Neurophysiologie Clinique 36 (2006) 207–218

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journal homepage: http://france.elsevier.com/direct/neucli

ORIGINAL ARTICLE / ARTICLE ORIGINAL

Measurement of skin temperature after infrared laser stimulation ☆

Mesure de la température cutanée après stimulations au laser infrarouge

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Received 29 November 2005; accepted 17 July 2006
Available online 23 August 2006

KEYWORDS
Laser;
Skin temperature;
Pyrometer;
Pain;
Heat;
Perception;
Laser evoked potentials

Abstract
Objectives. – Several types of lasers are available for eliciting laser evoked responses (LEPs). In order to understand advantages and drawbacks of each one, and to use it properly, it is important that the pattern of skin heating is known and duly considered. This study was aimed at assessing the skin temperature during and immediately after irradiation with pulses by Nd:YAP and CO_2 lasers.
Materials and Methods. – The back of the non-dominant hand was irradiated in 8 subjects. Temperatures were measured by a fast analogical pyrometer (5 ms response time). Stimuli were tested on natural colour (white) and blackened skin.
Results. – Nd:YAP pulses yielded temperatures that were correlated with pulse energy, but not with pulse duration; much higher temperatures were obtained irradiating blackened skin than white skin (ranges 100-194 °C vs 35-46 °C). Temperature decay was extremely slow in white skin, reaching its basal value in more than 30 s. CO_2 pulses delivered with power of 3W and 6W yielded temperatures of 69-87 °C on white skin, and 138-226 °C on blackened skin. Temperature decay was very fast (4-8 ms).
Conclusions. – Differences in peak temperatures and decay times between lasers and tested conditions depend on energy and volume of heated skin. The highest temperatures are reached with lesser degree of penetration, as in the case of CO_2 laser and blackened skin. Taking into account the temperature decay time of the skin, the minimum interstimulus interval

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doi:10.1016/j.neucli.2006.08.004

to get reliable LEPs should be no less than 10 s for Nd:YAP and 100 ms for CO_2 laser. Another important practical consequence of the heating pattern is that the Nd:YAP pulses will activate warmth receptors more easily than CO_2.

MOTS CLÉS
Laser ;
Température cutanée ;
Pyromètre ;
Douleur ;
Chaleur ;
Perception ;
Potentiels évoqués au laser

Résumé

Buts. – Des stimulateurs laser de différents types sont disponibles pour l'enregistrement des potentiels évoqués au laser (LEPs). Condition indispensable préalable à leur utilisation adéquate, une bonne compréhension de leurs avantages et inconvénients respectifs, nécessite qu'on ait déterminé et qu'on tienne compte du pattern d'échauffement de la peau. Cette étude a pour but de mesurer la température cutanée pendant et après irradiation au moyen de lasers Nd :YAP et CO_2.

Matériel et méthodes. – Huit sujets sont irradiés au niveau de la main non dominante. Les températures sont mesurées à l'aide d'un pyromètre analogique rapide (temps de réponse de 5 ms). Les stimulations sont effectuées sur la peau intacte (naturellement blanche) et après noircissement de celle-ci.

Résultats. – Les stimulations au moyen du Nd :YAP produisent une augmentation de température proportionnelle à l'énergie mais indépendante de la durée de la stimulation ; des températures bien plus élevées sont obtenues sur la peau noircie (100–194 °C) que sur la peau naturelle (35–46 °C). La température décroît très lentement sur la peau naturelle, retrouvant sa valeur basale en plus de 30 s. Les impulsions laser CO_2 à 3 et 6 W produisent des températures de 69–87 °C sur la peau naturelle et de 138–226 °C sur la peau noircie. La diminution de la température est très rapide (4–8 ms).

Conclusions. – Les différences entre les températures atteintes et leurs cinétiques de décroissance, selon les types de laser et les conditions expérimentales, paraissent liées à l'énergie et la puissance employées ainsi qu'au volume de peau chauffée. De fait, les températures les plus élevées sont atteintes lorsque la pénétration est moindre, comme c'est le cas avec le laser CO_2, mais aussi quand la peau est noircie. Compte tenu de la cinétique de décroissance de la température cutanée, l'obtention de LEPs fiables nécessite un intervalle minimum entre les stimulations supérieur ou égal à dix secondes pour le laser Nd :YAP et 100 millisecondes pour le laser CO_2. Une autre conséquence pratique importante du modèle d'échauffement cutané est que le laser Nd :YAP activera les récepteurs thermiques plus facilement que le laser CO_2.

Introduction

Pioneered by Mor and Carmon [17], laser stimuli are currently used in clinical and experimental neurophysiology since they allow a synchronized and fast activation of Aδ and C mechano-heat nociceptors (AMH) and provide the only objective means for exploring pain and heat sensation pathways [3,4,7,14–16,21,22] .The properties of the stimulus are such that a sufficiently synchronized volley is generated in the thin myelinated and unmyelinated fibres, so that evoked responses can be recorded from the scalp.

At first, the CO_2 laser was employed as a stimulator in neurophysiology, but later on, solid state lasers like neodymium-doped yttrium aluminium garnet (Nd:YAG) and finally neodymium yttrium aluminium perovskite (Nd:YAP) were preferred for their simplicity of manufacturing, reliability, better general and beam handling. Although all these lasers emit within the infrared spectrum, there are remarkable differences as to the wavelengths and properties of the radiation. CO_2 lasers emit between 9 and 11 μm, whilst Nd:YAP has wavelength of 1.34 μm. Such differences are relevant to tissue heating and receptor activation. Indeed, in order to understand how lasers excite thermal and pain receptors, it is important to know the temperature reached by the irradiated spot with as high a degree of accuracy as possible. Although accurate theoretical models have been calculated [9], experimental measurements are, of course, essential. There are, however, some difficulties. Intradermal thermocouples have been employed to measure the actual temperature of skin at various depths [5], but the method has several disadvantages. As small as they may be, thermocouples have a non indifferent caloric capacity and inertia. Therefore, their response time cannot equal the fast times of the laser pulse. Furthermore, their very presence inside the tissue or upon its surface locally modifies the thermal properties, thereby influencing measurements [8]. Some of these drawbacks can be overcome by thermocameras and pyrometers, which read the heat emitted by the tissue surface at a distance, without interfering with the heating and cooling process. Fast thermocameras have indeed been used to measure skin temperatures after solid state laser stimulation and some preliminary results have been published [2]. Thermocameras have the advantage of mapping the temperature over a given area, but their response is slowed down by the very process of building a matrix and even their best frame rate, which is comprised between 30 and 60 frames/s, is not fast enough to investigate events of few ms duration. Pyrometers detect the heat of just one spot, but can be much faster than thermocameras. Usually, commercial pyrometers do not have a time definition better than 100 ms. But nowadays, very fast pyrometers are available. Considerations about the wavelengths of infrared radiation are of paramount importance. The heat radiation emitted by the skin is in the 8-14 μm range, which comprises the CO_2 laser radiation wavelength. During the laser pulse, there will be some reflection, which, although minimal, will be sufficient to affect the thermocamera or the pyrometer pointed at the irradiated spot.

We endeavoured to overcome some of the above mentioned difficulties and to perform a detailed study of the surface temperature during or immediately after the laser pulse with a temporal definition as high as possible. Therefore, the aims of the research reported in this paper were 1) to measure the surface skin temperature before, during, and after laser infrared irradiation by means of a very fast pyrometer; 2) to compare the surface temperatures of natural and blackened skin after irradiation by CO_2 and Nd:YAP laser pulses, and 3) to seek the correlation between surface temperature and sensory perception.

Materials and methods

Lasers

A CO_2 (Neurolas® by El.En.) and an Nd:YAP (Stimul 1340® by El.En.) laser, both approved for medical use, were employed to provide infrared radiation at the respective wavelengths of 10.6 and 1.34 μm. The CO_2 laser could not be readily set to deliver pulses with constant energy, but only constant power. For this reason, we do not report here the temperature readings after pulses of different durations from the CO_2 laser. Energy and power emitted by our lasers were monitored by means of a power-energy meter (Nova Display®, with FL250A head, by Ophir). Diameter of the irradiated spot was measured on ZAP-IT® Target Paper (by Rockwell Laser Industries), a thermally sensitive paper which records the beam energy imprint.

Pyrometer and shutter

Temperature measurements of the skin surface were performed with an analogical KT22® pyrometer (by Heitronics) covering the range between 8 and 14 μm with complete suppression of other infrared spectral regions. The pyrometer has a reading spot with nominal diameter of 4 mm with the front lens set at a distance of 160 mm from the target. Our instrument, however, was thoroughly checked with the aid of an optical bench, and it was found that the actual reading spot had the diameter of 2 mm. Such feature further increased the reliability of our measurements, as it made easier to aim the pyrometer spot well within the laser irradiated area. In such way, there would be little chance of the pyrometer reading borderline zones of the laser irradiated area with lower temperatures. The wavelength range of the pyrometer response comprised the spectral emission of the CO_2 laser. If the pyrometer were to be active during CO_2 laser illumination, the reflected radiation would have been detected, and consequently put the pyrometer out of use for some time or altered the reading somehow. To prevent this, a shutter was constructed, that avoided temperature reading by the pyrometer during the time epoch of the laser emission. The shutter consisted of a brass blade butterfly shaped, pivoting on the central axis of a 4-phase stepper motor driven by a microchip with a dwell time of 760 μs per step and 400 steps per 360° rotation. An infrared barrier (emitting diode optically coupled to a sensor transistor) was set up to synchronize the shutter with the laser trigger, in order to start the pyrometer reading 1-2 ms after the laser had stopped its emission.

The laser head, pyrometer and shutter were placed on a steel frame by means of suitable holders allowing fine and reliable alignment to the target (Fig. 1). The target itself was positioned behind a steel screen, on which a 20 mm diameter hole had been made. In order to obtain accurate and reproducible positioning of the target area, it was sufficient that the subject pressed his or her hand on the back of the screen, leaving exposed the skin within the area of the hole.

The pyrometer nominal response time is 5 ms from 0 to 200 °C. In order to test the actual response time, experiments with black body and shutter were first performed. The black body, with emissivity factor of 1, was a round steel plate of 15 mm thickness and 120 mm diameter, painted black; it was electrically heated to 170 °C, with temperature monitored via conventional precision thermoresistor. Fig. 2 depicts the response from the pyrometer obtained by pointing it to a black body and blinding it for 40 ms with the shutter blade. The black body was screened so that only a spot with diameter of 6 mm could be read by the pyrometer, in order to simulate the real recording conditions (we remind here that the actual size of the pyrometer reading spot was 2 mm). The slope of the up-going front gives the measure of the response time of the pyrometer. In our experimental setting, the pyrometer reached the reading of 170 °C in less than 4 ms.

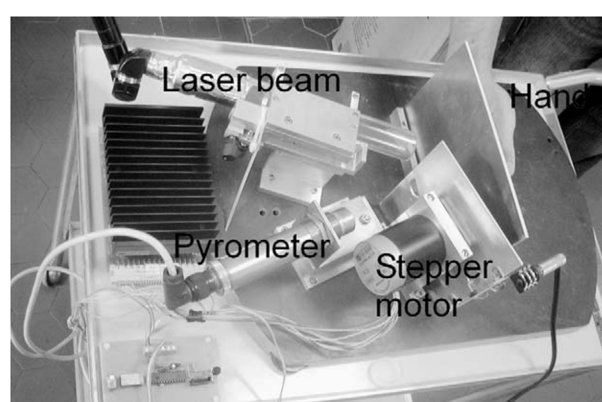

Figure 1 Set up of laser and pyrometer on a steel basement. The step by step motor drives the shutter blade, in front of the pyrometer. The subject's hand is firmly positioned behind the screen, where a hole with 20 mm diameter has been drilled, centred on the point of convergence of the laser beam and pyrometer reading spot.

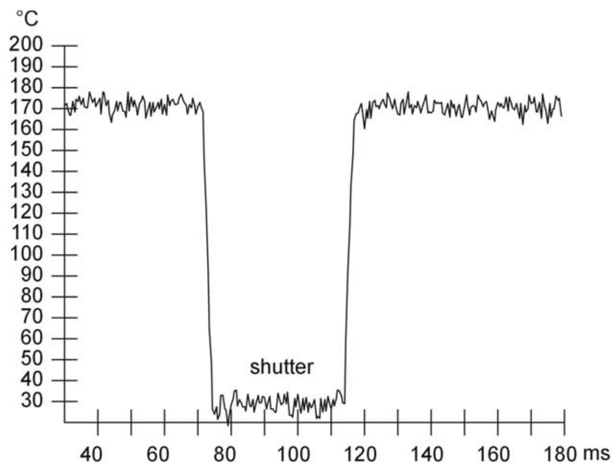

Figure 2 The pyrometer reading times in our experimental setting (with the shutter blade obscuring a 6 mm 170 °C hot spot).

Measurement of perception

Subjective perception of the laser pulse was quantified according to the visual analog scale (VAS) graded from 0 to 10, where 10 was the strongest imaginable pain. Grade 0 meant no sensation, grades 1-3 meant heat sensation, not painful. Subjects were also asked to describe qualitatively the perceived painful sensation, as pinprick or dull pain. We operated in a single blind fashion, as the subjects did not know the settings of the laser pulse.

Subjects and irradiated areas

The project had been approved by the competent body of our institution. The whole procedure was thoroughly explained to the subjects for their informed consent. They were eight Caucasian white skin subjects, all healthy volunteers, involved in the research. The investigated area was the hairy skin of the back of the hand of the non-dominant side.

Experimental setting and data analysis

Skin temperatures were measured under the following conditions. a) Nd:YAP irradiation with constant spot diameter of 6 mm, constant duration of 12 ms and variable energy of 0.5, 1.0, 1.5 and 4.5 J (with respective energy densities of 18, 35, 53, 159 mJ/mm^2). The procedure was performed on natural colour skin (white) and on skin blackened with India ink (with emissivity factor of approximately 1); in the blackened skin condition, the maximum delivered energy was 1.5 J to avoid skin damage. b) Nd:YAP irradiation with constant spot diameter of 6 mm, constant energy of 4.5 J on white skin and 1 J on blackened skin, and different durations of 4, 12 and 20 ms. c) CO_2 irradiation with constant spot diameter of 3 mm, duration of 15 ms and power settings of 3 and 6W (45 and 90 mJ, with resultant energy density of 6 and 13 mJ/mm^2) on white and blackened skin. In addition, one set of experiments with extended time base recording of 30 s was performed on 5 subjects using Nd:YAP pulses on a 6 mm spot, with 12 ms duration and 4.5 J energy on white skin, and 1.5 J on blackened skin. Recording of laser evoked potentials (LEPs) from the scalp, simultaneous with skin temperature recording, was performed in 3 subjects. The active recording electrode was placed at the vertex, referenced to electrically linked earlobes. Twenty responses were averaged per each set. Signals were amplified 100,000 times with high and low pass filters set at 0.3–100 Hz, then digitally converted (1024 points per 1 s), with amplitude resolution of 0.195 μV per digit (SystemPlus, Micromed, Treviso, Italy).

On all experiments, each temperature recording was performed twice, for reproducibility.

Results

Nd:YAP stimulation

Influence of stimulus energy

Pulse duration was 12 ms with a spot of 6 mm diameter. Energy was increased from a minimum of 0.5 (energy density 18 mJ/mm^2) to a maximum of 4.5 J (energy density 159 mJ/mm^2).

Fig. 3 shows the pyrometer reading of the skin temperature in one case. The direct relationship between energy and peak temperature is very obvious and is best represented in the aside graph, based upon the recorded mean values. Skin cooling occurs very slowly, with a temperature well above the basal values even after 800 ms from the stimulus.

Table 1 summarizes the mean absolute temperatures and differences from their basal values, together with subjective perception and VAS ratings. It can be noted that an increase of 13.7 °C above basal temperature is sufficient to give a fairly strong pinprick sensation (graded 5).

When stimuli with identical parameters were delivered to the same area blackened with India ink, much higher temperatures were recorded (Fig. 4); also the perception ratings were accordingly increased, with a definite strong pin prick sensation since the lowest energy setting (Table 2). Because of the high temperatures, energy levels had to be kept in the low range (maximum 1.5 J). A further difference from the white skin was the behaviour of temperature across time: the temperature decrease was much quicker with blackened than white skin. A comparison of temperature decay after Nd:YAP pulses of 0.5 and 1 J energy, delivered to a 6 mm spot (18 and 35 mJ/mm^2), with duration of 12 ms is shown in Fig. 5. The most striking differences in decay trend show up with the 0.5 J pulse, where 100 ms after the pulse, the blackened skin temperature drops to 20% of its maximum value, whilst the white skin only cools down to 77%.

In order to assess how long the temperature of white and blackened skin would take to return to its basal value, we also performed recordings in 5 subjects with an analysis time of 30 s (Fig. 6). Differently from the previous experiment, intensity of stimulation was graded so that the sensory perception of both stimuli was the same and rated 6 (VAS). A stimulus of 1.5 J (53 mJ/mm^2) 12 ms was employed for blackened skin, while a 4.5 J (159 mJ/mm^2), 12 ms

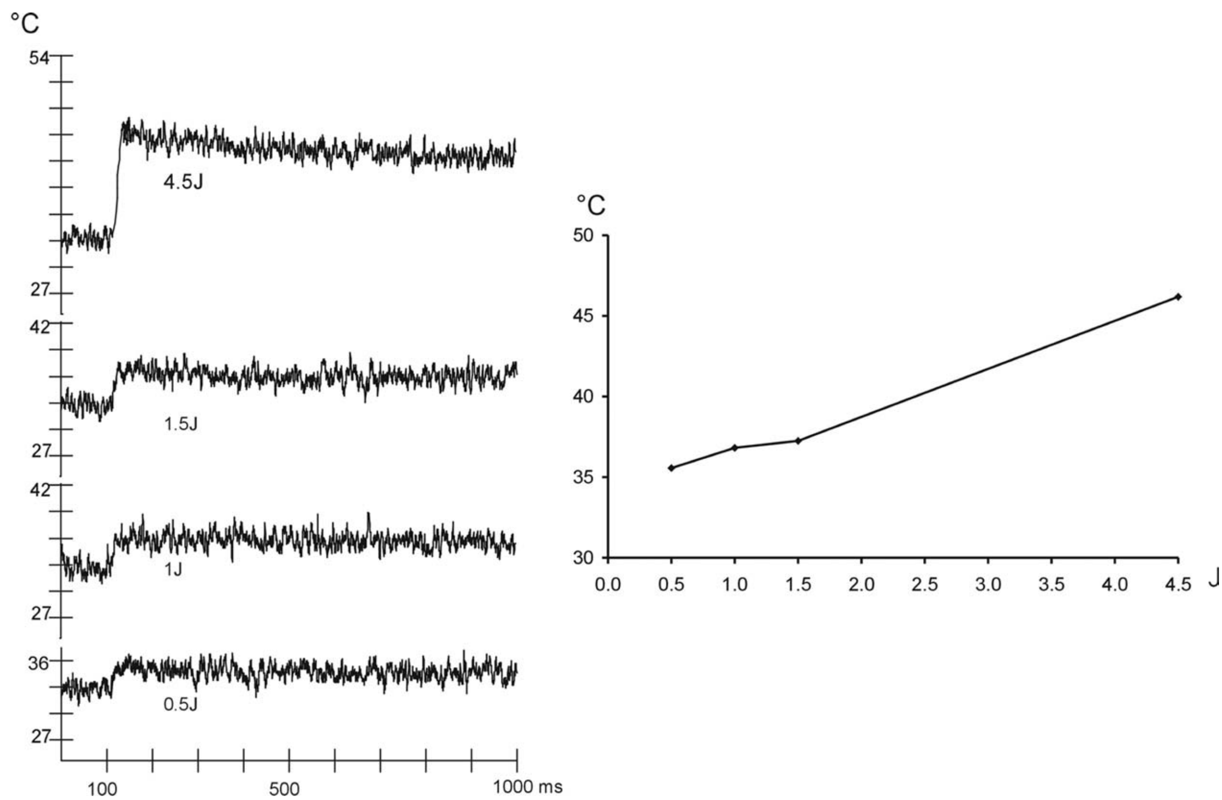

Figure 3 *Left:* Temperature recording after irradiation with Nd:YAP pulses of increasing energy. *Right:* mean temperatures vs pulse energy.

Table 1 Skin temperatures by Nd:YAP laser pulses on white skin, according to different energy levels, pulse duration 12 ms, 6 mm spot

Energy (J)	Energy Density (mJ/mm^2)	Mean absolute temperature °C	SD	Mean differential Temp °C	SD	Subjective perception	VAS
0.5	18	35.6	1.57	2.9	0.48	No perception	0
1.0	35	36.8	3.98	5.5	0.68	Warmth	1
1.5	53	37.2	2.14	5.4	0.38	Warmth	1
4.5	159	46.2	5.37	13.7	2.33	Pin Prick	5

pulse was used for white skin. In this example the maximum temperature was 195 °C for blackened skin and 49 °C for white skin. After 3 s, the skin temperature had reached 1.4% of the peak value for blackened and 40.8% for white skin.

Influence of stimulus duration

In these experiments the stimulus durations ranged from 4 to 20 ms. The same energy of 4.5 J and the same spot diameter of 6 mm were used on white skin (159 mJ/mm^2), whilst an energy of 1 J (35 mJ/mm^2) was used for blackened skin. Results for white and blackened skin are summarized in Tables 3, 4, respectively.

Statistical analysis showed no significant differences in absolute and differential temperature values among the various sessions, leading to the conclusion that temperature was not dependent upon stimulus duration, provided that the energy was kept constant, thus confirming a previous research [12].

Timing of peak temperature was strongly dependent upon stimulus duration (Fig. 7 left), and the amplitude of the peak was slightly reduced with longer duration, although the difference did not reach statistical significance. It should also be noted that the rise time of temperature is steeper when shorter stimuli are used.

Much higher temperatures developed after blackening the skin area (Fig. 7 right). There was, however, an inverse relationship with duration, as higher durations yielded significantly lower temperatures. Here too, a shorter stimulus time was associated with a steeper temperature rise time.

Table 4 reports details of peak temperature and rising times according to pulse durations. It is worth noting that whatever the pulse duration, all temperature rise times with blackened skin were far shorter than those with white skin.

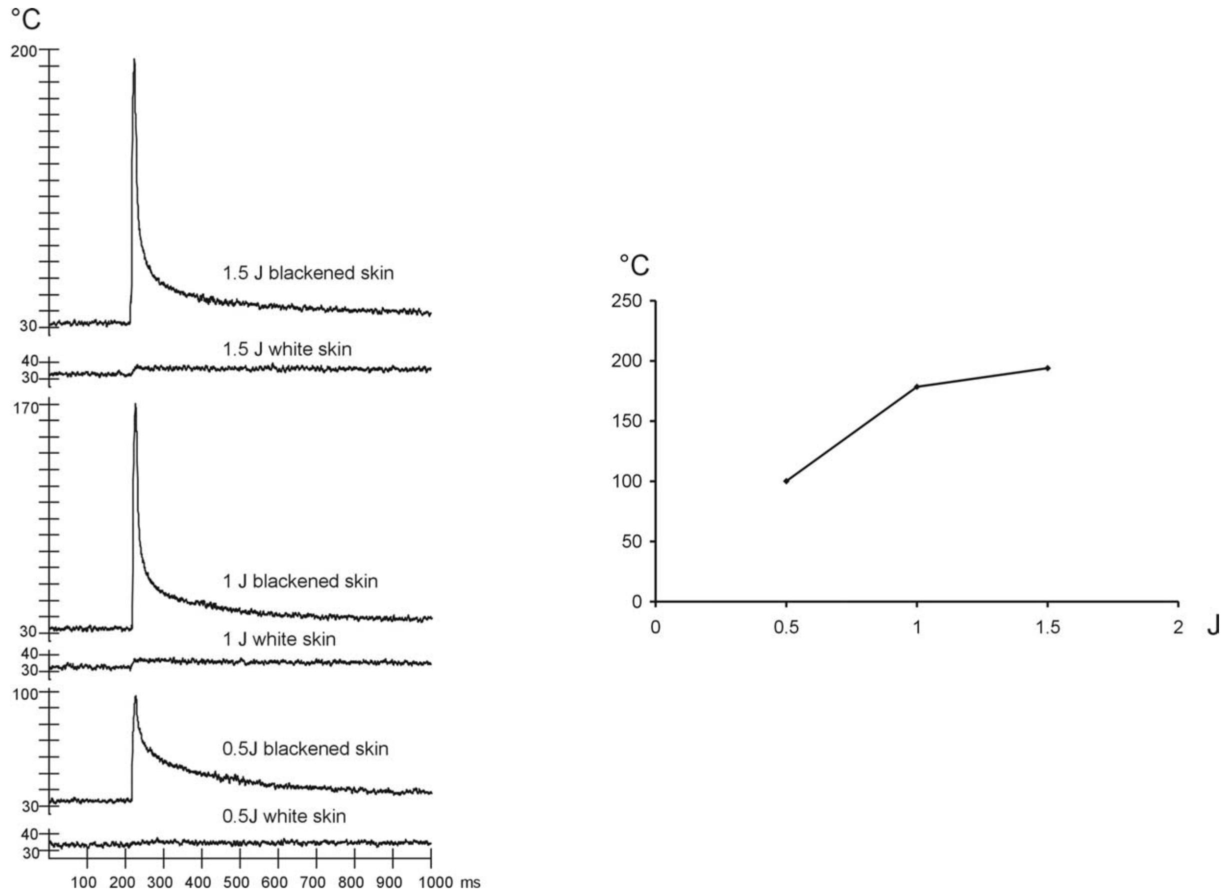

Figure 4 Irradiation with Nd:YAP pulses. *Left*: There was a much sharper rise in temperature when the skin was blackened, compared to the behaviour of white skin (lower trace of each pair of curves). *Right*: mean values of temperature of blackened skin as a function of pulse energy (note the different ordinate scales in the right parts of Figs. 3 and 4).

Table 2 Skin temperatures by Nd:YAP pulses on blackened skin, according to different energy levels, pulse duration 12 ms, 6 mm spot

Energy (J)	Energy Density (mJ/mm^2)	Mean absolute temperature °C	SD	Mean differential Temp °C	SD	Subjective perception	VAS
0.5	18	100.1	13.72	69.3	12.01	Pin Prick	4
1.0	35	178.5	17.00	148.8	15.24	Pin Prick	5
1.5	53	194.0	13.16	162.2	12.71	Pin Prick	6

CO_2 laser stimulation

Influence of stimulus power

A 3 mm spot was irradiated with 15 ms pulses, with power settings of 3 and 6W (6 and 13 mJ/mm^2), and under the usual two conditions of white and blackened skin (Fig. 8). The unavoidable use of the shutter prevented the recording of temperature during the laser irradiation. The time epoch during which the shutter obscured the pyrometer is marked by a grey area in the graphs of Fig. 8. Noteworthy, the rising slope of the response recorded by the pyrometer immediately after the shutter has opened, from baseline up to the peak, is only due to the response time of the pyrometer, and does not reflect the heating process of the skin. Return to basal values was comparatively quick, showing a much faster drop than in the Nd:YAP case. Blackening the skin had the effect of raising the temperature to a considerable amount, but had no effect on the skin cooling time.

Mean values of temperatures, decay times and perceptions are detailed in Table 5.

Laser evoked potentials (LEPs) after Nd:YAP and CO_2 stimulation

In 3 subjects LEPs were evoked by irradiating the hand back with Nd:YAP pulses, with energy of 4.5 J for white and 1.0 J for blackened skin. Duration of 15 ms and spot diameter of 6 mm were used (energy densities of 159 and 35 mJ/mm^2). In the same subjects CO_2 stimulation with power of 6W, duration of 15 ms and 3 mm diameter spot were used

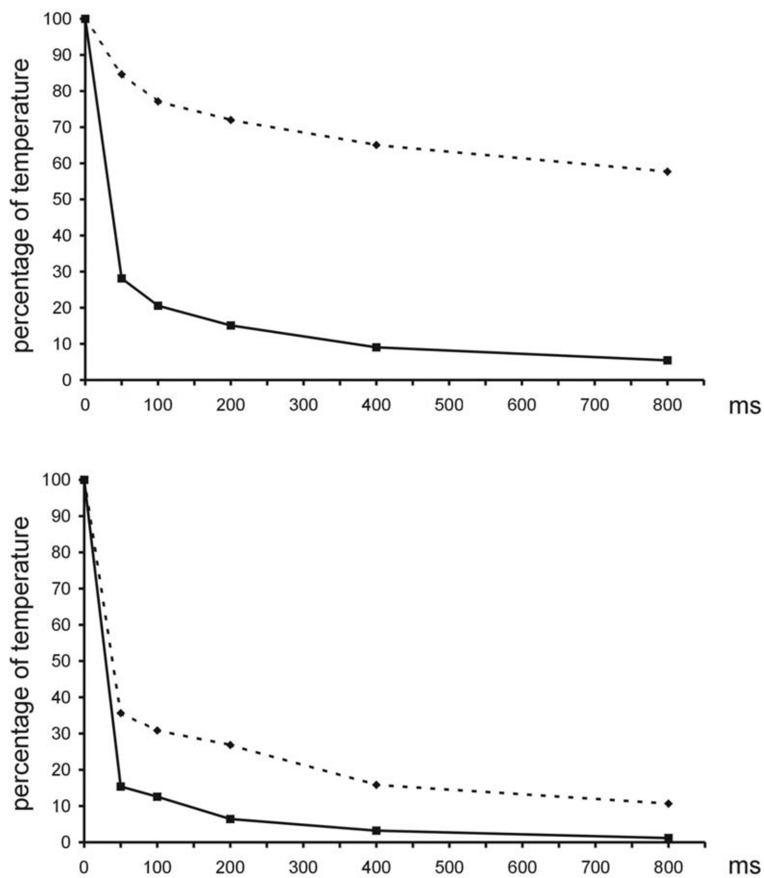

Figure 5 Graphs meant to show the temperature decay in the 800 ms epoch after a laser pulse of 0.5 J (*top curves*) and 1.0 J (*bottom* curves) energy pulse. Temperature is expressed as percentage of the maximum increase, normalized to 100. The dotted line represents temperature reading from white skin; the full line is from blackened skin.

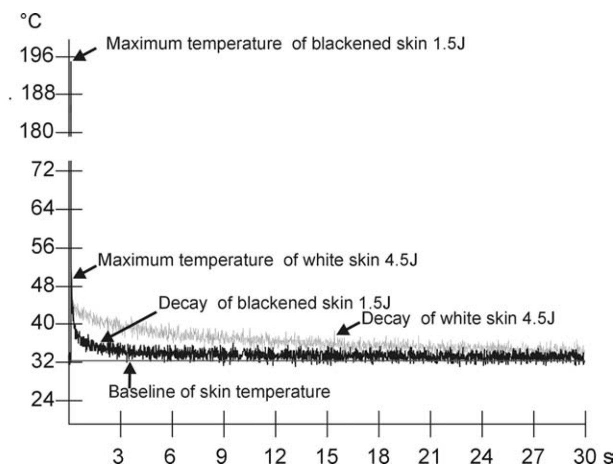

Figure 6 The use of a 30 s baseline shows that although the white skin reaches a maximum temperature of only 47 °C, it takes much longer than the black skin to cool down, as the baseline temperature is not reached yet after 30 s. Note that intermediate values of the temperature scale have been cancelled out (blank gap).

(energy density of 13 mJ/mm^2). The above parameters were chosen, as they were threshold values for pinprick perception. All stimuli gave visually similar responses and a sample of recording, with superimposed skin temperature graph, is shown in Fig. 9.

Adverse Events

No permanent skin damage was ever detected through our experiments. Nd:YAP stimuli producing high temperatures caused some reddening of the irradiated spot, which lasted for a few hours, whilst pigmentation of the spot, which lasted for a few days could be produced by CO_2 irradiation at 6W on white or 3 W on blackened skin, and by Nd:YAP 1.5 W irradiation on blackened skin.

Discussion

To our knowledge, this is the first successful attempt aimed at a high time definition and methodical measurement of skin temperature during or immediately after infrared laser radiation with a non-contact instrument. We recently published previous sets of experiments that were performed in our laboratory with the same equipment, reporting partial results focused upon the measurement of rising time of skin temperature [12].

Before discussing our results, a brief foreword may be helpful as a remainder of some principles of physics relative to the process of how radiation conveys heat to the recep-

Table 3 Temperatures by Nd:YAP, rise times and perception ratings after pulses of constant energy (4.5 J, 159 mJ/mm^2) but different durations on white skin

Pulse Duration	Mean absolute temperature °C	SD	Mean differential Temp °C	SD	Rise time (ms)	SD	Subjective perception	VAS
4	45.4	6.39	13.5	2.46	12.47	0.26	Pin Prick	6
12	46.0	5.41	13.4	2.72	19.13	0.35	Pin Prick	6
20	46.8	5.77	14.7	3.03	24.92	0.27	Pin Prick	6

Table 4 Temperatures, rise times and subjective ratings after 1.0 J (35 mJ/mm^2), 12 ms Nd:YAP pulse of different durations on blackened skin

Pulse Duration	Mean absolute temperature °C	SD	Mean differential Temp °C	SD	Rise time (ms)	SD	Subjective perception	VAS
4	211.6	8.91	181.0	8.88	4.63	0.48	Pin Prick	6
12	169.6	15.30	139.0	14.77	11.15	0.61	Pin Prick	5
20	99.5	6.59	69.4	5.40	11.78	1.09	Pin Prick	5

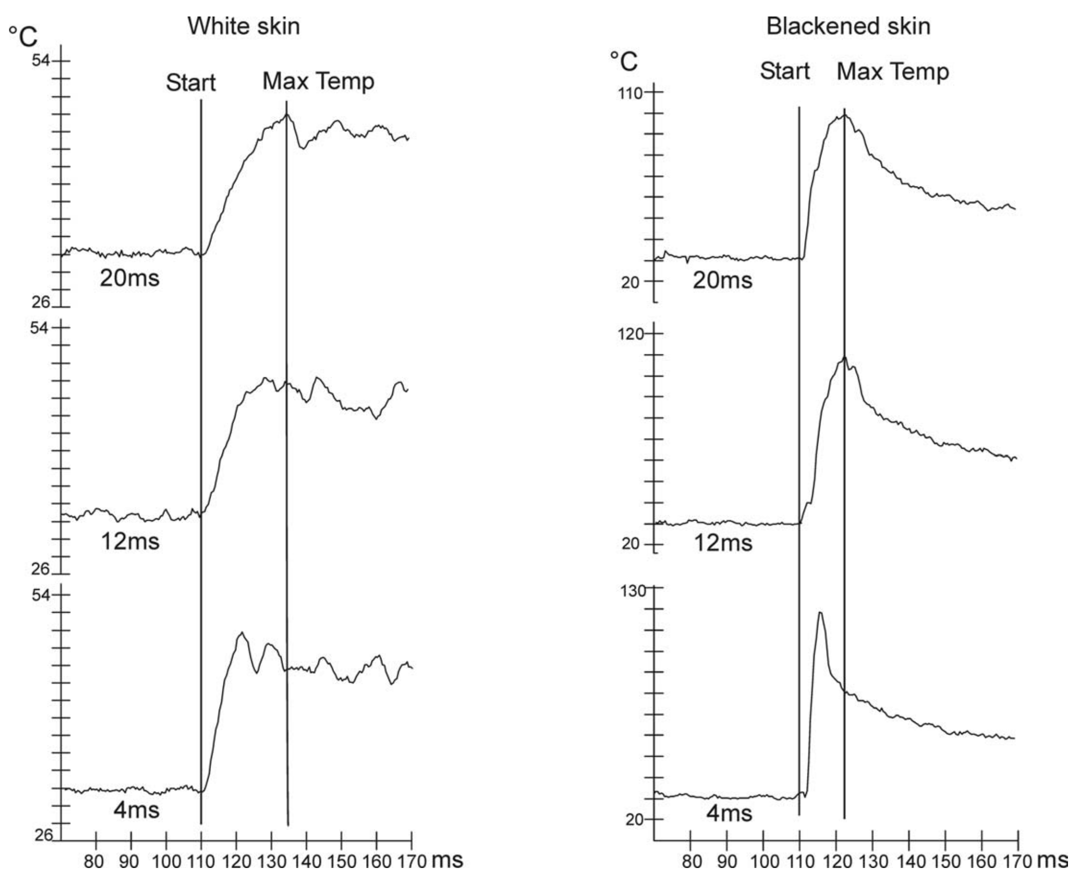

Figure 7 Effect of increasing the pulse duration, while keeping the energy constant, upon skin temperature. Irradiation by Nd: YAP laser, with 1 J energy, 4–20 ms pulse duration, focused on a 6 mm spot. *Left*: irradiation of white skin. *Right*: irradiation of blackened skin. The 2 vertical lines mark the start and end of the slowest rising slopes. Two interesting phenomena can be observed: 1) the duration of the rising slope of skin temperature is directly proportional to the duration of the stimulus, both with white and blackened skin and 2) the peak temperature on blackened skin is inversely correlated with duration of stimulus.

tors. Interactions between the laser radiation and excitable tissues are dictated by the optical characteristics of the skin. When infrared laser radiation hits the skin, the most important physical effect that takes place is absorption [20]. This is defined by the absorption coefficient (cm^{-1}) and varies according to the wavelength and the properties of the molecules present in the tissue. Water, which is the main constituent of biological tissues, has an absorption coefficient of 1.83 cm^{-1} for the Nd:YAP radiation (wavelength 1.34 μm) and of 792 cm^{-1} for the CO_2 radiation

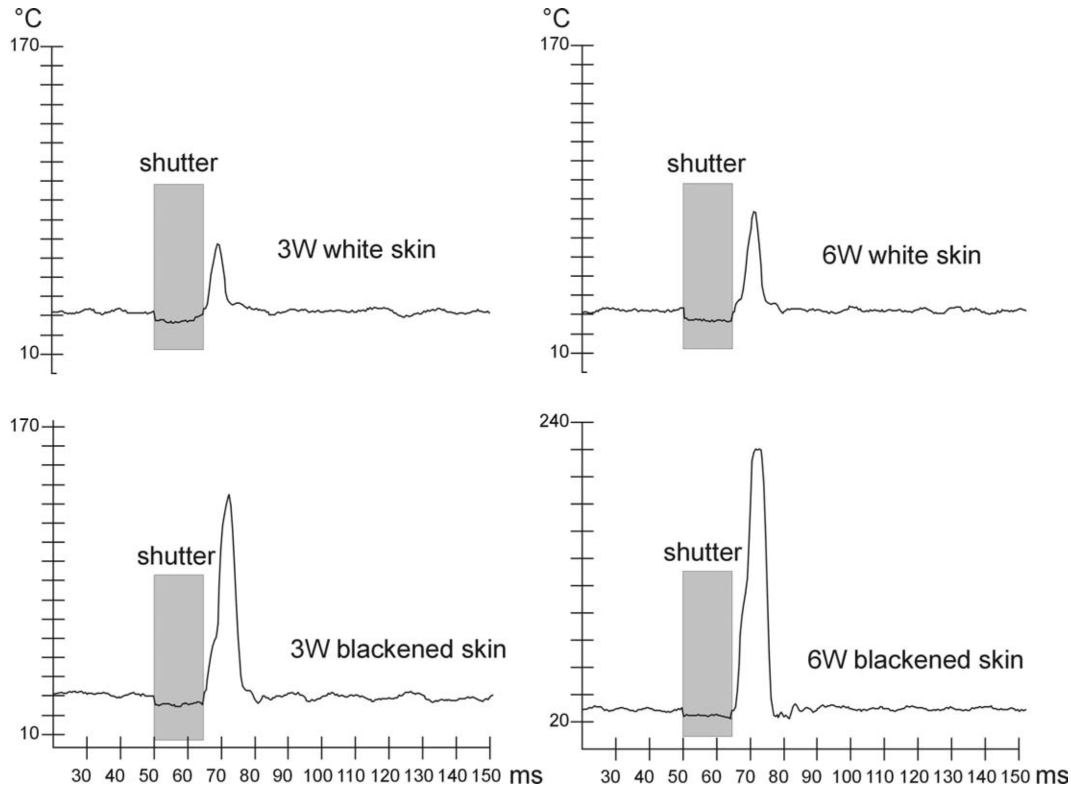

Figure 8 Skin temperatures measured immediately after CO_2 laser pulses 15 ms wide. During the laser pulse the shutter obliterates the pyrometer's view, as shown by the grey area. During that time, the pyrometer reads the temperature of the shutter blade, lower than the subject's skin temperature. On the right are the responses to 3W stimulation on white and blackened skin; on the left are the responses to 6W stimulation. It is worth noting that the temperature decay time is much faster than with Nd: YAP pulses.

Table 5 Effects upon skin temperature and subjective perception of CO_2 laser pulses

Power (W)	Energy Density (mJ/mm^2)	Skin colour	Mean absolute temperature °C	SD	Mean differential Temp °C	SD	Decay time (ms)	Subjective perception	VAS
3	6	White	69.4	1.77	33.7	1.72	4.29	Pin prick	4
3	6	Black	138.9	3.74	103.7	1.78	8.60	Pin prick	4
6	13	White	87.0	2.51	52.3	2.03	8.19	Pin prick	6
6	13	Black	226.6	2.06	193.5	1.91	4.26	Pin Prick	6

(wavelength 10.6 μm) [10]. Depth of penetration, which is the parameter of interest to the neurophysiologist who wishes to know which receptors are activated, depends upon intensity of radiation and absorption (beyond other factors, such as scattering, reflection and refraction, not as much relevant in the usual experimental conditions, and upon which we shall not dwell here). Depth of penetration is defined by the Beer-Bouger-Lambert law, or absorbance law, which, put down in very simple terms, states that $I_T = I_0 \exp(-\mu_a d)$, where I_T is the intensity at the site of interest within the substrate, I_0 is the intensity on entry of the substrate, μ_a is the absorption coefficient, and d is the distance through which the radiation is transmitted within the substrate. In rough terms, the law clearly shows that the more the radiation is absorbed by the substrate, the lesser it will penetrate the substrate, and so the more superficial the biological effects of laser will be.

Nd:YAP laser

Technically, measurement of skin temperature due to Nd: YAP laser with wavelength of 1.34 μm offered no difficulties, as the KT22 pyrometer was not sensitive to this radiation. It was therefore possible to use it throughout the laser emission without fear of artefacts or erroneous measurements. If we make some assumptions for the sake of simplicity, and consider that the absorption coefficient of the skin for Nd:YAP radiation is approximately $\mu_a \approx 10\ cm^{-1}$ [20] (greater than pure water, because of pigments and other chemicals), the Beer-Bouger-Lambert equation calculates that 65% of the energy is absorbed within the first 1 mm of skin (not considering scattering and reflection, which would account for a further slight reduction of energy). Assuming also that the penetration pattern is cylindrical, then the largest part of energy delivered will heat up a

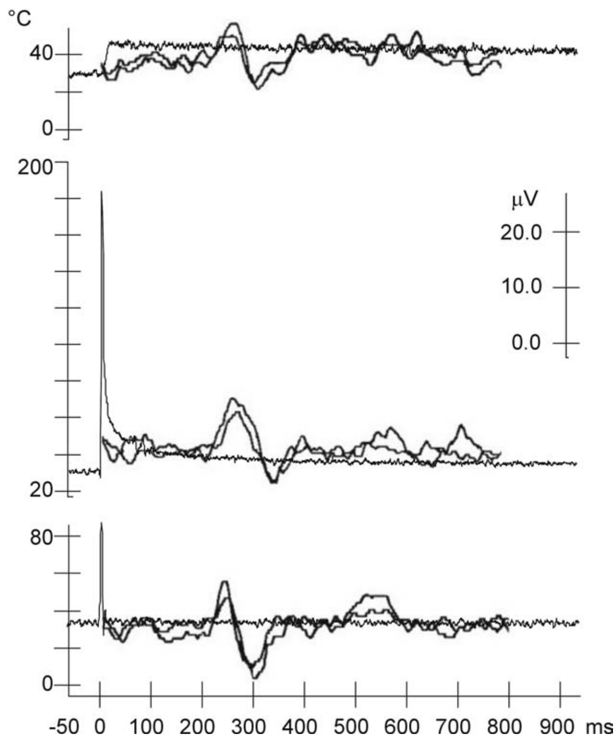

Figure 9 The first two traces show the temperature graph (thin line) and the scalp recorded laser evoked potentials (LEPs) (in bold) after laser stimulation with Nd:YAP on white and blackened skin (12 ms duration, 4.5 J energy for white and 1.5 J for blackened, 6 mm spot, respective energy densities of 159 and 35 mJ/mm^2). The third trace shows temperature and LEPs after CO_2 stimulation (15 ms, 6W, 3 mm spot, energy density of 13 mJ/mm^2). Perception grade and quality were the same for all the stimuli. Y axes on the left report the pyrometer measured temperatures, whilst the Y segment on the right is the amplitude calibration in μV relative to all LEPs. Time axis is common to all graphs, temperature and LEPs, and starts from –50 ms before the laser stimulus. Analysis time was 800 ms for LEPs, and 1000 ms for temperature reading.

skin volume of approximately 28 mm^3 in the case of a 6 mm spot. From our data, we have no way of knowing how the temperature was distributed within the irradiated volume. However, we do have some indirect evidence pointing towards an approximately uniform heating. We found that the perception threshold for Nd:YAP stimuli occurred in our subjects at a measured 5.5 ± 0.68 °C above the basal skin temperature (Table 1, 1.0 J row). This would fit with activation of C heat receptors, which is known to take place for a skin temperature increase of no less than 1 °C [11,13]. On the other hand, the pin-prick sensation, due to activation of Aδ–supplied receptors, which is known to take place between 41 and 46 °C [11,23], occurred at 46.9 ± 2.97 °C measured on the surface. Based upon this experimental evidence, one may draw the conclusion that the temperatures reached on the surface were quite similar to those activating the heat and pain receptors, with no large differences between surface and depth. The rather large volume of heated skin is probably responsible of the very long decay time of temperature, which in our subjects still retained a temperature comprised between 60 and 20% of the initial increment after 800 ms (Fig. 5). Such feature makes the Nd:YAP radiation on white skin unsuitable for double or repeated pulse experiments with intervals shorter than several seconds, as the residual heat will cumulate with that from the new stimulus. On the other hand such feature might prove useful in studies upon temporal summation effects, provided that the heating time is properly monitored.

A dramatic change in temperature measurement and in perception took place when the white skin was blackened with India ink. This procedure made the surface of the epidermis absorb the radiation, rather than the deeper layers. We have no means of knowing to which depth the radiation penetrated, but we can safely assume that it was no deeper than 100 μm. Therefore, in agreement with the Beer-Bouger-Lambert law, the heat was distributed to a much smaller volume, (approximately 10 times smaller than in the case of white skin) with a sharper rise in temperature and a much faster temperature decay time (residual temperature was always below 10% of the maximum value at 800 ms after the stimulus). The highest temperatures were well matched by the perception grading, which was definitely higher as well.

The differences between white and blackened skin, as to the maximum temperature reached, rise and decay time, can be explained by the different volumes that are heated by laser radiation. The smaller the volume, the higher the energy density, hence the higher the temperature reached. At the same time, the speed at which the process of heating and cooling takes place is much faster with a smaller volume, which has smaller thermal capacity. The effect of heating concentrated within a smaller volume implies that the receptors lying within the irradiated spot are more readily and synchronously activated, and it would be predictable that better LEPs could be obtained after irradiation of blackened skin. Such differences between white and blackened skin are in agreement with previous experiments using reaction times as a measure of absorbed laser radiation [1].

CO_2 laser

CO_2 radiation is considered to be absorbed by the skin as by water [18,19]. If we calculate the Beer-Bouger-Lambert equation for the water absorption coefficient (792 cm^{-1}), then we find out that 65% of the incident radiation is absorbed within the first 0.015 mm layer. Such theoretical result may not perfectly match the reality, as several factors may intervene in reducing the above absorption coefficient. Anyway, the result of calculation well supports the notion that CO_2 radiation heats a much smaller skin volume than the Nd:YAP (something between one hundred- and tenfold difference). When irradiated with a CO_2 laser pulse, skin is expected to act as a black body, independently from its actual colour, [18,19]. Our experimental evidence, however, demonstrates that, with the parameters of stimulus that we employed, such assumption cannot be held as completely true. CO_2 temperatures would be three to four times higher in the case of blackened than for white skin. This may be explained by assuming that the India ink made the CO_2 radiation to be absorbed by an even more superficial layer, so that the heated volume would be several times smaller than in the case of white skin. As for the behaviour of skin temperature in the time domain, our data are necessarily limited by the use of the shutter. The latter was programmed to open 1-2 ms after the laser had stopped emitting its radiation. The pyrometer suddenly started reading the residual skin temperature, and, in a time of approximately 5 ms recorded what appears to be the peak value in the graphs of Fig. 8. In fact, if the actual peak temperature had taken place some milliseconds earlier, it may have escaped detection. It is therefore possible that CO_2 radiation might have yielded even higher temperatures than those recorded, though we do not expect that large differences may have occurred. The reading of the decay time, however, was accurate, and it demonstrated that the skin cooled very quickly after a CO_2 pulse. Return to basal temperatures took place no later than 30 ms after the trigger pulse had been sent to the laser, hence presumably 15 ms after the temperature peak. Such quick cooling was obviously due to the very limited volume of skin heated by radiation. No differences in cooling time were detected between white and blackened skin. Such feature may appear to be in disagreement with the higher peak temperatures recorded in the case of blackened skin. An interpretation of these two phenomena may be that the higher peak temperature could be due not to a more superficial absorption of the radiation and an ensuing smaller heated volume (as in the case of Nd:YAP laser), but more simply to a further reduction of the already small reflection of the CO_2 radiation by the skin. This way, more energy would be available for heating the superficial layers of the skin, and temperature would accordingly increase, without any change in the heat capacity of the system.

Comparison between Nd:YAP and CO_2 effects on skin temperatures, perception, evoked potentials (LEPs) and biological consequences

When the white skin was irradiated, the differences between Nd:YAP and CO_2 effects were most striking: the increase from basal skin temperature that was induced by Nd:YAP approximately ranged to 1/10 of the values that was reached with CO_2, though the surface energy density used was far higher in the case of Nd:YAP. The minor increase in temperature in the case of Nd:YAP fits with the very large difference between volumes of heated skin. The more distributed and gentle heating provided by Nd:YAP has the effect of activating thermal heat receptors (C fibres) distributed in a fairly large volume, down to 1 mm depth. Consequently, the sensation of warmth is easier to obtain with Nd:YAP, whilst CO_2 laser will mainly evoke the sensation of pin prick, thought to be linked to activation of AMH receptors [6,23]. If such mechanism is going to be experimentally confirmed, the thermal process induced by Nd:YAP could have the theoretical advantage of allowing a sort of selective activation of C fibre receptors. On the other hand, one has to consider that such heating process brings the possible drawback of a rather asynchronous and slow activation of all receptors (nociceptive and not) contained in the skin volume under examination. Such condition might not be deemed ideal in order to obtain a laser evoked potential, although our recordings did not show relevant differences. It is not clear why we recorded almost identical scalp evoked responses following laser stimuli which gave so striking differences as to skin temperatures. It may be speculated that the similarity in scalp responses parallels the similarity in perception. All these issues will be addressed in a future research aimed at studying the correlation between skin temperatures and evoked potentials.

Conclusion

Infrared lasers are by far the most reliable method of exciting Aδ and C fibres for investigation by neurophysiological or neuropsychological methods. The key question that our study tried to answer was: what happens to the temperature of the irradiated spot during and immediately after the very short laser pulse that we deliver? In the introduction section we discussed the problems of accurate and fast temperature measurement and explained why, so far, no or very little reliable information could be gathered on the issue. The question is important also because several types of lasers, emitting radiation with different wavelengths, have been used through the years. All of them emit infrared radiation and “heat” the target, but, are there any relevant differences in the heating pattern? We have found that CO_2 and Nd:YAP radiations yield a very much different temperature at the skin surface, and that the cooling down of the spot is much slower in case of Nd:YAP. All this is due to the very much different volume of skin that is being heated: in the case of CO_2 a very thin cylinder, and in the case of Nd:YAP a ten or more fold thicker cylinder (we took the liberty, in our study, in order to perform some simple calculations, to assume that the radiation spreads within the tissue in a cylindrical shape, though this is not strictly correct). Not surprisingly, superficialization of the Nd:YAP heating by blackening the skin makes its thermal effect similar to that of CO2 laser. Of course, all these phenomena have implications in activating receptors of different kind, at different depths and with different temporal patterns. Correct knowledge of the characteristics of the stimuli that we have so far used rather empirically, may lead to better use and interpretation of the effects of the currently employed lasers. For example, we are now aware that a minimum interval of 10-15 s between stimuli should be observed when using a Nd:YAP laser on the same spot, to allow the receptors to go back to their basal temperature. The same laser will more easily activate C receptors. Conversely a CO_2 lasers is suitable even for double pulse studies, with intervals as short as 100 ms, and it will be easier to selectively activate AMH receptors.

Acknowledgements

The authors are grateful to Dr. Stefano Modi from El.En. Industries for reviewing the technical issues concerning the laser equipment.

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