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Microneurographic study of C fiber discharges induced by CO₂ laser stimulation in humans

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Abstract

We investigated C-fiber discharges and cerebral potentials evoked by weak CO₂ laser beams applied to a tiny skin area in five healthy subjects. Microneurography was performed from the peroneal nerve in the right popliteal area. Cerebral potentials were recorded from the Cz electrode referred to linked earlobes. The mean conduction velocity of five stable single units was 1.1 ± 0.3 m/s. The mean latency of the positive peak of cerebral potentials was 1327.4 ± 46.2 ms. These findings indicated that this new stimulation method selectively activated C-fiber nociceptors of the skin.

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Since Mor and Carmon demonstrated that a brief CO₂ laser pulse can produce brain potentials related to A δ -fiber activities [7], many studies have used laser beams to study the pain system in humans [6]. However, laser pulses have been known to activate both A δ - and C-fiber nociceptors [3] and it has been very difficult to stimulate the latter selectively using this method. Recently, Plaghki's group developed a method of selectively stimulating C-fiber nociceptors [2]. They demonstrated that CO₂ laser stimulation of a tiny skin surface area (0.15 mm²) produced ultra-late brain potentials related to C-fiber activation. This method is based on the physiological background that C afferent sensory terminals in the skin have a higher density and lower activation threshold than the A δ terminals [8,14,17]. A few recent studies have confirmed that similar methods can produce very late responses recorded by electroencephalography (EEG) [5,12,13,16] and magnetoencephalography (MEG) [5,11,15]. These studies reported peripheral conduction velocities (CV) of 0.8–1.5 m/s, which are in the CV range of C-fibers. However, since

that was an indirect method, a more accurate or direct method for measuring the CV of C-fibers using microneurography is necessary. This is the objective of this study. We also compared the findings with those of evoked EEG recorded simultaneously.

Five healthy male volunteers participated in the study. Their ages ranged from 28 to 39 (mean \pm SD: 35 ± 5) years and their heights from 165 to 172 (mean \pm SD: 169 ± 3) cm. None of the subjects had diseases that might affect normal somesthetic perception. All participants gave informed consent and the research protocol was approved by the Ethics Committee of our Institute.

To activate cutaneous A δ - and C-fiber nociceptors, a CO₂ laser stimulator was used (Nippon Infrared Industries Co. Ltd., Tokyo, JAPAN). The laser beam had a 10.6 μ m wavelength that was 2 mm in diameter and 20 ms in duration. To selectively stimulate C-fiber nociceptors, we used a thin aluminum plate (0.1 mm in depth, 40 mm in length and 60 mm in width) as described previously [10]. In a 25 \times 25 mm square on this plate, parallel lines were drawn every 1 mm, giving 26 \times 26 intersections. A total of 676 (26 \times 26) tiny holes were drilled at these intersections, each with a diameter of 0.4 mm, corresponding to an area of

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0.125 mm² for each hole. This thin plate was used as a spatial filter and placed the skin at the site to be stimulated. The array of holes allowed the 2 mm laser beam to pass through one to four holes to reach the skin. The stimulus intensity was approximately 2–4 Watts. Laser pulses were delivered with interstimulus interval of 20–40 s on the dorsal foot.

Single C- and A δ -unit activity was recorded from the peroneal nerve. A tungsten microelectrode (tip diameter, 1 μ m; shaft diameter, 100 μ m; impedance, 10–12 M Ω) (Frederick Haer & Co, Bowdoinham, ME) was inserted percutaneously into the peroneal nerve in the popliteal area without local anesthesia. Neural signals were amplified and filtered (500–5000 Hz) and its responses were displayed on an oscilloscope. Data were collected at a sampling frequency of 10 kHz using an analogue to digital converter recorded on digital audio tapes (DAT) with a DAT recorder (PC216Ax; Sony Precision Technology, Tokyo, Japan). Data were analyzed off-line using published software (Spike 2; Cambridge Electronic Design Limited, Cambridge, UK). Mechanical search stimuli were applied to the dorsum of the foot. When a single unit was found, its conduction velocity was measured by transcutaneous electrical stimulation (0.25 Hz, 120–150 V) within the receptive field to classify the recorded fibers, namely, A β -, A δ - and C-fibers. When A δ - or C-fiber unit was found, we stimulated the receptive fields with CO₂ laser pulses and recorded nerve discharges. We analyzed the present results based on previous microneurographic study that CV of C- and A δ -fibers was 0.4–1.8 and 4–30 m/s, respectively [18].

Evoked EEG was recorded simultaneously. Exploring electrodes were placed at the Cz, C₃ and C₄ (according to the international 10–20 system) and were referenced to linked earlobes (A₁ + A₂). Impedance was maintained below 5 K Ω . Amplifier frequency response ranged from 0.1 to 50 Hz with 10 μ V/cm sensitivity. The analysis time was 2 s and the sampling rate was 512 Hz. The room temperature was 25°C and sound and light were regulated. Skin temperature was kept above 30°C.

Five individual afferent C-fibers by electrical stimulation were identified in four subjects (Fig. 1, Table 1). The mean CV was 1.1 ± 0.3 m/s. All these five C-units were also activated by CO₂ laser pulses. The latency difference

Table 1
Stable single unit recording in each subject

Subject	Receptor type (total of no.)	Conduction velocity (m/s)	Latency (P) of LEPs (ms)
S1	C (1)	1.44	1248
S2	C (2)	0.71 and 1.30	1360 and 1327
S3	C (1)	1.25	1346
S4	C (1)	0.72	1356
Mean		1.1 ± 0.3	1327.4 ± 46.2

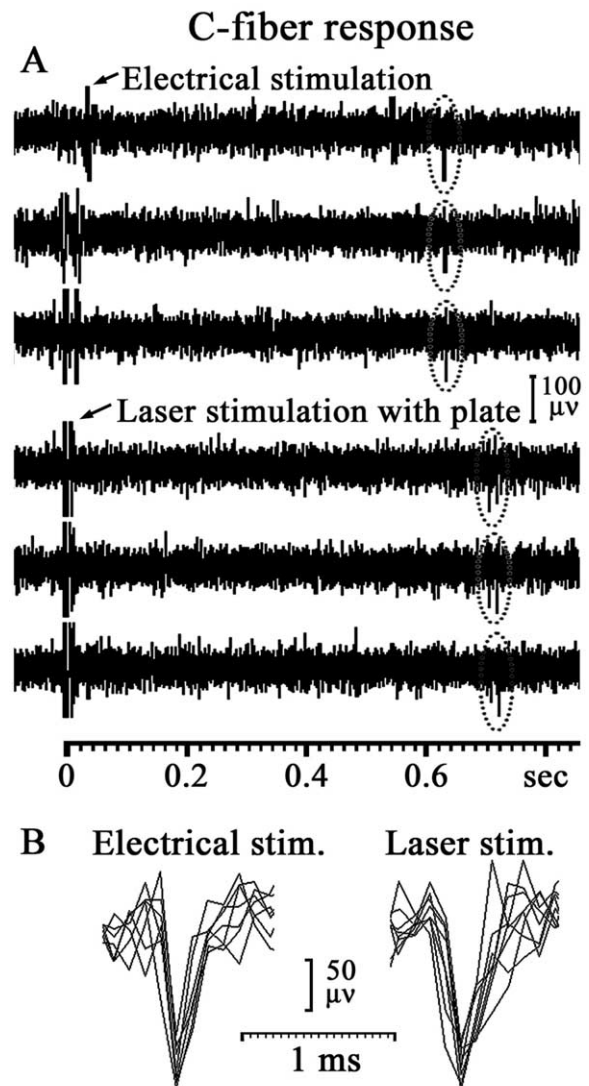


Fig. 1. C-fiber action and cerebral potentials in subject 2. (a) Raster display of C-fiber responses to transcutaneous electrical stimulation and laser stimulation through an aluminum plate on the dorsal foot. C-fiber action potentials (in the circle of dots) were recorded from the peroneal nerve in the popliteal area. (b) Single C-fiber responses to seven electrical and eight laser stimuli were superimposed, respectively.

between electrical and laser stimulations ranged from 25 to 88 ms with a mean of 56.6 ± 44.6 ms. As for EEG responses, the main positive deflection peaked at 1248–1360 ms.

CV of the two afferent A δ -fibers observed by electrical stimulation was 11.7 and 14.1 m/s, respectively. However, CO₂ laser pulses through the thin aluminum plate could not produce spikes in these two fibers (Fig. 2). This finding indicated that our C-fiber stimulation method with a plate selectively activated C fibers.

The CV of C-fibers measured in the present study was 1.1 ± 0.3 m/s, which was similar to the CV reported in earlier microneurographic studies (0.4–1.8 m/s) [18] and CV estimated by averaged EEG (0.8–2.6 m/s) [10,16]. The CV of A δ -fibers in the present study, 11.7 and 14.1 m/s,

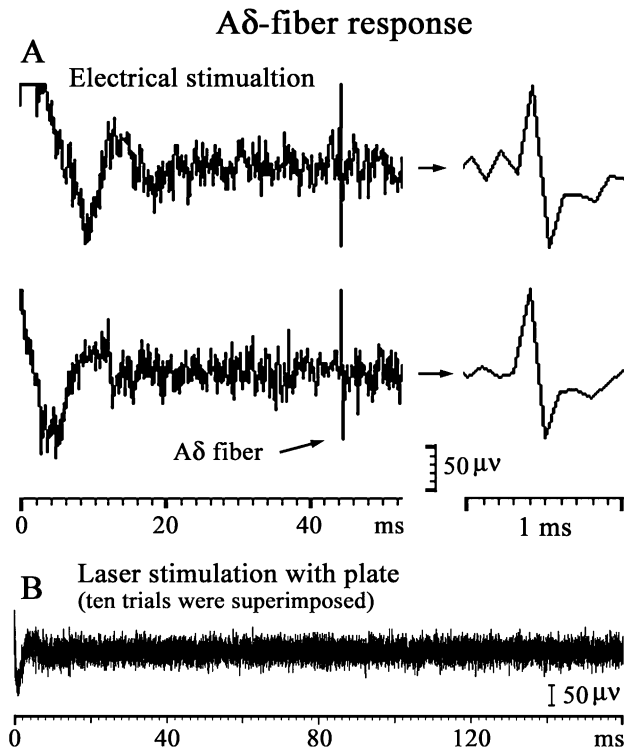


Fig. 2. (a) A δ -fiber action in subject 2. Arrows point to the A δ -fiber responses to the transcutaneous electrical stimulation on the dorsal foot. Right hand traces are expanded views. (b) There were no A δ -fiber responses found after laser stimulation through an aluminum plate. Ten single responses were superimposed. These findings indicated that this new stimulation method selectively activated C-fiber nociceptors of the skin.

were similar to the CVs measured by microneurography: 4–30 [18] and 19.2–9.4 m/s [1]. The CV was also similar to the values estimated on averaged EEG studies [6]. In the present study, all C-units were activated by CO₂ pulses to a tiny skin area while A δ -fibers were activated only by conventional laser stimulation, indicating that our method using a special spatial filter could selectively stimulate C-fiber sensory terminals. Results of simultaneously recorded EEG responses also supported this view. CO₂ laser pulses through an aluminum plate evoked EEG response components (1327 ms). Since the signals of C-fibers ascend through the spinothalamic tract with a CV of 2.2 m/s [12], a gross calculation indicates that it takes about 1318 ms to reach the cortex from the foot (115 cm/1.1 m/s plus 60 cm/2.2 m/s), which is compatible with the present EEG findings.

Since laser beams activate skin receptors via temperature conduction, there is a latency difference between the onset of laser stimulation and actual activation of receptors. The latency difference between electrical and laser stimulation-evoked spikes of C-fibers in the present study was 56.6 ± 44.6 ms. With similar methods, the value was reported to be 50 ms in monkeys [4] and 37 ms in humans [3]. Such a period is necessary to activate C-fibers by CO₂ laser stimulation after discharge.

There are three types of C-fibers: (1) polymodal or

mechano-heat responsive C-fibers; (2) warm or heat responsive C-fibers; and (3) silent or mechano- and heat-insensitive C-fibers. Second pain is transmitted by (1) [9, 14]. In normal subjects, CO₂ laser stimulation can activate (1) and (2) [3]. However, it was found that (2) have a higher heat threshold (48°C) than (1) (40°C) [19]. In the present study, we used a CO₂ laser to stimulate a tiny area of skin at weak intensity. Therefore, this stimulation probably activated polymodal C-fibers. The activity level in nociceptors may or may not be associated with pain, depending on whether the stimulus intensity is above of below the pain threshold. However, there is a clear causal relationship between the two [20]. There is probably no difference between cerebral activation of C-fiber responses in the present study and second pain-related cerebral activation.

In conclusion, the present microneurographic study provided direct evidence that C afferent fibers are selectively activated by CO₂ laser stimulation of a tiny area of skin. In addition, the present study showed that the occurrence of EEG responses components well matched the presence of nerve fiber discharges in C-fibers.

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