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Direct isolation of ultra-late (C-fibre) evoked brain potentials by CO_2 laser stimulation of tiny cutaneous surface areas in man

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Abstract

In this study, it is reported that CO_2 laser heat stimulation of tiny skin surface area (0.15 mm^2) provides a unique method to directly and selectively activate C-fibre as assessed by the ultra-late brain potentials (peak latencies: N810, P996) evoked consistently across a set of stimulus energy levels. On a larger surface area (15.5 mm^2), low energy stimulation also resulted in minute ultra-late potential, while higher intensities induced only late potentials related to A-delta fibre activity (peak latencies: N247, P394). The selective activation of C afferent sensory terminals in the skin by stimulation of tiny surface area is explained by their relative high density and lower activation threshold.

Keywords: Nociception; Thermal perception; CO_2 laser; Surface area; Evoked potential; C-fibres; A-delta fibres

Unmyelinated fibres (C-fibres) constitute the major part of peripheral afferent nerves. They are involved in perception of thermal, mechanical, chemical or noxious stimuli. Selective activation of these small afferents allows examination of their functional integrity and to assist clinical differential diagnosis. However, these fibres are very difficult to study differentially. Thermal stimulation with CO_2 laser has been shown to activate specifically both A-delta and C-fibres [4, 8]. Classical EEG recordings reveal the occurrence of evoked potentials (EPs) with long latencies (N250–P390) [24] clearly related to the activation of afferents with peripheral conduction speeds around 14 m/s, i.e. A-delta fibres. Hitherto, no ultra-late, C-fibre-evoked, brain potentials have been directly isolated without invasive techniques or conduction block of myelinated fibres.

Nine healthy volunteers who gave their informed consent (age range 24–49 years) were presented with brief CO_2 laser heat stimuli [18]. A large diameter beam (20 mm) was directed to the dorsum of the left hand so that only the skin area between the first and second metacarpal bones was exposed to the stimuli. A thin aluminium disk operating as a shutter and drilled with calibrated holes was interposed just above the skin surface. The resulting stimulus then presented a quasi-constant energy profile at any surface areas. Two different surface areas were used: 0.15 mm^2 (small surface area, SSA) and 15.5 mm^2 (large surface area, LSA). Stimulus intensities were chosen so that sensations reported ranged from 'barely detectable' to 'slight pain'. Spread over several sessions (3–5 blocks of 20 stimuli per session), subjects received a total of about 600 stimuli in SSA condition (10 ms, 0.15 mm^2, six subjects) and about 300 in LSA condition (40 ms, 15.5 mm^2, five subjects).

Cerebral evoked potentials (EPs) were recorded along with reaction time, qualitative and quantitative aspects of stimulus perception. EP, electrooculogram and laser power output signals were digitised at 1000 cps from −100 to 2900 ms according to stimulus onset and stored for off-line analysis. EPs were recorded with platinum needle electrodes at the vertex (Cz−) referenced to the right ear (A2+), with ground to the forehead. Stimulus-locked time averaging was realised from all recordings

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corresponding to unartifacted sweeps and detected stimuli (SSA: $n = 1554$, 40% accepted; LSA: $n = 781$, 55% accepted). Averages were first computed within subjects and afterwards between subjects. The relationship between stimulus intensity and EPs was assessed in the frequency domain. Individual EP frames were downsampled (from 1000 to 100 cps) and divided into 11 time segments of 32 points between −100 ms and 1810 ms. Segments were transformed by a raised cosine window in order to avoid truncation errors and zero-padded in order to increase frequency resolution of FFT to 0.1 Hz [7]. The power spectral density ($\mu V^2/s$) was computed in the 0.5–7.5 Hz frequency band in each segment. A time overlap of 160 ms between two successive segments was imposed to compensate the application of the raised cosine window. Time segments were grouped in four windows, representative of potentials observed in the time domain: preactivation reference (pre; segment 1, centered on 55 ms), late potential (LP; segments 2–4, centered on 215, 375 and 535 ms), ultra-late potential (ULP; segments 5–9, centered on 695, 855, 1015, 1175 and 1335 ms) and postactivation reference (post; segments 10 and 11, centered on 1495 and 1655 ms). Electric activity in these four windows was estimated by summing power spectral densities of each related segments, taking into account a background activity proportional to the number of segments added. Statistical analysis was carried out by means of a one-factor repeated measures ANOVA and Bonferroni/Dunn tests for contrast analysis.

When stimulating the skin with SSA, EPs appear with very long latencies. These ULPs extend between 750 and 1200 ms in the five intensity classes (Fig. 1, top, and Table 1). Up to now, similar ULPs have been reported only after selective peripheral A-fibres block [5,6,10] or in patients showing impairment of afferent myelinated fibres [13,25]. Arguments advanced to relate ULPs to activation of unmyelinated afferent C-fibres are: (a) the long latencies are consistent with peripheral conduction velocities around 1 m/s; (b) the related sensations are reminiscent of second pain (diffuse, long-lasting, burning sensation); (c) the potentials disappear after anaesthetic block known to act preferentially upon C-fibres [6]. When stimulating with LSA (Fig. 1, bottom), low intensity laser stimulation also shows minute ULPs. In contrast, higher intensities only induce the typical LPs, i.e. a negative-positive complex (peak latencies N247 and P394 ms, Table 1) well documented in several studies [1,11,16,24]. Latency and amplitude of LPs are related to the distinct A-delta fibre-mediated first pain (sharp, pricking sensation) [6].

Skin stimulation with fine CO_2 laser beam, 100 times smaller than those commonly used, evokes consistent ULPs without any conduction block of afferent fibres. These stimuli evidently activate very few cutaneous receptors. Skin has a high innervation density of unmyelinated afferents. In humans, they are 3–4 times more numerous than A-delta fibres [17,21]. Their absolute density

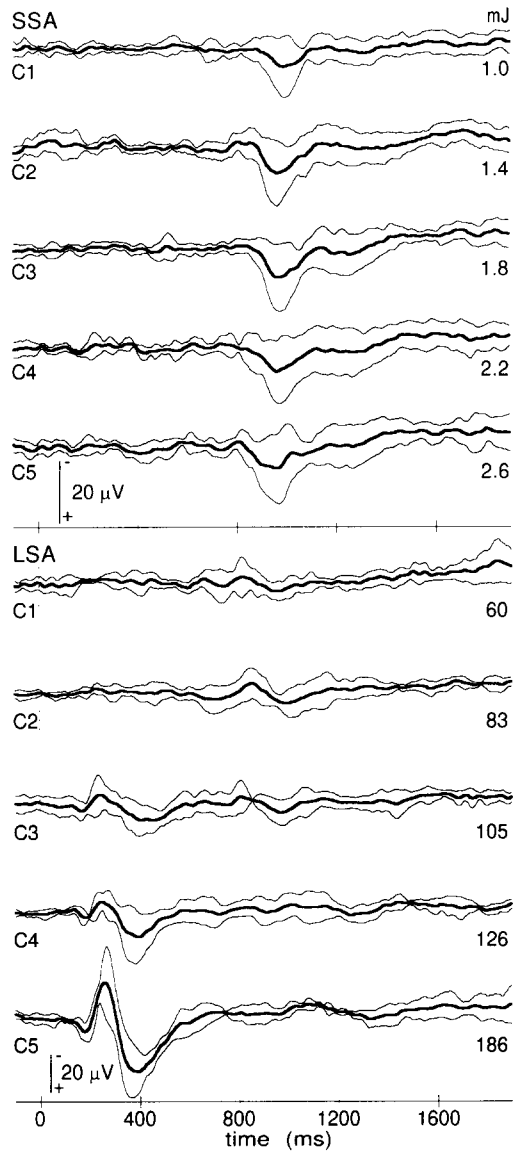

Fig. 1. Time-domain averaged EPs (mean ± 1 SD) separated into five classes of increasing stimulus intensities (C1–C5) and two surface area conditions (SSA, 0.15 mm^2, six subjects; LSA, 15.5 mm^2, five subjects). The column at the right part of the figure indicates the mean stimulus intensity (mJ) for each trace. With SSA, ULPs appear consistently in all classes as a negative/positive complex with respective mean peak latencies of 810 and 996 ms. In LSA conditions, low intensity stimulation show minute ULP while higher intensities induce typical LPs (mean latencies: negative peak 247 ms, positive peak 394 ms). See also Table 1 for individual parameters of LPs and ULPs.

distribution is unknown but estimated at 2–8 terminals/mm^2 for the skin innervated by the sural nerve in the rat [14]. Accordingly, SSA selectivity for unmyelinated fibres may result from a higher probability to hit C-fibre than A-delta sensory terminals. The 100-fold increase of receptors exposed to the stimulus in LSA condition alters dramatically EP profiles. ULP appears preferentially with low intensity stimulation while LP is evoked only in higher stimulus energy classes. This may reflect that C-

Table 1
Individual parameters and grand mean of LPs and ULPs

Subject	SSA (0.15 mm^2)			Subject	LSA (15.5 mm^2)					
	ULP				ULP			LP		
	LVN	LVP	ΔA		LVN	LVP	ΔA	LVN	LVP	ΔA
1	848	980	34.9	1	630	715	10.9	250	383	33.5
2	782	943	21.6	2	746	889	13.8	223	381	42.8
3	783	939	7.2	7	876	1007	14.0	240	371	14.9
4	862	1003	16.9	8	814	931	23.9	267	429	44.9
5	741	840	5.0	9	879	1030	18.4	275	416	31.5
6	–	1227	–							
M	810	996	18.7		805	933	17.9	247	394	36.5
SD	50	126	11.3		89	106	7.8	19	25	18.9

Intra-individual averages and grand mean (M) are computed in two conditions, SSA and LSA. LVN is the latency for vertex negativity (ms), LVP is the latency for vertex positivity (ms) and ΔA is the peak-to-peak amplitude (μV). Subjects 1 and 2 performed both experimental conditions. The negative peak was impossible to determine for subject 6.

mechano-heat receptors have a lower activation threshold for laser thermal stimuli than A-delta-mechano-heat receptors [26].

Time jitter induced by desynchronisation of slow conducting afferents (within subjects) and variation in stature (between subjects) may distort EPs obtained with time domain averaging procedures so that the observed peak amplitudes and latencies may be biased [20]. In this case, the relationship between stimulus intensity and single EP responses is more adequately assessed in the frequency domain [1]. With SSA, the power spectral density increases significantly ($F3 = 5.17$, $P = 0.008$) in the ULP window as compared to the activity in the pre-activation, LP and post-activation windows (Fig. 2, top). Furthermore, these latter three windows do not differ one from another. A similar increase is observed in LSA condition for the lowest stimulus intensities. As laser energy increases, the power spectral density in ULP window decreases and, reciprocally, reappears centred on LP window (Fig. 2, bottom). As already reported [1,18], power spectral density of LP appears significantly correlated with stimulus intensity ($F4 = 8.69$, $P = 0.0003$). In contrast, there is no such correlation for ULP window ($F4 = 0.13$, $P = 0.969$). However, C-fibre terminals are known to code stimulus intensity [2,9,12,23]. This discrepancy may be related to (a) the weak synaptic efficacy of C-fibre afferents requiring an important temporal and spatial summation to evoke consistent perception as compared to A-fibres [23,27], (b) the possibility, despite similarity in shape between LP and ULP [6], that these two potentials originate from different sources with different intensity coding properties, and (c) a less well synchronised afferent volley in unmyelinated fibres.

Ultra-late brain potentials are recorded when A-fibre activity has been suppressed by a selective nerve pressure block [5,10] and, as shown in the present study, by stimulating the skin with brief CO_2 laser stimuli upon a tiny area. Besides, it seems that with LSA, the LP evoked by A-delta activation represses the C-fibre-related ULP. Based on quantitative or qualitative aspects of perception, inhibition of C-fibre inputs by A-delta afferents at the spinal level, as proposed by the gate control theory, has already been suggested [3,19]. On the other side, the

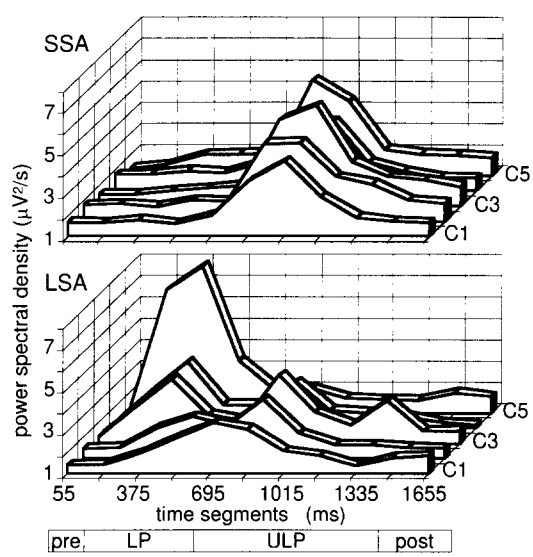

Fig. 2. Evolution of power spectral densities in SSA (upper panel) and LSA (lower panel) conditions as a function of five increasing stimulus intensity classes (C1–C5) for 11 time segments (referred by their central values, from 55 to 1655 ms). The lowest bar indicates correspondence between the 11 time segments and the four time windows used to describe the different components of the evoked response (pre, LP, ULP and post). In SSA condition, power spectral density increases only in time segments between 695 and 1335 ms (ULP window). A similar situation is observed with LSA for the lowest stimulus intensities. As stimulus strength increases, cerebral activity appears sooner (from 215 to 535 ms; LP window) while vanishing in the later time segments.

negative/positive complex may be considered as a physiological correlate of the detection process. The negativity has been related to attention orienting and the positivity to early sensory processing [22] or to a pain-related cognitive function [15]. It is then inferred that the physiological correlate of the C afferent-related detection process is repressed or absent when preceded by the detection of A-delta-mediated information.

Based on the findings of the present study, we conclude that CO_2 laser stimulation over small surface areas may pragmatically provide a non-invasive, new and direct way to examine the function of unmyelinated somatosensory afferents in humans and animals.

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