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Surface Temperature Change, Cortical Evoked Potential and Pain Behavior Elicited by CO_2 Lasers

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Abstract

The performance of a self-designed CO_2 laser stimulator, TL#2, was evaluated against a commercial product, model DE20XL of the Direct Energy Inc. (Irvine). The major items evaluated were the temperature change of the irradiated surface and the electrophysiological and behavior changes in the rat elicited by single laser pulse irradiation. Single shots of TL#2 produced a profile of surface temperature change similar to those of the DE20XL, as quantified by their maximal temperature change, rate of rise (half time to maximum) and rate of temperature drop. TL#2 and DE20XL elicited the same pain behaviors and the same pattern of cortical evoked potential in awakeful, behaving rats. TL#2 differed from the DE20XL in its laser beam shape and focal depth. The cross sectional energy profile of the TL#2 was a Gaussian shape, i.e., most intense at its center point, whereas that of the DE20XL with the FL20XL attachment had a shape of an inverted Gaussian, i.e., most intense in the periphery. Consequently, the peak energy of the center of the TL#2 laser beam grows rapidly with an increase in the pulse intensity. Caution must be taken not to use this machine at high intensity or for long duration less permanent damage should be produced on tested animal or human subject. In summary, TL#2 when used properly, should be a useful tool in the study of pain mechanism. (Chinese J. Physiol. 37: 193-199, 1994)

Key Words: CO_2 laser, cortical evoked potential, pain behavior, rat, surface temperature, thermal radiation

Introduction

Low intensity lasers with their beams deliberately diffused have been used successfully to selectively activate pain sensation in human and elicit pain behavior in laboratory animals. (2,7) The advantage of the laser stimulation lies in its ability to raise the surface temperature of the skin very rapidly (in milliseconds (3), thereby activating cutaneous thermal nociceptors synchronously. A nociception related evoked potential can thus be elicited and used as an index for the studying of pain in human (9) or nociceptive pathway and pain behavior in animals (4,5).

The design and building of a laser stimulator is sophisticated. However, modification of such machine can only be made with detailed knowledge of its internal design and circuity. Furthermore, laser machines are fairly expensive. From our experience, home made machine cut the cost by more than 50%. With future modification and cost-effectiveness in mind, we designed and build a CO_2 laser machine by modifying a laser surgery unit, Tjing Ling #2. We documented in this communication thermal and biological effects of this laser, and compared the performance of this machine with a commerical laser system often used in the study of pain. (1,8)

Materials and Methods

The Laser Machines

Two carbon dioxide laser machines were used.

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The first one was modified from a laser surgical system, the Laser Knife #2 (TL#2) by the Tjing Ling Industrial Research Institute of National Taiwan University. The second laser machine was a model 20XL surgical carbon dioxide laser system with the FL20XL optical attachment (DE20XL) produced by the Direct Energy Inc. (Irvine, USA). The important specifications of the laser beam and the optical system of the two lasers are listed in table 1.

The laser beam of the TL#2 and the DE20XL differ in two important ways. The intensity of the TL#2 laser beam is a Gaussian distribution, i.e., it is most intense at the center, while the intensity falls off sharply towards the periphery. The intensity of the DE20XL beam with the FL20XL attachment, however, is an inverted Gaussian, i.e., the beam has a ring of the most intense places, surrounding a less intense center, a shape like a donut. The second difference is the size of the laser beam. The beam diameter of the TL#2 is slightly smaller than that of the DE20XL, and the angle of the divergence of the beam is smaller.

There are also two important differences in the optical system. The DE20XL has an adjustable spot size at the focal point. In contrast, the spot size of the TL#2 is fixed. The second difference is the focal length and divergence. The TL#2 has a long focal length and small optical divergence, whereas the DE20XL has a much shorter focal length and consequently a larger divergence.

Table 1. Comparison of the two laser systems

	TL#2	DE20XL[a]
I. Laser beam		
peak output	35 Watts	30 Watts
mode	TEM[b]	TEM
wavelength	10.6 μ	10.6 μ
beam diameter	8 mm[c]	11 mm
beam divergence	2 mrad[c,e]	8.5 mrad
II. Optical system		
focal length	125 mm	(26 mm)[d]
divergence	3.7°[c]	16°
spot size	0.2 mm[c]	0.3-2 mm

[a]with FL20XL attachment
[b]TEM: transverse electromagnetic
[c]estimated values
[d]working distance
[e]3140 mrad = 180°

Comparison of Laser Irradiation with Heat Sensitive Paper

Heat sensitive paper (Mitsubishi K65HM thermal paper) was used to capture the cross sectional distribution of the peak energy of the laser beam. The laser head piece was hand-held, and the beam directed perpendicularly towards the paper. Laser lights of different intensity and duration were shone onto the paper from different distances with the aid of a foot switch.

Comparison of Laser Irradiation with Thermosensors

A thermocouple (Harvard microprobe IT-18) with a sensor tip 0.1 mm in diameter (0.45 mm with insulation) was used to record surface temperature changes of a piece of paper by laser radiation. The plastic insulation of the sensor was partially scratched off, therefore, the time constant of the thermal couple should have a time constant between 5 ms (without plastic cover) and 100 ms (with cover). The thermocouple sensor was placed flat on a piece of thick black paper with smooth surface. The laser gun was hand-held and directed perpendicularly towards the sensor, and an effort was made to aim the center of the laser beam at the sensor tip. Temperature changes were amplified to 8 mV/°C. A 12 bit analog-to-digital converter card was used to digitize the temperature change, and a software program[6] was used to calculate the maximal change and the time required for the temperature to rise to half of its maximal change ($t_{1/2}$).

A second method was used to record more accurately the surface temperature changes after laser irradiation. An infrared photoconductor (PCI-M, Vigo Sensor SA Lab, Warsaw) and a high frequency amplifier (bandpass 500 to 1M Hz) was used to record the thermal radiation produced from surface temperature change. The most sensitive wavelength of the PCI-M photoconductor was in the range of 2 to 8 μ. To further reduce the interference from the laser source (10.6 μ), a 1.5 mm thick sapphire filter was used to limit the incoming infrared wavelength to a range below 6 μ.

Comparison of Cortical Evoked Potential and Pain Behavior

Cortical evoked potential (EP) by laser pulse irradiation of the tail was recorded in conscious, behaving rats with cortical electrode implanted. The rat was anesthetized with intraperitoneal sodium pentobarbital (50 mg/Kg). The skin and connective tissues over the skull was cleaned. One or a pair of stainless steel screws were drilled into the skull as the recording electrode at the tail region of the primary somatosensory cortex, i.e., 2.5 mm lateral to the midline and 2.5 mm caudal to the bregma. The screw used as the ground lead was implanted over the olfactory bulb (8 mm rostral to the bregma and 2 mm lateral to the midline). Dental cement was used to secure and insulate the wire and the screws. The skin was sutured and the rat returned to its cage. The rats were housed individually in the cages, 46 × 24 × 21 cm in length, width and height respectively.

A month later, the rat was placed in an acrylic restrainer (52-0916, Harvard Apparatus Ltd., Kent) with its tail completely exposed. Commercial cosmetic depilator was used to remove most of the hairs over the tail. The laser beam was used to stimulate the right side of the tail at the base, in the middle or at the tip. Stimulation parameters were: 10 ms duration, 6 to 18W intensity, beam diameter 3 mm. The laser irradiation when directed to the experimenter's thenar eminence produced slight heat sensation at 12W and was moderately painful at 15W.

Recording was either monopolar (for TL#2) or bipolar (for DE20XL). Conventional electrophysiological setup were used to record laser induced cortical evoked potential with an amplification of 2,000 and a filter bandwidth of 0.3 to 300 Hz. The cortical evoked potential was averaged 10 times with the repetition rate less than once every 10 seconds.

The pain behaviors of the animals following laser irradiation were also recorded. These behaviors were divided into 3 categories. They were reflex behaviors, such as tailflick and local twitches, movements of the body, for examples, leg movements, whisker movements... etc., and complex movements, such as grooming, urination and defecation. A score of 1 and only 1 was given for each type of behaviors seen with each laser shot. For example, if the rat flicked its tail after laser irradiation, the score was 1; if the rat flicked its tail and twisted its head, the score was 2; and if the rat showed grooming behavior following tailflick and head twisting, the score would be 3. Stimulation was repeated 10 times, the total score in the ten trials was added as the behavior intensity. Threshold intensity was defined as the intensity which had a 50% chance to elicit a reflexive pain behavior.

Statistical Analysis

Paired and unpaired Student's *t*-test and one-way ANOVA were used to compare paired, unpaired or groups of samples respectively. Data were expressed as mean ± standard error where not specified. P value less than 0.05 were considered statistically significant.

Results

Laser Effect on Thermal Paper — Varying Distance

The effect of varying the distance of the laser gun from the irradiated target is shown in figure 1. The TL#2 laser burned a small hole through the thermal paper at its focal point (upper left). When held at 3.5 cm away from the focal point, the laser beam affected a circle approximately 3 mm in diameter with the center ashened but not burned through. When held at 7.2 cm away, the intensity of the heat decreased markedly, so that only a very small area burned in the center. The area of the blackened circle did not increased very much, however, probably due to the small divergent angle of the laser beam and the rapidly falling intensity.

The DE20XL laser had been adjusted to a diffused focal point (2 mm). The donut shape of the beam could be clearly seen at all distances. At its focal point, the laser beam burned a ring in a blackened circle. This ring became less intense as the laser gun was held further away from the thermal paper. Another important difference between the two laser system was the faster divergent rate (beam diameter change/cm) of the DE20XL.

Laser Effect on Thermal Paper — Intensity and Pulse Duration

TL#2 and DE20XL were tested at 6, 9 and 12W and 5, 10 and 20 ms duration at a comparable beam diameter of 3 mm. This was done by holding the DE20XL gun slightly off focal point and by holding the TL#2 gun 3.5 cm away from its focal point. Intensities were calibrated with a thermopile (Laser point 8021, Italy). The results are shown in figure

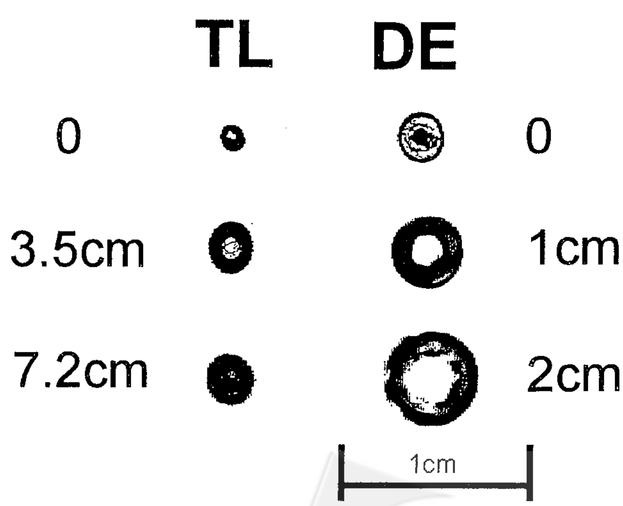

Fig. 1. Effect on thermal paper by 12W, 10 ms single pulse laser irradiation from different distance. Zero represented distance at focal point. Other distance (in cm) were the distances further away from the focal point. TL: TL#2. DE: DE20XL.

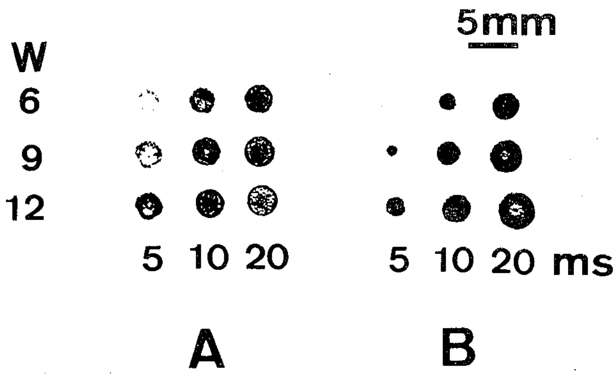

Fig. 2. Effect on thermal paper by combinations of 3 different intensities (in W) and 3 pulse durations (in ms) single pulse laser irradiation. The laser guns were held slightly away (DE20XL, A) or 3.5 cm away (TL#2, B) from their respective focal points.

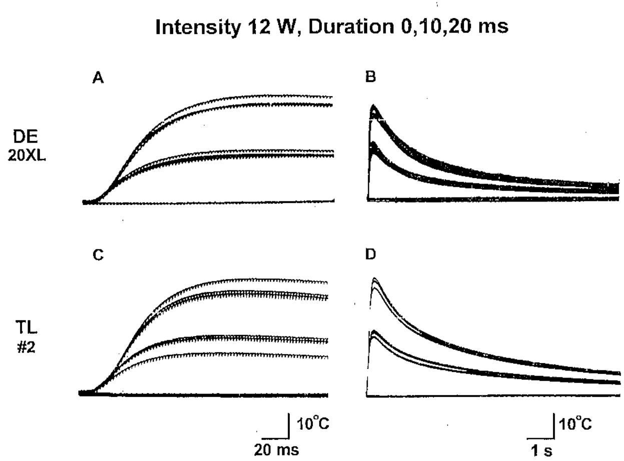

Fig. 3. Surface temperature changes as recorded with a thermocouple produced by single pulse laser irradiation (pulse duration 10 and 20 ms, pulse intensity 12 W). (A), (B): Effects by DE20XL. (C), (D): Effects by TL#2. Notice similar rising and falling profile of temperature change.

2. Noticeable changes of the thermal paper appeared at 6W and 5 ms for both laser machines. By placing the same thermal paper in the oven, the same shade of gray could be obtained at 60-70°C. The difference in beam shape was also apparent. With either an increase in intensity or an increase in pulse duration, the DE20XL beam produced a circle of heat change. The energy of the beam was stronger at the periphery and weaker in the middle. However, the total area of the circle remained relatively unchanged. The TL#2 beam produced a small dot on the thermal paper at 6W and 5 ms. The dot grew larger as the intensity or the pulse duration of the beam became stronger or longer. The center of the beam produced, however, the most intense change. The white center at 12W and 20 ms in B was the trace of burned paper, whereas the white center at 9W and 10 ms in A was the relatively unchanged white paper.

Laser Effect on Thermosensor — Dynamic Change

An example of the temperature change produced by laser irradiation recorded with the thermocouple is shown in Fig. 3. The profile of the rise and the fall of the surface temperature were similar for the two lasers. The average change of surface temperature with 3 pulse durations (5,10 and 20 ms) and from 3 to 20W intensity of the two lasers is shown in figure 4. The DE20XL laser seemed to produce a larger temperature change than the TL#2 laser. The rate of temperature rise was similar for the two lasers. The half times to maximal temperature of the two machines were not significantly different (Fig. 5).

Because of the slow responsive property of the thermocouple (time constant between 5 to 100 ms), the profile of the temperature change as shown in the Fig. 3 was a severely distorted recording, with the half time almost 2 to 4 times longer than the pulse duration, and the maximal temperature reached nearly 100 ms after the laser irradiation. We used a fast responsive photoconductor (PCI-M) to record the thermal radiation of the surface irradiated with a 12W, 10 ms pulse of the TL#2. It is shown in figure 6. Notice that the surface thermal radiation started to rise immediately with the laser irradiation and reached its maximum at the end of the pulse (10 ms). The absolute change of temperature, however, was uncertain.

Laser Effect on Animal — Evoked potential and Behavioral Responses

Laser intensities from 6 to 18W with 10 ms pulse duration and 3 mm beam diameter were tested on the tail of the same group of 8 rats. The animals started to show behavioral change at approximately 9W. The threshold intensity was lowest at the base of the tail, and significantly higher at the middle of the tail or the tailtip (table 2). Comparing the threshold intensity of the two laser machines, DE20XL seemed to be more effective at low intensity. For

Table 2. Threshold intensity (W·msec) of 10 msec laser pulse in eliciting reflexive movements

Laser system	N	Position on tail stimulated: base	middle	tip
TL#2	8	102.2 ± 3.9	118.2 ± 3.2	124.1 ± 6.9
DE20XL	8	99.3 ± 5.9	140.2 ± 8.3*	136.3 ± 13.2*

*Significantly different from values obtained at tailbase by Student-Newman-Keuls test

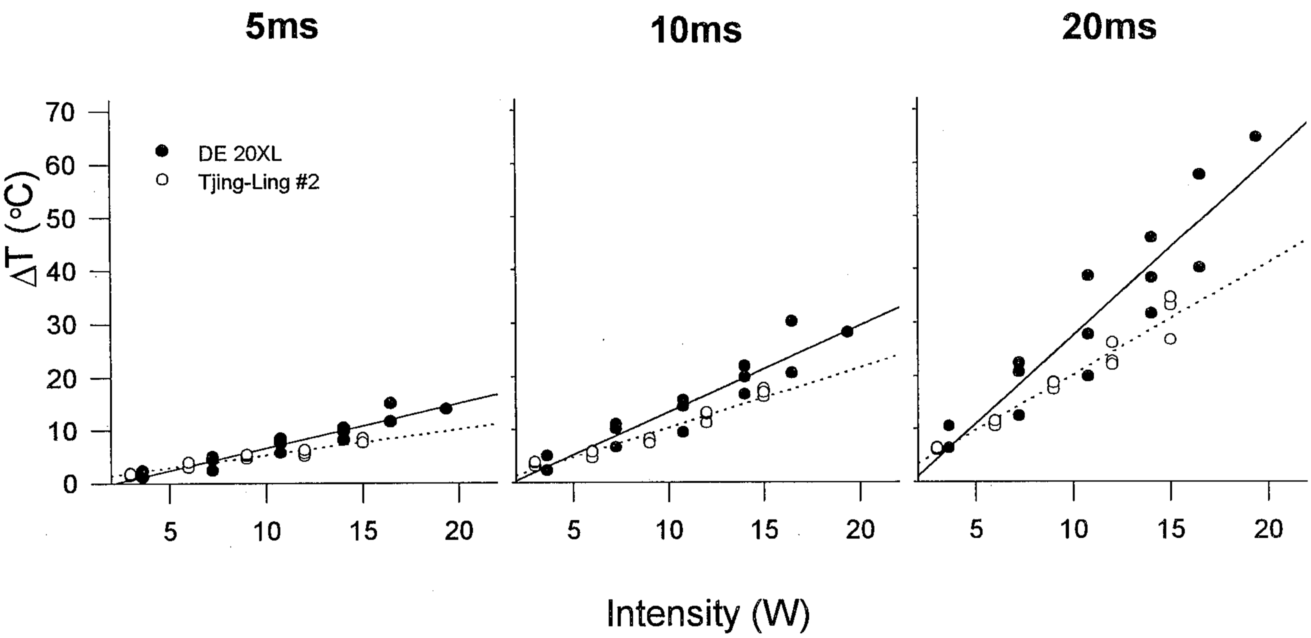

Fig. 4. Maximal surface temperature changes recorded with a thermocopule. Each point represents the average maximal changes from 5 single laser pulses of a fixed pulse duration and intensity. The solid lines are linear regression curves for DE20XL and the dotted lines are for TL#2.

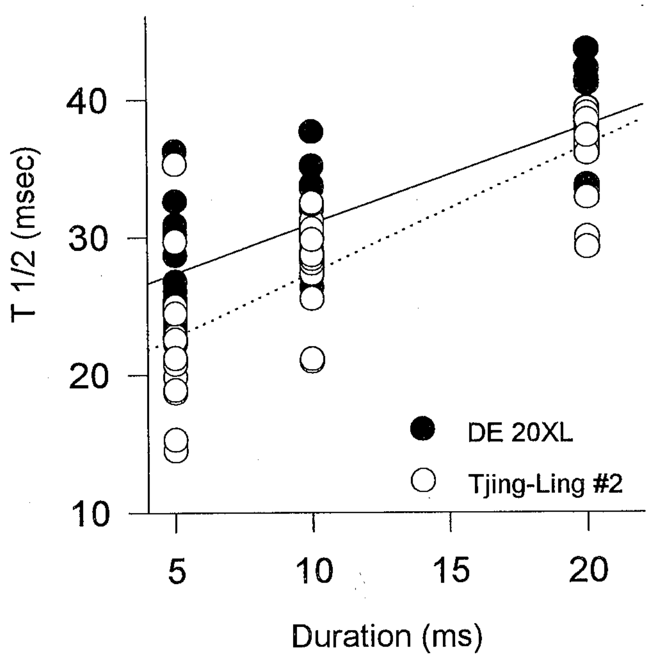

Fig. 5. The rate of rise of surface temperatures (half time to maximal temperature) recorded with a thermocouple. Within each pulse duration, half time from all intensities were polled. There was no significant difference for half times measured at either different durations or different intensities for the two lasers. Note a linear increase of the half time with the increase in pulse duration.

Fig. 6. Surface thermal radiation recorded with an infrared photoconductor (2 to 6 μ wavelength). A single 10 ms, 12W laser pulse was directed to the thermal sensitive paper with the input window of the photoconductor aimed perpendicularly to the plane defined by the laser path and its reflection. Arrowpoint to the beginning of the pulse.

places where stronger intensities were needed, the TL#2 was more effective (Fig. 7). Pain behaviors were stronger at comparable intensity with the laser irradiation at the tailbase than at the midtail or the tailtip (Fig. 8). The TL#2 and DE20XL showed no significant difference in their intensity and behavior relationship (Fig. 8). No significant difference was found with regards to the sequence of the machine used. The thresholds and the behavior intensities were not significantly different whether the TL#2 or the DE20XL was used first on the rat.

Both the DE20XL and the TL#2 laser machines were able to elicit cortical potentials. An example is shown in Fig. 9. The principle pattern was a short latency response and a long latency response, which corresponded to A and C fiber activations

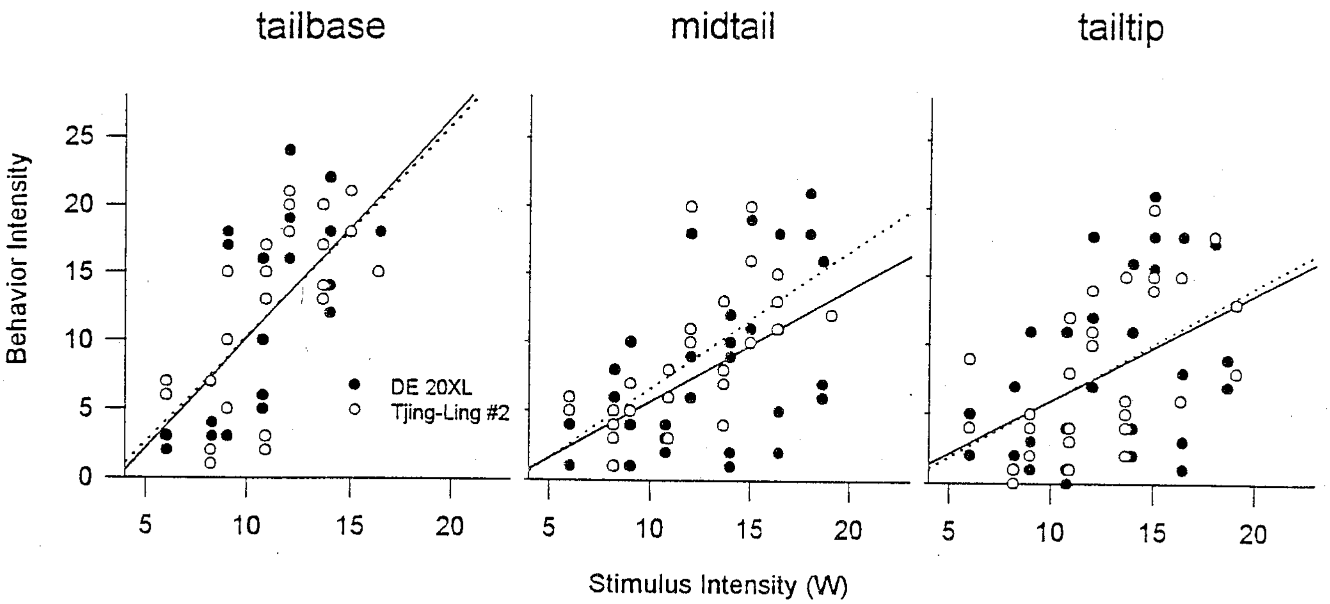

Fig. 8. Behavior intensity (defined in text) elicited by 10 ms single pulse laser irradiation of different intensities. Note the stronger stimulus-behavior responses at the tabilbase. No significant difference was seen with the two laser machines at any of the stimulation sites.

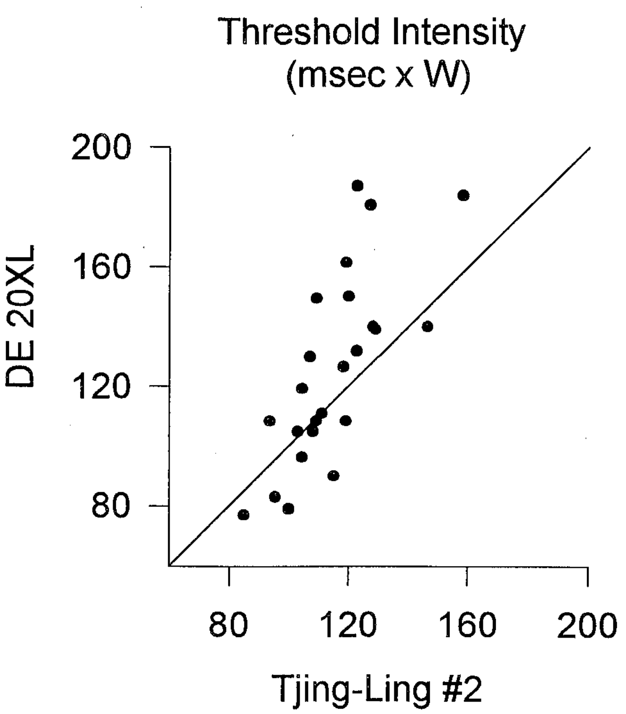

Fig. 7. Comparison of threshold intensities which had a 50% chance to elicit a reflexive behavior in the rat for the two lasers. Each dot represents the matched threshold intensities on the same body part of the same rat for the two lasers. Straight line represents the line of equality. Notice that at low intensities, the threshold intensities of DE20XL were smaller; at high intensities, those of TL#2 were smaller.

respectively (8). DE20XL was tested on 6 rats and TL#2 was tested on 5 rats. No significant difference was found as to the pattern, latency and polarity of the short and long latency responses.

Discussion

The modified Tjing Ling #2 laser could produce similar rapid surface temperature change as the commonly used DE20XL laser system. When directed to the skin of the rat, similar cortical evoked potentials and pain behaviors were produced by both laser systems. Thus, the TL#2 could be used as a tool to activate cutaneous thermal nociceptors synchronously and to study the pain mechanism of the rat.

The TL#2 laser system has one major difference compare to the DE20XL. The beam profile of TL#2 is a Gaussian distribution, i.e., the beam is most intense in the center. As the intensity goes up, the temperature of the center of the beam rises rapidly. This might explain the low pain threshold to this machine at higher intensities. Also the very small center might have made it difficult for the small thermocouple to capture the highest temperature rise. Therefore, the slightly lower maximal temperature change compared to the DE20XL should not have any physiological importance. This most intense center point does have a serious consequence, however, care must be taken not to use too high an intensity on human or animal subjects, less permanent damage of the skin may be caused.

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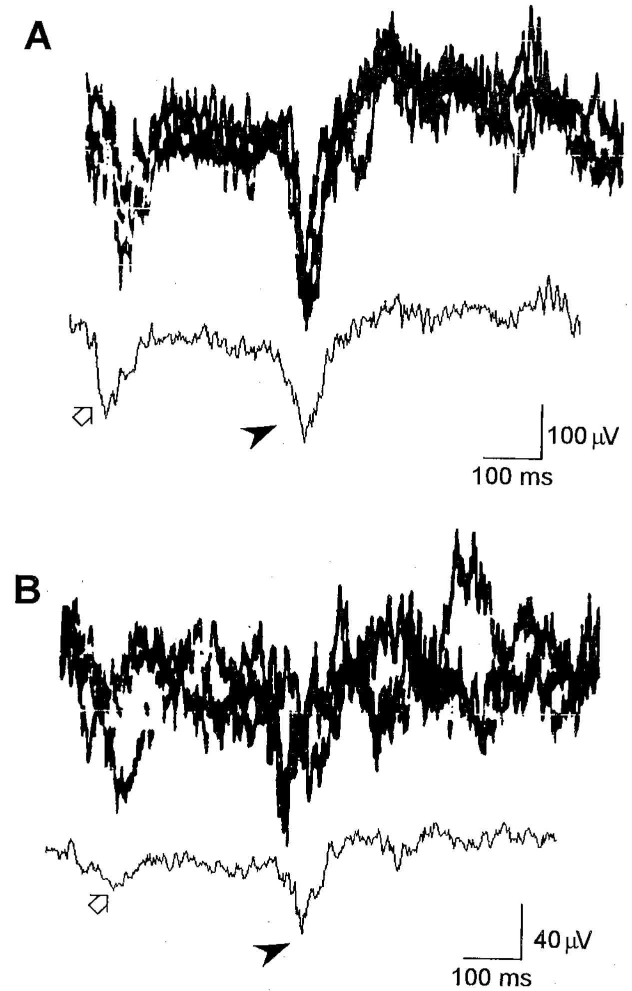

Fig. 9. Cortical evoked potential elicited by 15W, 10ms single laser pulse directed to the midtail. Laser shots were given at the beginning of the traces. A: TL#2. B: DE20XL. Within each figure, the top trace was superimposed original traces (three), and the lower trace was the averaged curve from 10 original traces. Note in both figures there were an early response (open arrows) and a late response (arrowheads).

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