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Original article

How do we selectively activate skin nociceptors with a high power infrared laser? Physiology and biophysics of laser stimulation

Comment activer selectivement des nocicepteurs cutanés avec un laser infrarouge de forte puissance ? Physiologie et biophysique de la stimulation laser

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Abstract

This review presents and discusses the leading arguments justifying the use of high power laser stimulators to explore the nociceptive system. To grasp the particularity of such stimulators, fundamentals concerning the interaction of low-energy radiation with the skin will be recalled and focused on the optimal match between the wavelength of the emitting source and the thermophysical properties of the skin. This knowledge shall allow us to discuss critical characteristics of laser stimulators. Study of the cutaneous spectrum of receptors showed that laser stimulators allow the selective activation of Aδ-and C-fiber nociceptors. We will present different methods, which increase the selectivity of the laser stimulation, restricting the activation to isolated C-fiber nociceptors. These methods open new perspectives in the study of the cerebral processing of signals ascending through Aδ and/or C nociceptors and should contribute to a better understanding of their central interaction and integration in normal and pathological states.

Résumé

Dans cette revue nous allons présenter et discuter les principaux arguments pour justifier l'utilisation de stimulateurs laser à puissance élevée afin d'explorer le système nociceptif. Pour saisir les particularités de ces stimulateurs, il est utile de rappeler les principes de base qui gouvernent l'interaction de radiation à faible énergie avec la peau. Ces principes vont nous introduire à la relation entre les propriétés thermophysiques de la peau et la longueur d'onde de la source émettrice. Ces données nous aideront à discuter le choix du meilleur type de laser. L'étude du spectre des récepteurs cutanés activés par le stimulateur laser montre que cette méthode permet l'activation sélective des nocicepteurs Aδ- et C. Finalement, nous présenterons plusieurs méthodes permettant d'augmenter la sélectivité de la stimulation laser à l'activation isolée des nocicepteurs C. Ces méthodes ouvrent de nouvelles perspectives pour l'étude du traitement cérébral de signaux transmis par les nocicepteurs Aδ- et/ou C et devraient pouvoir contribuer à une meilleure compréhension de leur interaction et intégration centrale tant dans les états normaux que pathologiques.

Keywords: Laser; A-fiber nociceptor; C-fiber nociceptor; Laser evoked potentials; Reaction times

Mots clés : Laser ; Nocicepteurs Aδ ; Nocicepteurs C ; Potentiels évoqués laser ; Temps de réaction

1. Why should we consider using a laser stimulator to investigate the nociceptive system?

Both in fundamental and clinical research, study of sensory systems require a perfectly controlled stimulus to activate the system under investigation. It is probably because such devices have been available to the scientific and medical community, that current knowledge about visual and auditory systems has reached such a remarkable level today. Regarding nociception, a device with sufficiently sophisticated and reliable characteristics is not readily available. This technical drawback has probably hampered the progress of knowledge within that domain.

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Any experimental noxious stimulus should be quantifiable (defined in physical units as to intensity, time and spatial distribution), reproducible and safe. In order to selectively elicit the nociceptive system, the stimulus should predominantly activate Aδ- and C-fibers [16] with no or only low concurrent activation of other sensory modalities. Electrical, mechanical and thermal stimuli fulfill some of these requirements, but they all have their specific shortcomings.

Electrical stimuli are easy to control and consequently are the most commonly used procedure to induce nociception both in animal and human studies. When one activates a peripheral nerve through transcutaneous electrical stimulation, various sensations are triggered as a result of the non-selective activation of the full spectrum of peripheral afferent fibers (unless part of the fibers are blocked). Furthermore, the afferent pathway is activated in an unnatural and synchronized manner. Finally, electrical stimulation bypasses the generator compartment of nerve terminals. All information concerning transduction processes is therefore lost.

In addition to tactile sensations, when increasing intensity of the stimulation, a growing aversive component is added. This aversive component is called pain when it exceeds an individual central threshold, which depends on factors such as attention, vigilance, anxiety, personality and socio-cultural background [23]. The simultaneous activation of fast conducting fibers, mostly of the mechano-receptive system, is unavoidable as the large diameter of these fibers is responsible for their low electrical impedance and hence their very low electrical activation threshold as compared to the thin myelinated Aδ- and non-myelinated C-fibers. It is thus unlikely that nociceptive afferents can be activated electrically without coactivation of mechanoreceptors. Some attempts have been made to evade this problem by using intracutaneous stimulus electrodes [11,24] or intraneural microstimulation pioneered by Torebjörk and Ochoa [44]. These methods have technical limitations (for a critical review see [19,46]).

Mechanical stimuli delivered with needles and stimulation with pressure algometers are the most common forms of clinical pain testing. However, these methods also lack selectivity as mechanoreceptors are excited in addition to nociceptors. Furthermore, conventional mechano-stimulators do not provide the fast and precisely controlled stimuli required for the psychophysical study of time-locked neural events. The use of high-energy ultrasound is problematic as the biophysical effects are not well understood and it is not always clear which form of energy (thermal or mechanical) exerts the sensory effect [17]. Controlled impact of small metallic cylinders can provide fast onset but have not been extensively used in psychophysical studies [27].

Heat stimulation is the most frequently used form of natural noxious stimulation. However, if the source of energy is radiant heat from a light bulb or heat conducted from a contact thermode, usefulness of the method is limited. Indeed, the increase in cutaneous temperature is too slow (in the range of seconds). For this reason, the peripheral and central neuronal responses cannot be synchronous. These stimulators are thus unsuitable for studying neurophysiological phenomena such as reaction times (RTs), muscle reflexes, neuronal responses, and brain evoked potentials. An additional disadvantage is that conventional radiant heat sources emit in the visible and near infrared spectral band. At these wavelengths, human skin energy absorption is poor, reflectivity is important and depends, among other factors, on skin pigmentation. The overall control of heat transfer is thus quite unpredictable.

Thermodes, which require contact with the skin, activate concomitantly low threshold mechanosensitive afferent fibers. These fibers modulate the spinal transmission of both nociceptive and heat information [36]. Furthermore, the energy transfer is not easily controlled as it greatly depends on the pressure of the thermode applied against the skin. Finally, the fixed and rigid surface areas of thermodes sometimes limit their use, as most cutaneous surface areas are not flat. Even if the heat source is controlled by feedback of the skin surface temperature, stimuli applied with different radiation devices and thermodes are not necessarily comparable. The intracutaneous temperature profiles may be very different, depending, among other parameters on the wavelength of the source in radiative devices or on the type of interface between the thermode and the skin.

We shall now review why monochromatic radiant heat sources of high power density such as infrared lasers are able to circumvent most of the shortcomings encountered by conventional heat stimulators.

2. The laser as a powerful heat stimulator of skin

A laser (acronym for *Light Amplification by Stimulated Emission of Radiation*) is a very special light source. In comparison to classical incandescent light sources, which emit their radiative energy in all spatial directions and in a large spectrum of wavelengths, the laser energy is confined to a narrow beam of nearly parallel monochromatic electromagnetic waves. This results in a high energy density (radiation per unit area). The combination of these characteristics make the laser a light source with a spectral energy density (radiation per unit wavelength) several orders of magnitude higher than any known light source. This is an important characteristic if fast and high transfer of radiation energy is needed. To achieve a perfectly defined geometrical configuration of the laser beam, the laser is usually built to operate according to the fundamental mode TEM_{00} (transverse electromagnetic mode). This mode exhibits the so called Gaussian amplitude profile: the distribution of calorific power in a beam section perpendicular to the optical axis follows a Gaussian distribution centered on that axis. Fig. 1 illustrates a method for measuring the beam diameter from the irradiance profile and Fig. 4 (lower panel) shows the temperature profiles recorded at the target with a very fast IR-camera.

As pioneered by Mor and Carmon [34] high power CO_2 lasers are very appropriate heat stimulators (for other classes

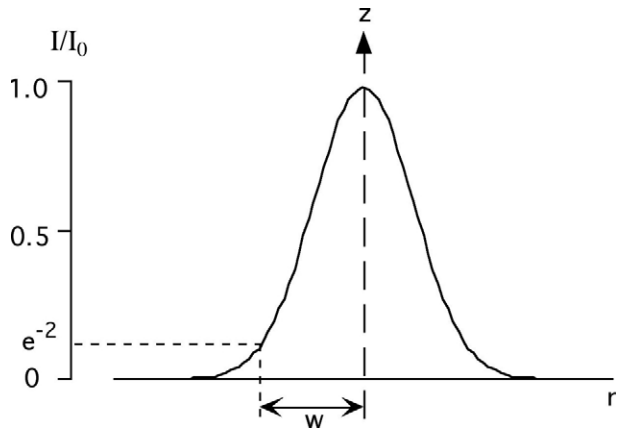

Fig. 1. Relative power distribution across the beam at the target zone is plotted versus radial distance (r) from the maximum in the optical axis z. The laser beam exhibits an irradiance profile according to the square of a Gaussian, characteristic for the TEM_{00} mode. The radius of the beam (w) is thus defined as the distance from the axis where the radiant power is reduced to 13.5% of the maximum power I_0 on that axis. A radiation pyrometer equipped with a small aperture (pinhole) in front of the detector is placed in the focal plane and the beam is scanned in two orthogonal directions to that plane. The first one determines the position of maximum irradiance (I_0). This position defines the optical axis of the beam. Then the pyrometer scans again the beam in an axis r perpendicular to the optical axis and in steps of ±0.2–0.5 mm. The result of such a scanning procedure is shown. Compare this curve with the temperature profile of Fig. 4B, recorded at the target with a very fast IR-camera.

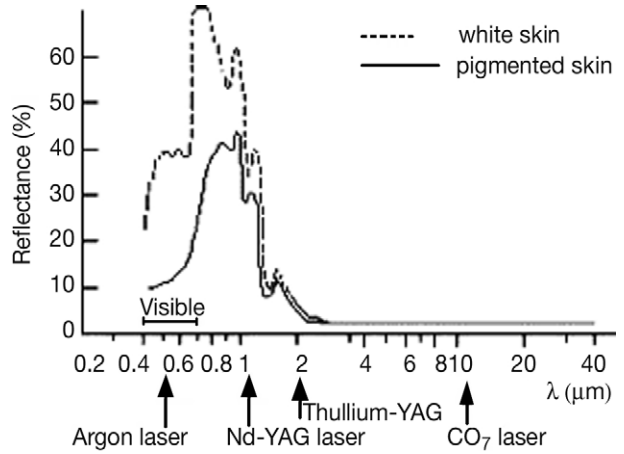

Fig. 2. Skin reflectance as a function of wavelength is shown for both white skin (dashed line) and pigmented skin (solid line). Notice that at wavelengths greater than 2 μm, skin reflectance is independent of pigmentation (adapted from [20]).

of infrared lasers see next section and for a full discussion see the review by Arendt-Nielsen in this volume). There is no contact with the skin and the light beam is outside the human visible spectrum. The short- and long-term stability of the output power can be made very constant. A CO_2 laser generally emits in a continuous mode. For the purpose of skin stimulation, the radiant heat must be emitted as a pulse defined by instantaneous power (e.g. 0.2–20 W), duration (e.g. pulses from 10 to 150 ms to continuous) and surface area (e.g. diameter 2–20 mm). The temperature ramps may rise to several thousands degree centigrade per second allowing to reach skin temperatures that activate nociceptors within a few milliseconds. The resulting activation of afferent nerves is thus very synchronized, and therefore, allows the recording of time-locked neural responses [15]. At wavelengths in the far infrared (10.6 μm), the skin acts as a black body [20], absorption is nearly complete and transparency is very low (see next section for more details). For these reasons, the calorific energy remains confined to the upper skin layers where transducer nerve terminals sensitive to thermal variations are located. There is no need for intracutaneous temperature measurements with indwelling thermocouples, which are unreliable, as the probe itself modifies the thermal field. Indeed, laser stimulus intracutaneous temperature profiles may be easily modeled and simulated in time and space (Fig. 3 and compare with Fig. 4 upper panel).

The margin between the energy needed to evoke noxious responses and the energy capable of injury is very thin [32]. Application of laser heat pulses to the skin sometimes evoke a slight punctuate erythema (first-degree superficial burn). This effect is difficult to avoid as small blood vessels extend almost as close to the surface as nociceptors and are thus inevitably heated up to noxious temperatures. However, the high cutaneous absorption of the CO_2 laser radiation restricts heating to the most superficial skin layers. This characteristic greatly reduces the danger of causing intradermal damage (see next section). Most importantly, skin heating above the boiling point of water should be absolutely avoided. At that point, water, constituting 85% of soft tissues, acts as a sink for further heat input. If additional energy is supplied, it provides latent heat for the water to boil explosively [32]. Within the usual range of operating power, radiation, and stimulus duration, tiny spots (≤2 mm diameter) will at most produce local burns in the most superficial skin layers (<150 μm skin depth). The small spots of tissue injury, which may become hyperpigmented, vanish within 3–5 days without residue [6]. Beydoun et al. [5] observed that a stimulus intensity of 0.14–0.21 W mm^{-2} produced only delayed and temporary dislocation in the *striatum corneum* and proposed that 0.35 W mm^{-2} should be the upper limit for testing patients. Finally, sufficient time (minutes) must be allowed between successive stimulation of the same cutaneous area in order to avoid accumulation of calorific energy but also sensitization and/or habituation of the underlying nervous structures.

In Section 3, we shall review the fundamentals of calorific energy transfer to the skin. This introduction shall allow us to discuss the optimal match between the physical characteristics of different classes of lasers and the thermophysical properties of the skin.

3. The transfer of low-energy radiation to the skin

When radiation reaches a surface it is reflected or transmitted. In the bulk of the material, the radiation may be

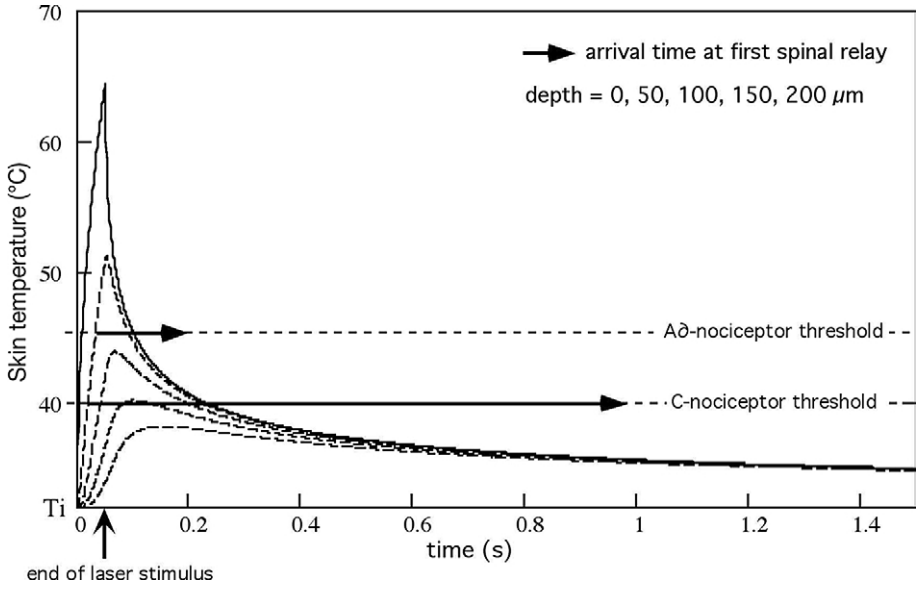

Fig. 3. Simulation of skin temperature as a function of time for various skin depths (x) according to the model described in [10,14,18]. The parameters for the simulation were set as follows: power, 0.23 W mm^{-2}; beam diameter, 10 mm; stimulus duration, 50 ms; $r = 0$; initial skin temperature, 30 °C. The horizontal arrows show the arrival time of nociceptive information at the first spinal relay taking a peripheral nerve conduction distance of 1 m and a nerve conduction velocity of 8 m s^{-1} for Aδ nociceptors and 1 m s^{-1} for C nociceptors.

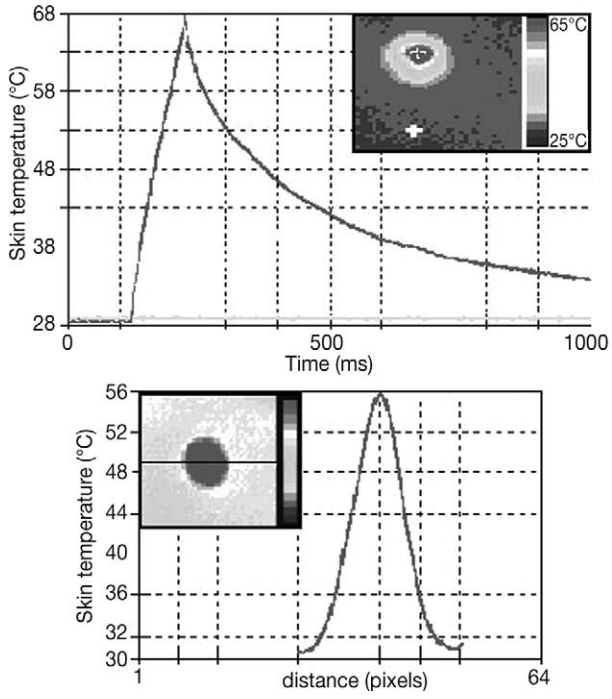

Fig. 4. Temporal (upper panel) and spatial (lower panel) profile of a laser heat pulse (stimulus parameters similar as for the simulation shown in Fig. 3) applied to the dorsum of the hand and recorded with a very fast (1 kHz) IR-camera (in collaboration with Dr. D. Le Bars).

absorbed or deviated. Skin is inhomogeneous and a great fraction of the light transmitted through it is scattered in transit [21]. Scattering increases the average path traversed by the light and causes light to re-emerge as reflected light by turning it through more than 90°. Skin reflection is thus a function of depth and not only a surface phenomenon.

If I_0 is defined as the intensity of incident radiation and I_R as the intensity of reflected radiation then reflection is defined by the *reflectance* ($R = I_R/I_0$).

Similarly if I_T is defined as the intensity of transmitted radiation then transmission is defined by the *transmittance* ($T = I_T/I_0$).

In homogeneous material, the *absorption coefficient* (μ) or its reciprocal the *space constant* (λ) is computed from the transmittance according to the equation $I_T = I_0 \exp(-\mu x) = I_0 \exp(-x/\lambda)$; where, the space constant is the thickness of tissue required to reduce the intensity of incident radiation to 37% ($1/e$) of its original value.

The reflectance, transmission and absorption characteristics of a material are critically dependent of the wavelength of the source. Human epidermis transmits most of the incident light in the waveband of 0.4–1 μm (the visible and near infrared) and a variable amount in the farther infrared. Much of the incident radiation is transmitted with little epidermal scattering [21]. The epidermal transmission of 1–3 μm infrared radiation is appreciable, but is reduced by strong absorption bands. This has been assigned to water absorption. Above 3 μm, absorption is nearly complete [20].

The dermis absorbs or reflects nearly all the radiation transmitted through the epidermis. Only a small fraction of the red and near infrared radiation is transmitted through 2 mm of skin and presumably reaches the subcutaneous tissue. The radiation transmitted through thick dermis is perfectly scattered. The absorption coefficient of dermis is less than that of the surface layers of skin for visible light, but is the same for infrared radiation [20].

The spectral reflectance of white human skin is maximum (60–70%) at 0.7 μm corresponding to the boundary between the visible and the infrared (Fig. 2). As the wavelength is

increased above 4 μm, the slight reflection (<1%) usually found probably results of a surface reflection as any radiation at these wavelengths, which penetrate into the skin, would likely be absorbed before it could be reflected. While pigmented skin reduces the reflectance in the visible spectral band (Fig. 2), at wavelengths greater than 2 μm, the skin reflectance is no more dependent of pigmentation. The series of absorption bands in the near infrared seen both in transmission and reflection can all be assigned to the absorption of water. Since absorption of infrared radiation is mainly due to water, it may be assumed to take place uniformly throughout all hydrated structures, irrespective of their histological content. This agrees with the observation that the absorption coefficient of dermis is the same as that of epidermis in the near infrared, although they differ in the visible waveband [20]. In heavily pigmented skin most radiation is absorbed near the surface so that neither penetration nor its concomitant reflection can occur. In the near infrared, skin water absorbs strongly, limiting penetration and reflection. In the far infrared, skin water causes nearly perfect absorption within the superficial epidermal layers.

A complete theory of heat transfer during and after radiation pulses has been developed and tested by Buettner [14] and Hendler et al. [22] and has been applied to laser pulses by Bromm and Treede [10] and Haimi-Cohen et al. [18].

4. Matching a laser stimulator with the thermophysical properties of skin

The preceding discussion concerning low-energy radiation transmission through the skin brings out that the most relevant parameter of a skin laser heat stimulator is the wavelength of the laser source. Indeed, this parameter defines the thermophysical behavior of the skin with regard to the laser emitted energy. As shown in Table 1, the CO_2 laser, emitting at the far infra-red wavelength of 10.6 μm, has the most interesting features as reflectance is quasi negligible (absorbance = 99.7%) and transparency is very low. At this wavelength the skin acts practically as a black body [20], which absorbs all the energy of the incident radiation independent of skin pigmentation [3]. The energy deposited in the skin per unit volume decreases approximately exponentially with depth. The absorption coefficient is 200 cm^{-1} (space constant λ = 50 μm; [4]).

This allows confining the energy to the most superficial layers of the skin, where the transducer nerve terminals sensitive to thermal variations are located (i.e. at the dermo-epidermal junction, between 20 and 500 μm deep). Modeling and simulation of the laser–skin interaction is easily feasible (Fig. 3). Indeed at this wavelength the thermophysical properties of the epidermis and dermis are similar to those of water and the exposed skin may be considered a homogenous structure as it may be assumed that heat absorption takes place uniformly throughout all hydrated structures, irrespective of their histological content.

The CO_2 laser stimulator produces a slightly divergent beam with a well-determined temporal, energetic and spatial profile. Any of these parameters can be modified depending on needs (see Section 2). The beam power is such that cutaneous temperature increases very fast. The stimulator thus allows a selective and synchronous activation of nerve afferents with no cutaneous contact and outside the human visible spectrum.

Arendt-Nielsen and Bjerring [1] used CO_2 and Argon laser stimulators to investigate the influence of skin reflectance on RT. For a given RT, more power is needed with the Argon laser (wavelength 0.488 and 0.515 μm) as compared to the CO_2 laser stimulator (wavelength 10.6 μm). This difference disappeared when blackening the skin with carbon black. Similar results were obtained for sensory and pain thresholds. When Argon laser stimuli are applied to normal skin, 35–45% of incident light is reflected (Fig. 2 and Table 1). Argon laser thresholds determined on blackened skin were significantly lower than that on non-blackened skin. Argon laser thresholds on blackened skin were similar to CO_2 laser thresholds estimated on both non-blackened and blackened skin [2]. Reflectance dropped to approximately 12% across the visible spectrum when the skin was blackened with carbon black.

If a mean reflectance of 38% is assumed for non-blackened skin the ratio of absorbed energy for blackened and non-blackened skin is 1.4. It should, therefore, be expected that 1.4 times more energy was needed to reach threshold on non-blackened as compared to blackened skin. However, ratios of 1.75 and 2.75 were found for sensory and pain thresholds, respectively. This diverging result could indicate that in addition to reflectance, other factors are involved [2]. Argon light penetrates through the epidermis and is absorbed by both the melanin in the basal epidermal layers and the hemoglobin in the papillary capillaries and superficial vascular plexus. Part of the energy is thus carried away by vascular circulation [39]. Furthermore, the infrared

Table 1
Laser radiation wavelength and corresponding biophysical properties of skin

Laser class	Color	Wavelength (μm)	Reflectance (%)	Absorbance (%)	Absorption coefficient (cm^{-1})
CO_2	Invisible	10.6	<1	99.7	200
Thulium-YAG	Invisible	2.01	<10	High	28
Neodymium YAG	Invisible	1.06	<10	High	?
Argon	Blue-green	0.488–0.515	>40	Low	?
He–Ne	Red	0.6	>60–70	Very low	?

radiation from the heated carbon on the skin surface could also explain this discrepancy.

From these considerations one can conclude that, with short wavelength lasers (<3 μm), energy transfer to the skin is difficult to model even when superficial reflectance of the skin is reduced by blackening. The penetrance and absorbance is not easily controlled and it is thus quite impossible to predict to which dermo-epidermal structures the energy will be confined.

However, an important technical advantage of lasers with shorter IR wavelengths (e.g. Argon and YAG lasers) is that the radiant energy can easily be transmitted to the target zone through standard optical fibers. CO_2 lasers usually require that the beam be guided by a set of high quality coated metallic mirrors, as optical fibers or beam guides for that wavelength (10.6 μm) are expensive and fragile.

5. The cutaneous fiber spectrum activated by the laser

With relatively large (>100 mm^2), brief (tens of ms) and powerful (±10 mJ mm^{-2}) CO_2 laser stimuli directed to the dorsum of hand or foot, subjects (even unfamiliar with the stimulus) spontaneously report a characteristic double pain sensation; a sharp pricking sensation, well localized in time and space that does not long outlast the stimulus, followed by a burning or aching sensation that spreads far beyond the spatial and temporal limits of the evoking laser stimulus [40]. This double sensation is remnant of the first and second pain characterized by Lewis and Ponchin [29]. Psychophysical (reviewed in [41]) and neurophysiological (reviewed in [42]) studies have clearly shown that these two different perceptual responses are, respectively, related to the activation of Aδ and C primary afferents and that these two groups activate very different central neural processes [43].

Several studies [9,12] have revealed that the double perceptual component induced by high power laser stimulation can indeed be ascribed to the selective activation of Aδ- and C-fibers above the dermo-epidermal junction. The high power of the laser produces very fast heat ramps, which activate quasi-synchronously the terminals of the thin afferents [13]. The large difference in conduction velocity of both afferents (±10 m s^{-1} for Aδ-fibers and ±1 m s^{-1} for C-fibers) determines the difference in arrival time at the spinal level (Fig. 3). Depending on the peripheral nerve conduction distance (d) and assuming a similar receptor activation time for both groups, the difference in arrival time can range from about 100 ms (for stimulation in the trigeminal area; d = ±0.15 m) up to more than 1 s (for stimulation on the dorsum of the foot; d = 1.15 m). Thus, well-controlled and brief powerful laser stimuli of remote skin areas should provide an experimental means of producing selective and time-locked activation of small fiber afferents [15]. Most importantly, it should allow discriminating electrophysiological (e.g. laser evoked brain potentials) and behavioral responses (e.g. simple RT) evoked by activation of Aδ-fibers from those evoked by C-fibers in humans.

6. Selective activation of amyelinic C-fiber afferents

By exploiting the different physiological properties of Aδ- and C-fiber nociceptors, it is possible to conceive experimental conditions that would allow to selectively activate C-fibers without activating Aδ-fibers. To our knowledge, four methods are currently available. These are based on the following differential characteristics: resistance to ischemia, heat threshold, distribution density in the upper skin and selective pathology.

6.1. Method 1: difference in resistance to ischemic compression block

The first method is based on the fact that slightly myelinated Aδ-fibers are less resistant to ischemia than non-myelinated C-fibers. Using this differential property, Bromm et al. [9] were the first to record ultra-late laser evoked potentials (LEPs) at latencies compatible with the conduction velocities of C-fibers. The method consisted in compressing the superficial branch of the radial nerve using two weights of 700 g attached to a 2 cm large pad during more than 1 h. The disappearance of the electroneurogram, the elevation of the threshold for mechanical pressure, the loss of cold perception, the persistence of warm perception, and the persistence of pain to pinprick monitored the ischemic bloc. In a similar study, focused on perceptual variables, Nahra and Plaghki [35] observed that, once the conduction block established, first pain sensation evoked by laser stimulation vanished while second pain was preserved. An ultra-late LEP was recorded which disappeared and was replaced by a late-LEP within minutes after removal of the block. In addition, ultra-late LEPs disappeared completely upon local anesthetic blockade of the remaining conducting nerve fibers. Thus, changes in perceptual variables, RTs and LEPs were clearly linked to the direct and selective activation of C-fibers.

Application of this method is difficult. The ischemic block requires a compression of at least 1 h to be effective. Furthermore, the block can be applied to only a few restricted skin areas (those where a sensory nerve is very superficial and crosses a bone giving rigid support for the pressure block): peroneal, ulnar, superficial radial nerves are the only ones.

6.2. Method 2: difference in heat threshold

The second method takes advantage of the difference in heat threshold between Aδ-fibers (ca. 46 °C) and C-fibers (ca. 40 °C). Magerl et al. [31] devised an ingenious experimental design based on a feedback controlled CO_2 laser stimulator. At a base temperature of about 33 °C, the skin was exposed to two successive heat ramps at 5 s interval. A first heat ramp, of 50 °C s^{-1} and lasting 150 ms, brought skin temperature to 40 °C allowing selective activation of polymodal C-fibers. Skin temperature was kept constant at 40 °C for another 5 s. The skin was then exposed to a second similar

heating ramp bringing the skin temperature to 48 °C and allowing activation of Aδ-fibers. The first heat ramp evoked sensations and neurophysiological correlates compatible with selective C-nociceptor activation while the second heat ramp evoked responses compatible with Aδ-nociceptor activation. Even though C polymodal nociceptor response adapts within seconds [33], it should be noted that the Aδ burst of action potentials arrived at a dorsal horn already exposed to a tonic C-fiber barrage. In addition, C polymodal nociceptors were activated at near threshold levels (as stated by the authors, the temperature used in their study may correspond to the lower limit for eliciting reliably ultra-late LEPs). Another way to exploit the difference in threshold to activate selectively C nociceptors has been developed by Tran et al. [45]. They used a low intensity CO_2 laser beam and interposed a very thin aluminum plate with numerous tiny holes (0.125 mm^2) as a spatial filter.

6.3. Method 3: difference in distribution density

The third method takes advantage of the fact that C-fibers have a higher density distribution than Aδ-fibers [37]. Depending on the species and the methods for quantification, the density distribution of C terminals is approximately 2–8 mm^{-2}. That of Aδ-fibers is <1 mm^{-2} [30]. Let us assume that the spatial distribution of Aδ- and C-fiber terminals has a Poisson distribution with average occurrence of $\lambda_D = 0.5\ mm^{-2}$ and $\lambda_C = 5\ mm^{-2}$, respectively. Suppose we are planning to selectively activate C-fiber terminals with a laser beam of diameter d and want to know the probability that there are no Aδ-fiber terminals in the target area. For example, what laser beam diameter can guarantee that no Aδ terminals are inside the stimulus surface area (A) with a probability of 90%?

The probability that no Aδ-fiber terminals are in area A is given by $P[N(A) = 0] = \exp(-\lambda_D A)$

Thus for a probability of 0.9 that no Aδ-fiber terminals are in the skin surface area exposed to the laser beam we must have $P[N(A) = 0] = 0.9$

This implies that $\exp(-\lambda_D A) = 0.9$ or $-\lambda_D\left(\frac{d}{2}\right)^2\pi = \ln 0.9$

Solving for d, we obtain a laser beam diameter of $d \approx 0.5$ mm or a surface area of $A = 0.21\ mm^2$.

If this is applicable to humans, then we can interpret the following experiment [7]. The dorsum of the hand was ex-

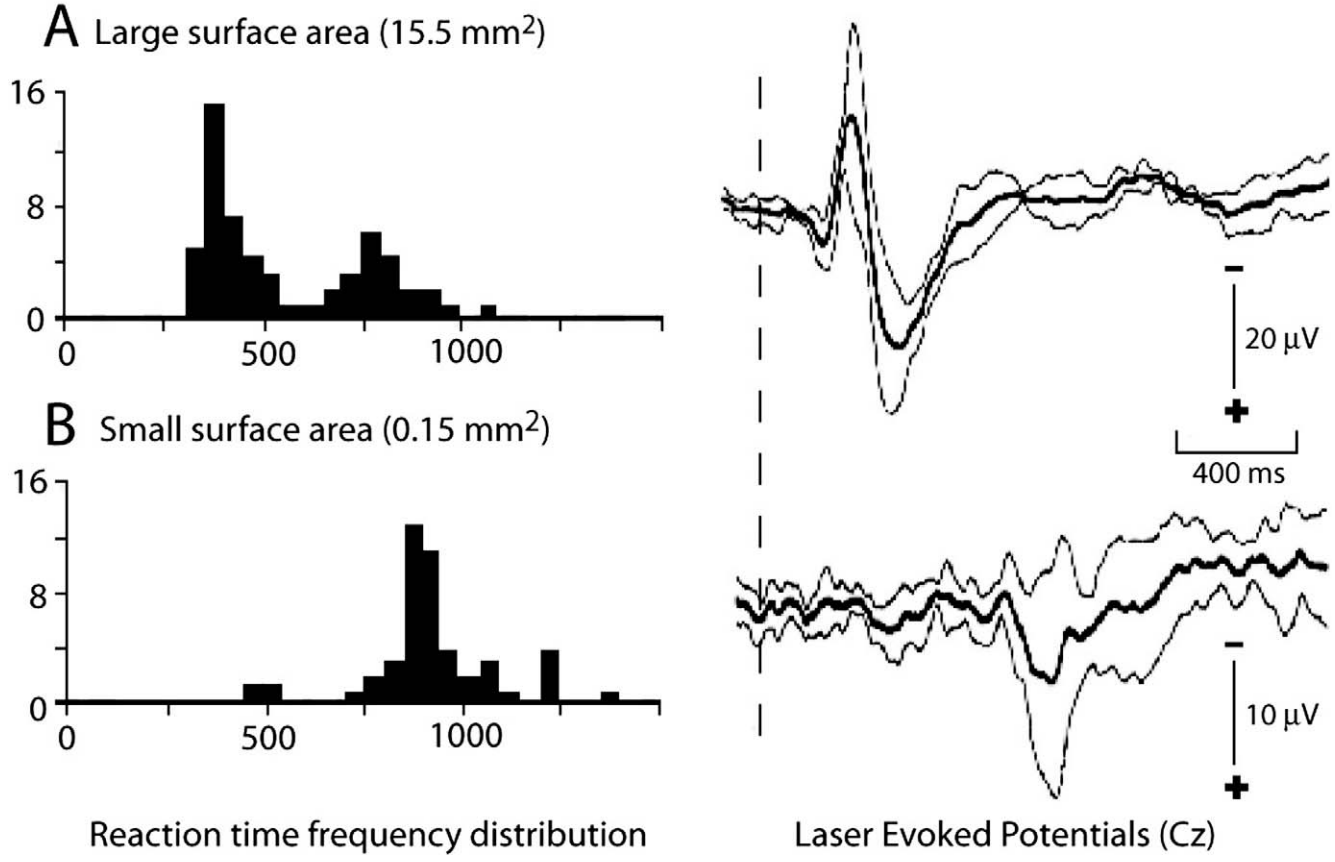

Fig. 5. Effect of stimulus surface area with constant energy density (40 ms duration and 7.6 mJ mm^{-2}, i.e. near first pain threshold) on RT and LEPs recorded at Cz after laser stimulation of the dorsum of the left hand. With a relatively large surface area (panel A), RT distribution was clearly bimodal and in favor of shorter RT (63% <600 ms). With a tiny surface area (panel B; i.e. 100 times smaller than the large one), RT distribution was still bimodal but clearly in favor of longer RTs (4% <600 ms). Large laser stimulus surface areas evoked a well-defined late-LEP; (grand mean ± 1 S.D. of six subjects). However, when stimulating the skin with the tiny beam, LEPs appeared with an ultra-late latency (between 750 and 1200 ms). Note the difference in amplitude scale between panel A and B (adapted from [7]).

posed to smaller and smaller laser stimulus surface areas. The energy density, however, was kept constant and about the threshold for first pain (7.6 mJ mm^{-2}). To measure RT, subjects were asked to respond as fast as possible by pushing a button held in the other hand. With relatively large surface areas (>1.5 mm^2), RT distribution was clearly bimodal and in favor of shorter RTs (Fig. 5). With tiny surface areas (0.15 mm^2 i.e. more than 100 times smaller than the large ones), RT distribution was still bimodal but clearly in favor of longer RTs. More than 90% of RTs recorded using the smallest laser beam were above 600 ms. There is converging evidence [38] allowing to say that the first RT distribution peak corresponds to detection of Aδ-fiber activation while the second distribution peak corresponds to detection of C-fiber activation in the absence of Aδ-fiber activation.

Large laser stimulus surface areas evoked a well-defined late LEP (Fig. 5). However, when stimulating the skin with very tiny beams, LEPs appeared with an ultra-late latency (between 750 and 1200 ms).

6.4. Method 4: selective pathology of Aδ-fibers

In well-documented studies, Kakigi et al. [25] reported pathological conditions where loss of Aδ-fibers occurred. These conditions were characterized by a concomitant loss of late-LEPs. Lankers et al. [28] described for the first time ultra-late cerebral potentials in a patient with hereditary motor and sensory neuropathy type I indicating a selective preservation of C-fiber function. For the interested reader, we recommend the following reviews focused on the clinical application of laser stimulation in neuropathies ([8,26,46], see also Treede in this volume).

These direct and non-invasive methods open new perspectives for the study of central processing of signals ascending through Aδ and/or C nociceptors and should allow us to better understand their interaction and integration in normal and pathological conditions. Also promising is the possibility to study the differential action of analgesic drugs on both pathways, as they are equipped with different receptors, transmitters and neuromodulators.

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