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Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 2000, Vol. 24, pp. 385–395

0278-5846/00/$–see front matter

PII: S0278-5846(99)00106-2

PAIN THRESHOLD CHANGES FOLLOWING ACUPUNCTURE, MEASURED WITH CUTANEOUS ARGON LASER AND ELECTRICAL TOOTH PULP STIMULATION, A COMPARATIVE STUDY

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(Final form, March 2000)

Abstract

Olausson, Bengt and Johan Sagvik: Pain Threshold Changes Following Acupuncture, Measured with Cutaneous Argon Laser and Electrical Tooth Pulp Stimulation, a Comparative Study. Prog. Neuro-Psychopharmacol. & Biol.Psychiat. 2000, 24, pp. 385–395. ©2000 Elsevier Science Inc.

1. The present study compared the argon laser technique for pain threshold measurements with the previously often used threshold measurements with electrical tooth pulp (TP) stimulation.
2. Conditioning stimuli with acupuncture was given since it previously has been shown to induce pain threshold changes. Acupuncture needles were inserted in the hands and arms and electrically stimulated.
3. The experiments were performed in a single blind fashion comparing pain threshold effects after either acupuncture or placebo. Pain threshold measurements with a method of limits were used with both electrical TP and the argon laser method.
4. In comparison to placebo the pain thresholds increased after acupuncture, both on the face and on the hand, with the argon laser method, while the TP thresholds were not influenced. No significant pain threshold change was detected with either the argon laser or the TP method following placebo treatment.
5. The results indicate that the argon laser used for sensory testing with a method of limits is a useful method for pain threshold measurements. The difference in pain threshold effects induced by acupuncture measured with the argon laser and the TP stimulation, are discussed and related to mechanisms that may differ between the two types of pain threshold measurements.

Keywords: acupuncture; argon laser; nociceptive; pain threshold; tooth pulp

Abbreviations: tooth pulp (TP),

Introduction

The laser technique with its ability to specifically activate heat sensitive nociceptors is a promising tool in psychophysical pain threshold measurements (Bromm et al. 1984, Arendt-Nielsen and Bjerring 1988a,b). Both the CO_2 laser (Mor and Carmon 1975) and the argon laser techniques (Arendt-Nielsen and Bjerring

1988a,b) have been used for objective as well as subjective pain measurements and the specificity of the former was controlled with microneurography (Bromm et al. 1984). A high specificity of the argon laser method has also been shown in a recent study (Olausson 1998). The advantage with the CO_2 laser is a low reflection of light from the skin (Bergeron et al. 1981), while a disadvantage is that the infrared light is absorbed in superficial skin layers (Cummins and Nauenberg 1983) with a high risk of inducing tissue damage. On the contrary the argon laser light penetrates deeper into the skin (Parrish and Anderson 1983,Cummins and Nauenberg 1983) with less risk of inducing burns. Disadvantages with the argon laser are the high percent of reflection from the skin (Parrish and Anderson 1983, Arendt-Nielsen and Bjerring 1988b) and its inability to activate nociceptors in dark skin.

Psychophysical pain threshold measurements with argon laser have used the forced choice method (Pertovaara et al. 1988, Arendt-Nielsen et al. 1991, Brennum et al. 1993, Van Der Burght et al. 1994), which has been regarded as more reliable than the method of limits. Recent studies of thermal and mechanical thresholds (Levy et al.1989, Bertelsmann et al. 1994) which compare the methods of limits and the forced choice method, indicate that they are equally reliable but the method of limits is faster to perform.

Electrical tooth pulp stimulation has been assumed to only induce a sensation of pain (Sessle 1979). Methodological problems with high variability in the electrical TP thresholds as well as dependence of healthy teeth limit the use of the method, especially in elderly patients (Widerström et al. 1993a). Since electrical stimulation is not a natural stimuli criticism of the TP pain threshold measurements has been raised (Sessle 1979, Matthews 1979). The assumption that TP afferents mediate pure nociceptive information has also been questioned based on the prevalence of myelinated fibres from the TP (Matthews 1979).

Analgesic effects induced by acupuncture in healthy subjects (Andersson et al. 1973) and in patients (Widerström et al. 1993b) have been described previously. The impact of placebo effects induced by acupuncture has also been evaluated and available data support specific analgesic effects induced by acupuncture compared to placebo (Haker and Lundeberg 1990).

Methods using natural stimuli for pain threshold measurements are essential for proper evaluation of influences on the nociceptive pathways. The purpose of the present study was to evaluate the argon laser

technique on physiological effects induced by acupuncture, effects that previously has been evaluated with TP pain threshold measurements.

Methods

Subjects: Eight healthy paid volunteers (4 male, 4 female, ages 22-37) were included after informed consent. All subjects were naive to acupuncture treatment. The human ethical committee, at the University of Göteborg approved the study protocol.

Pain Threshold Measurements

Cutaneous Laser Stimulation: An argon laser (LEXEL 95-4 Ion Laser, USA) with 75% of its power in the blue (488.0 nm) and green (514.5 nm) wave length spectra was used. The output energy of the laser beam could be adjusted between 0.5 mW to 4W. The laser beam was transmitted through a fiber-optic cable and passed through a lens system giving a homogenous intensity of light on a 3-mm diameter large skin area. Square wave light pulses with duration of 100ms were given with a frequency of 5 Hz. The amplitude of the pulses were controlled by a computer to generate four different linear ramps of stimuli starting from 0 and rising up to 100% of the maximal laser intensity. The rise times of the ramps were pre-set to either 25,30,35 or 40 s. At each skin location one of each rise time was tested once and the sequence of the different rise times were selected by the computer in a random order. The individual ramp stimuli were started randomly within a time interval of 5-15 seconds from the previous stimuli. The subjects were asked to interrupt the laser pulse with a handhold stop button as soon as they detected the first sensations of pain. They were also instructed to ignore other sensations than pain. In order to produce similar conditions during the whole experimental procedure a tone with a constant loudness appeared 5-15 s before the ramps were started. The subjects were informed that the painful stimulus could be expected any time as long as the summer tone was on. After the experimental session a calibration of the energy output from the laser was made with a calorimetric devise (Ophir, Israel).

Tooth Pulp Stimulation: The electrical stimuli as delivered through a handhold coal rubber electrode (cathode, 2X2 mm) which was applied on a healthy incisor of the subject. Care was taken to prevent moisture to spread the current to the gingiva. The same pressure and the same spot on the tooth were used at the different measurements. A constant current stimulator delivered 2.2ms long unipolar pulses with a frequency of 5,5 Hz. The current of the pulses (0-100uA) was controlled from the computer, giving ramps with different rise times and in random order (same as the cutaneous laser stimulation). Each set of

measurement consisted of 4 ramps starting randomly 5-15 seconds after the electrode was applied on the tooth. The subject was instructed to indicate the first sensation perceived (normally reported as a tingling sensation) by breaking the current with a hand held switch (anode).

Acupuncture Treatment

Two different types of treatments (Type 1 and 2) were given in random order (randomisation from envelopes) to each subject.

Type 1 Treatment. (Acupuncture): Four sterile stainless steel electrodes (Hwato) 30 mm long and with a diameter of 0.3mm were used. The needles were inserted bilaterally, perpendicular through the skin to a depth of approximately 8-10 mm, into two traditional acupuncture points Li 4 (hand) and Li 11 (elbow). The location of Li 4 is in the muscle of interosseus dorsalis I, at its highest point above the midpoint of the second metacarpal bone. The location of Li 11 is in the brachioradial muscle in the middle of a line drawn from the lateral epicondyle and the medial border of the brachioradial muscle at the elbow. After inserting the needles they were rotated and until the subject reported a cramp-like sensation from the region of the needles (in traditional Chinese medicine described as a so-called Chi-sensation, reported as deep unpleasant but not painful). From each pair of needles (Li4-Li11) electrical cables were connected to a constant current pulse generator (Electronic Acupunctoscope, Model WQ-6F) which delivered bipolar square wave pulses with a frequency of 2 Hz. The current strength (5-10 mA) was adjusted so that the subject reported a strong but not painful sensation from the needle region. During the 30-minute electrical stimulation period the amplitude was controlled every 5 minute and adjusted to induce the maximal stimulation possible without the subject reporting any unpleasant sensations. A loudspeaker was connected to the electrical stimulator, which delivered a clicking sound with the same rate as the one given to the needles. After 30 minutes the electrical stimulation was reduced to 0, the loudspeaker turned off and the needles removed.

Type II Treatment (Placebo): The aim of this treatment was to give as little stimuli to the tissue as possible at the same time as the subject received needles through the skin with a belief of a real acupuncture treatment. Short and thin sterile stainless steel needles were chosen (Hwatu, 15 mm long and 0.25 mm in diameter). The needles were inserted subcutaneously above the Li 4 and Li 11 points bilateral. Most subjects did not feel the insertion of the needles. The needles were connected to the electrical stimulator in the same way as in the Type I experiment but no electrical stimulation was given to the

needles. The loudspeaker in the stimulator delivered the same click sound as in the Type I experiment (same frequency and loudness). The loudspeaker was turned off and the needles removed after 30 minutes.

Experimental Procedure: Each subject participated in two sessions at the same time of the day on both occasions, each lasting about 2.5 hours and separated by at least 48 hours. The subjects were informed that the intention of the experiments was to test two types of acupuncture treatment, and compare their effects on the dental and cutaneous heat pain thresholds.

Subjects were supine on a comfortable bed. To prevent damage to the eyes from the argon laser, all people in the investigation room wore protection glasses during the experiment. Thermocouples (Exacon thermometer, MC 9200) were applied on the skin on the left cheek and hand, for continuos temperature monitoring. An infrared lamp was used to prevent the skin temperature to fall below 32 degree Celsius. The temperature range was 32-35 degree Celsius.

To familiarise the subjects with the test procedures at the first occasion, TP-thresholds were measured on an incisor on the right side. The laser thresholds were also tested on the skin on the right hand and on the right cheek.

After the learning procedure the first session started with TP-threshold measurements on an incisor on the left side, which was used for all tests. The laser stimulation was given within a 4 x 4-cm area on the dorsal side of the left hand (non-glabrous skin). Thereafter the laser stimulation was given within a 3 x 3-cm large area on the left cheek. The order of the measurements was preserved and repeated with 15 minutes interval. Great care was taken to avoid the same skin spot to be stimulated twice in each set of measurement.

After the initial 3 sets of measurements (each set took 5-6 minutes) the type I or type II treatment began. The subjects received either the type I or type II stimulation as first treatment in a single blind fashion. The same information of presumed effects of the treatment was given at both occasions.

When the needles had been removed, 4 sets of threshold measurements were executed with 15 minutes interval.

Data Analysis and Statistical Methods: The threshold measurements of the tooth pulp and the laser stimulation were fed on line into a microcomputer (PC 386). Students t-test was used to compare the

results after treatment with the preceding control values. In the analysis adjustments were made for multiple comparisons according to the Bonferroni method. In an analysis of variance (MANOVA) for repeated measurements, the data following the different treatments (type I and the type II) including the different methods (TP and laser) at the four different time intervals, were included. The analysis was then focused on contrast such as; acupuncture/placebo and TP/laser. Differences with p values below 0,05 were considered significant.

Results

No objective or subjective side effects following the laser heat threshold testing were reported by the subjects. Tooth pulp stimulation induced a tingling cold after sensation from the stimulated left tooth in a few subjects, a sensation that disappeared spontaneously within a day. The skin temperature was in all experiments at levels above 32 but not exceeding 35 degree Celsius. No significant change in skin temperature, in the face or on the hand, could be detected during the test period, after either acupuncture or placebo.

Heat Pain Thresholds: In relation to the preceding control a significant increase in heat pain thresholds was detected on the hand after acupuncture but not after placebo treatment (Fig 1a). In a multivariance analysis (MANOVA) the pain threshold increase following acupuncture was significantly higher compared to placebo treatment ($p=0.026$). No significant effect on heat pain thresholds measured at the cheek, was detected after either acupuncture or placebo treatment, compared to the control (Fig 1b). However, the multivariance analysis showed significantly higher pain thresholds on the cheek were found following acupuncture compared to placebo treatment (MANOVA, $p=0.048$).

Tooth Pulp Thresholds: No significant change in electrical TP-thresholds was detected following either acupuncture or placebo treatment compared to the control (Fig 1c). No significant difference in TP-thresholds was detected between acupuncture and placebo in the mutivariance analysis (MANOVA, $p=0.178$).

Comparison of Effects on Heat and Tooth Pulp Thresholds: A significant difference following acupuncture was detected, comparing TP-pain thresholds with heat pain thresholds on the cheek (MANOVA, $p<0.001$).

No significant difference was detected in laser effects between hands and cheeks (MANOVA, p=0.889). Placebo treatment did not induce any significant effects.

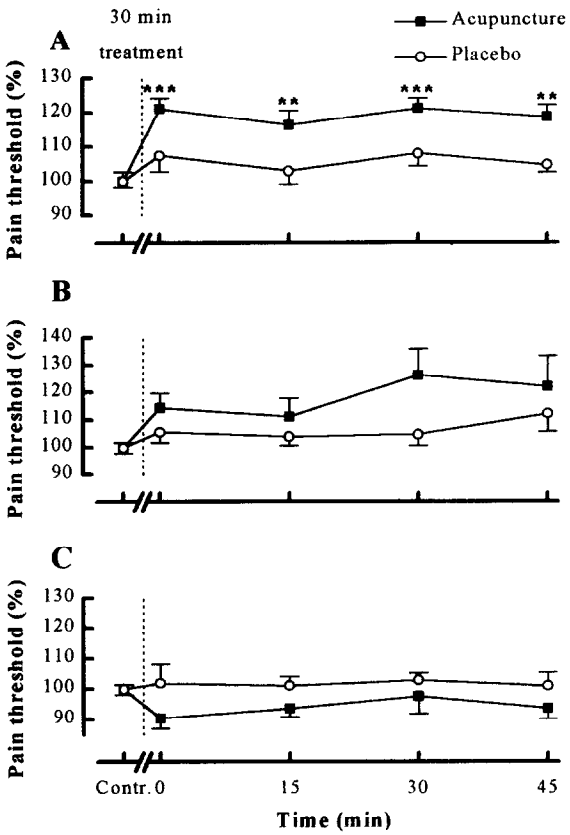

Fig. 1 Relative pain threshold changes following 30 minutes of preceding acupuncture or placebo stimulation. The threshold values are presented as means and standard error of the mean. Significance levels (t-test) indicate differences compared to control with adjustments for multiple comparisons according to Bonferroni (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Pain threshold effects measured with argon laser on the left hand (A) and on the left cheek (B) after acupuncture (filled squares) and placebo (circles). A significant increase in pain thresholds was detected at the hand but not in the face following acupuncture. No significant effects were induced by placebo. Pain thresholds measured with electrical TP stimulation (C) showed no significant change following neither acupuncture nor placebo.

In the overall statistical analysis (MANOVA) by looking at the method, treatment and time (Table I), highly significant differences were found for the method ($p<0.001$) and method/treatment ($p=0.022$) factors. This indicates that the effects measured by the different pain threshold measurement methods (TP/laser) could be separated and that they showed different effects depending on the treatment (acupuncture/placebo). No significant difference was found for the time factor ($p=0.051$), indicating that the time after ending treatment had less importance to the overall effects.

Table 1

Effect	F-value	P-level
Method	8,56	0.00076
Treatment	2.94	0.94
Time	2.67	0.051
Method/Treatment	4.20	0.022
Method/Time	1.10	0.36
Treatment/Time	0.97	0.41
Method/Treatment/Time	0.56	0.76

A 2-Way MANOVA of Repeated Measurements with 4 Dependent Variables. Significant differences were found for the method and for method/treatment. See text above for further information

Discussion

Increased pain thresholds was in this study detected with the argon laser but not with the TP method following acupuncture. Pain thresholds (with laser) were most elevated segmentally but also extra segmentally following acupuncture compared to placebo. Factors related to anatomical differences in stimulating and recording sites must be considered, since previous studies by Olausson and coworkers (1986) show significantly elevated TP pain threshold following stimulation in hands and arms. The thresholds were further elevated when the stimulation was also given to the face, which may be one of the reasons why threshold changes was not detected with the TP method in this study.

Physiological Effects: Since the pain threshold methods described use different physiological substrates, the diverging results may be related to different peripheral sites of action for the electrical and heat stimulation. The action of the heat stimuli mediated by the cutaneous heat sensitive nociceptors, while the action of the electrical stimulus could be either via the receptor endings or at the distal axon. If the electrical stimuli are presumed to activate the distal axons, the difference in results between heat and

electrical pain threshold measurements reflect their different sites of activating the nociceptive pathway. Alternatively the difference between electrical and heat stimulation is that electrical stimulation presumably activates also large myelinated fibre's. Since these fibres may influence and presumably inhibit transmission in the nociceptive pathway an analgesic effect may be hidden by the effects induced by the test method (Olausson et al. 1994). A third hypothesis is the differences in the central processing of different types of nociceptive signals evoked by either heat or electrical stimulation since analgesic effects induced by acupuncture have been proposed to have a central origin (Andersson and Lundeberg 1995).

Placebo Treatment: Studies of acupuncture effects have an inherent problem of finding a proper placebo treatment group. In this investigation the study design used are previously described by Haker and Lundeberg (1990). Their study showed a significant difference between their active treatment and placebo group. However, this design can be criticised since the placebo type of stimulation theoretically can activate afferent fibres with the risk of influencing the nociceptive pathway. In favour of a proper study design speaks the lack of pain threshold changes following placebo treatment.

Effects of Stress: No effects on TP pain thresholds were detected after acupuncture in this study. Similar effects have previously been described by Widerström and co-workers (1993b) who reported that so called "responders to acupuncture" had their increased TP thresholds reversed if subjected to mental stress simultaneous to acupuncture. The results from other studies have varied, with a majority showing an increase (Andersson et al. 1973,Mayer et al.1977, Widerström et al.1993b, Chapman et al. 1983) while others showing no change in the TP pain threshold following acupuncture (Widerström et al. 1993)

Conclusions

This study show that the argon laser technique coupled with the method of limits, could in a safe and reproducible way measure increased pain thresholds both intra and extra-segmantally to the sites of the conditioning stimuli. The results are contradictory with increased pain thresholds measured with argon laser in and outside the stimulated segments and uninfluenced pain thresholds measured by electrical TP stimulation (extra-segmentally), following acupuncture. Hypothesis has been proposed but with the results from the present study no distinction between a peripheral and a central origin of the diverging results can be done.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (project no. 11009), the Swedish Society of Medicine, Greta and Einar Askers foundation, the Swedish Royal Academy of Science and Tore Nilsons foundation. We thank Mr Tomas Karlsson and Mr Göran Pegenius for technical assistance and Drs Sven Andersson, Mikael Elam, Ulf Norrsell and Gunnar Wallin for helpful comments on the manuscript.

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