole model yielded a 3.7% residual variance. Other possible dipolar locations, including the primary somatosensory area (SI), were tested and all yielded a far higher residual variance.

When we applied the grand-average model to traces obtained from individual subjects, allowing the dipoles to move, neither the dipolar waveforms nor the source locations differed markedly from those yielded by the grand-average model (Table 2). The inter-individual variability of source locations was similar to that found for trigeminal Aδ-related LEPs (Bromm and Chen, 1995; Kazarians et al., 1995).

Discussion
Laser pulses with adequate characteristics, directed to the facial skin, selectively evoked pinprick or warmth sensations and brain potentials in latency ranges compatible with Aδ- and C-fibre conduction. The estimated conduction velocity of the afferents for C-related brain potentials was ~1.2 m/s, i.e. in the range of unmyelinated fibre conduction. Exemplary cases, serving as experimenta naturae, helped us to demonstrate that these scalp potentials are of unequivocal neural origin, are mediated by unmyelinated afferents, and follow the thermal-pain pathway in the brainstem. The most probable generators lie in the anterior cingulate gyrus and the bilateral opercular-insular regions.

Selectivity of stimulation
Ample evidence shows that CO2 lasers exclusively excite the most superficial free nerve endings that mediate thermal-pain sensations. Solid-state lasers, such as the Nd:YAP laser used in this study or the more widely used Nd:YAG or Tm:YAG lasers (Spiegel et al., 2000; Lefaucheur et al., 2001), having a shorter wavelength, inevitably penetrate deeper through the skin. These lasers also yield LEPs with a slightly shorter latency. Hence, the real ‘selectivity’ of solid-state lasers remains debatable.

A series of findings enable us to be certain that our Nd:YAP laser stimulation selectively excited Aδ or C thermal-pain receptors and that we recorded genuine neural signals related to this selective input. First, evoked sensation and LEP latency after Nd:YAP laser pulses set for Aδ-fibre activation matched those yielded by CO2-lasers in previous studies (Cruccu et al., 1999, 2001). Secondly, with low-intensity and large-spot CO2-laser pulses directed to the upper lip close to the sulcus nasus-genius (an area which in lower mammals is particularly rich in warmth receptors) (Dickenson et al., 1979), we evoked warmth sensations and clear LEPs at a latency similar to that yielded by the Nd:YAP laser (Table 1). The drawback of the CO2-laser was that pulses directed to skin spots outside the upper lip inevitably co-activated Aδ-fibres, as the appearance of the Aδ-related N160 component in the brain signals confirmed. Thirdly, with Nd:YAP laser pulses set for C-fibre activation all subjects invariably reported a purely warmth sensation. Fourthly, source analysis of the brain signal and our findings in the patients excluded a possible contamination by reflex activity (Patient 1) and confirmed that the afferent input was conveyed by unmyelinated afferents (Patients 2 and 3), and that it was mediated by the trigeminal thermal-pain nucleus in the medulla (Patients 4 and 5).

Although the stimuli we used certainly selectively activated unmyelinated afferents, we cannot exclude the possibility that nociceptive mechanio-heat receptors innervated by unmyelinated fibres (CMH units) contributed to the input. We

Fig. 4 MRI and neurophysiological findings in a patient with Wallenberg syndrome (Patient 4). T2-weighted MRI scans showed a small infarction in the left dorsal-lateral medulla. Laser stimuli, whether set for Aδ-fibre (76 mJ/mm², 4.9 mm²) or C-fibre activation (25 mJ/mm², 177 mm²) when directed to the ipsilateral face failed to evoke LEPs, whereas those directed to the contralateral face evoked normal Aδ-related and C-related LEPs. Stimulation of left (L) and right (R) sides.
believe that most of the afferent volley arose from warmth rather than CMH receptors because of the low rise in skin temperature induced by the laser pulse (always lower than 40°C) and because our subjects explicitly reported a non-burning warmth sensation. The brain signals provide no help in this case, because thermal and nociceptive sensations seem to have similar brain generators. Indeed, in Patient 2—who, as expected in trigeminal neuropathy (Lecky et al., 1987) lacked myelinated fibres—we could increase the stimulus intensity without eliciting any Aδ-related potential: the patient felt clear burning sensations and the brain signals did not differ from those seen with the stimulus characteristics optimal for eliciting warmth sensations (Fig. 3). Similarly, in the other patient with trigeminal neuropathy (Patient 3), we could increase the stimulus intensity without eliciting any Aδ-related potential, but this patient felt only burning sensations regardless of stimulus characteristics.

**Unmyelinated trigeminal afferents**

The measurement of conduction velocity must be considered an estimate, because, instead of always stimulating the same spot and recording the afferent volley at different sites along the nerve (which cannot be done even with microneurography), we stimulated different spots and measured the latency of the brain potentials. By taking care to provide a quantitatively similar input, thus minimizing differences in spatial summation at central synapses, we obtained LEPs of similar amplitude. Calculating the slope of the regression line evaluates statistically the reliability of the estimate of velocity and, by minimizing the weight of individual measures, increases their statistical power (Cruccu et al., 2000; Iannetti et al., 2003).

The conduction velocity that we found, 1.2 m/s, clearly comes within the 0.4–2.5 m/s range of unmyelinated fibres. In Patient 2, the unmyelinated fibres of the supraorbital nerve ranged from 0.2 to 1.4 μm in diameter, with one peak between 0.4 and 0.8 μm, consistent with our estimate of conduction velocity in the supraorbital nerve. Calculated using Gasser’s ratio (1.7), the expected velocity for that diameter peak would be 0.7–1.4 m/s (Gasser, 1950, 1955).

The available information on unmyelinated-fibre conduction in the trigeminal nerve comes from two studies only: one using electrical stimulation and microneurographic recordings in man (0.6–1.4 m/s; Nordin, 1990) and the other investigating polymodal nociceptors in the monkey (0.8 m/s; Beitel and Dubner, 1976).

The results of studies in the human limb nerves suggest that velocities of thermal and nociceptive C fibres overlap. Studies using C-related LEPs after laser stimulation of the upper arm reported a similar mean velocity of 1.2 m/s and 1.3 m/s for the nociceptive (Opsommer et al., 1999; Tran et al., 2002) and 1.3 m/s for the thermal fibres (Towell et al., 1996). In a human study still using C-related LEPs, Magerl et al. (1999) estimated a velocity of 2.4 m/s, but these investigators left open the question whether the afferents were thermal or nociceptive. Studies using warm stimuli (contact thermode) and measuring reaction times estimated a velocity of 0.5–1.5 m/s (Yarnitsky and Ochoa, 1991; Opsommer et al., 1999). Microneurographic data on human thermal afferents are based on very few fibres, probably because of the small number of this kind of afferents. In a large number of mechano-insensitive (CH) and mechano-thermal (CMH) C-fibre nociceptors, the mean velocities were 0.8 and 1.0 m/s (Weidner et al., 1999).

In studies that compared thermal and nociceptive afferents in monkeys, in contrast, the conduction velocity was
consistently higher in thermal than in nociceptive unmyelinated afferents (1.1–1.2 m/s versus 0.8 m/s) (Darian-Smith et al., 1979; LaMotte and Campbell, 1978).

Although these data suggest that the conduction velocity found in this study fits more with thermal than with nociceptive afferents, we must consider that the bulk of the available literature refers only to limb nerves. The conduction velocity of the trigeminal afferents is also only one of their peculiar physiological properties (Cruccu et al., 1987; Leandri et al., 1998).

Pathways
The time spent for receptor activation with laser stimulation (~40 ms) has been measured with microneurographic recordings from the human superficial radial nerve (Bromm and Treede, 1991). The activation times for myelinated and unmyelinated receptors after laser stimulation of the facial skin are unknown. The times for receptor activation may be longer on the hand than on the facial skin, which is thinner and probably has a higher receptor density (Whitton and Everall, 1973; Agostino et al., 2000).

The small thermal-pain afferents project to the trigeminal nucleus caudalis. From measurements on adult skull and stereotactic atlas, the route from the supraorbital region to the caudalmost trigeminal complex at C1 level (the maximum possible distance along the primary sensory neuron) amounts to 140 mm (Schaltenbrand and Wahren, 1977; Paxinos et al., 1995; Leandri et al., 1998). In our experiment on conduction velocity, we estimated a velocity of 1.2 m/s for the unmyelinated trigeminal afferents (Fig. 1). This velocity would yield a conduction time of 117 ms along the primary neuron. The distance from C1 to the thalamus is ~65 mm; the conduction time is usually higher in the central tracts than in the periphery: that for the laser-elicited C input along the spinal cord has been estimated as 2.2–2.9 m/s (Iannetti et al., 2003; Tran et al., 2002); at this velocity 22–30 ms should be spent along the secondary neuron. No more than 180–187 ms (receptor 40 ms, primary neuron 117 ms, secondary neuron 22–30 ms) should elapse from the stimulus onset to the arrival of afferent impulses at the thalamus. The peak latency of the main negative component of C-related LEPs was 289 ms, which leaves some 100–110 ms for conduction and processing within the brain, a delay certainly unjustified by thalamocortical conduction time alone.

Given the reported estimates of afferent conduction velocity along the primary neuron (9–15 m/s, Kenton et al., 1980; Bromm and Treede, 1991) and secondary neuron (10–20 m/s, Kakigi and Shibasaki, 1991; Cruccu et al., 2000) for the laser-elicited Aδ input, the Aδ impulses would take 22 ms with the lowest velocities and 12 ms with the highest on the same route as that calculated for the C input. Considering the time spent for receptor activation (40 ms), 52–62 ms elapse before the impulses reach the thalamus. The peak latency of the main component of the Aδ-related LEPs (corresponding to the so-called N2 after hand stimulation) was 166 ms. Hence the intracerebral times of the Aδ-related LEPs (166 minus 52–62 ms = 102–112 ms) were similar to those of the C-related LEPs.

According to the source analysis (Fig. 5), the generator of the main components (N2–P2) of our C-related LEPs was located in the posterior part of the anterior cingulate cortex; the P2 wave also being supported by bilateral generators in the insula, i.e. in the same regions that generate the N2–P2 components of the Aδ-related LEPs after hand, foot, and face stimulation (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996, 2000). The generators of the early waves (N1–P1) of the Aδ-related LEPs after hand or face stimulations have been located in lateral areas—mostly the secondary somatosensory cortex (SII) (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996, 2000). The long intracerebral times that we estimated (>100 ms) suggest that the C afferent input, before reaching the cingulate cortex, is conveyed and processed (like the Aδ input) elsewhere. Although we did not identify an early activity preceding the N2 wave in the standard individual recordings, the grand-average did reveal a negative wave at ~200 ms in the temporal leads and source analysis yielded the highest probabilities for a generator in the SII region (Fig. 5).

Consistently, in recent studies on functional MRI (fMRI) activation by laser pulses applied to the hand (Bornhovd et al., 2002; Buchel et al., 2002), the anterior cingulate cortex (and the dorsolateral prefrontal cortex) discriminated between warm and non-perceived stimuli; however, the SII-insular regions also seemed to show activation by warm stimuli. Although magnetoencephalography (MEG) succeeds in detecting field sources in SI generated by Aδ painful inputs (Ploner et al., 1999, 2002; Kanda et al., 2000; Inui et al., 2002; Tran et al., 2002), the MEG results about a possible field source generated by C-fibre input in SI are still

### Table 2 Talairach coordinates of C-LEP sources (mean ± SD)

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<th>Dipole 4</th>
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<th>Dipole 5</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
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<td>z</td>
<td>x</td>
<td>y</td>
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</tr>
<tr>
<td>Grand-average</td>
<td>3.7</td>
<td>-47</td>
<td>6</td>
<td>11</td>
<td>54</td>
<td>9</td>
<td>12</td>
<td>-6</td>
<td>27</td>
<td>36</td>
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<tr>
<td>Individual subjects</td>
<td>7.96</td>
<td>-46.7</td>
<td>6.4</td>
<td>9.8</td>
<td>52.3</td>
<td>7.2</td>
<td>11.1</td>
<td>-2.7</td>
<td>24.2</td>
<td>31.7</td>
</tr>
<tr>
<td>SD</td>
<td>2.53</td>
<td>0.5</td>
<td>6.9</td>
<td>8.3</td>
<td>2.2</td>
<td>4.1</td>
<td>1.3</td>
<td>6.4</td>
<td>12</td>
<td>8.1</td>
</tr>
</tbody>
</table>

RV = residual variance.
controversial (Ploner et al., 2002; Tran et al., 2002). Most investigators agree that the main generators of the LEPs after C-nociceptor stimulation are the same brain regions (anterior cingular cortex and SII-insular areas) that we activated with warm stimuli (Opsommer et al., 2001; Ploner et al., 2002).

Involvement of the same brain regions in processing warm and noxious laser stimuli lends support to the view that heat-pain perception requires integrated information from both nociceptive and thermal channels (Defrin et al., 2002).

**Conclusion and clinical significance**

The distinctive feature of this study is that it provides objective evidence of brain signals certainly related to the activation of unmyelinated trigeminal afferents. It also describes their physiological properties, including afferent conduction velocity and brain generators.

Because the short conduction distance minimizes the problem of signal dispersion along slow-conducting unmyelinated afferents and probably because the area has a high receptor density, the trigeminal territory is advantageous for studying C-related LEPs. Few trials are sufficient to obtain reproducible brain signals and no damage at all is caused to the skin. Under fibreoptic guidance, the laser beam can be directed to any facial region. Although the brain signals that can be measured easily in patients (i.e. the main N2/P2 complex), rather than representing the arrival of the afferent input at the sensory-discriminative cortex probably reflect the activity of non-sensory-modality-specific processing in the cingulate cortex, they nonetheless provide a reliable assessment of the unmyelinated fibre pathways. As we verified in our patients, these responses are sensitive to peripheral and central lesions of the trigeminal thermal-pain pathways. Our technique may prove clinically useful in any condition affecting these pathways.

**References**


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