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Research Article

Inhibitory Effects of 658 nm Laser Irradiation on Skin Temperature in Anesthetized Rats: Preliminary Results from a Controlled Study

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Red laser light stimulation can have many physiological effects. The goal of this animal experimental study was to investigate how red laser stimulation influences the temperature of anesthetized rats at different acupuncture points and nonacupoints. For that reason 12 adult male Wistar Han rats (300–380 g) were investigated. Six anesthetized rats underwent red laser stimulation (wavelength 658 nm, output power 40 mW, diameter 500 μm, and duration 10 min) at the Baihui (GV20) acupoint, the Zusanli acupoint (ST36, bilateral), and a control point on the forelimb. The other six rats underwent the same procedure; however, the laser remained switched off. Significant decreases in temperature were found at the acupoints Baihui, Zusanli left, and Zusanli right. In addition there was no significant temperature effect at a control point. During placebo laser irradiation (deactivated laser) there were also significant temperature changes. The mechanism underlying the results is currently unknown, but brain stimulation (via laser or mechanical pressure) and mainly direct central mechanisms may be responsible for the local and peripheral temperature decrease.

1. Introduction

Laser acupuncture is a trendsetting alternative method to the stimulation with metal needles. Many basic studies on this topic are available; however, there is still a lack of details concerning basic principles and mechanisms. It is also well-known that different kinds of lasers can produce different effects on skin temperature. For example, violet laser stimulation with a wavelength of 405 nm is able to produce significant increases in skin microcirculation and surface temperature [1]. Temperature can increase from 33.9°C to 36.6°C within 10 minutes in healthy volunteers using a laserneedle with a violet wavelength, 110 mW output power, and 500 μm diameter [1].

The research team at the Medical University of Graz has performed several animal experimental studies assessing the effects of laser acupuncture on basic physiologic parameters [1]; however, up till now the possible effects of laser acupuncture on skin temperature have not been given the necessary attention. A search for "laser acupuncture" and "rat skin temperature" in the most important scientific database PubMed yields only three scientific papers [2–4].

The goal of the present study was to investigate how red laser stimulation influences the skin temperature of anesthetized rats at different acupuncture points in comparison to a control point and to placebo stimulation.

2. Materials and Methods

2.1. Animals. Twelve adult male Wistar Han rats (300–380 g) were purchased from Harlan Laboratories (AN Venray,

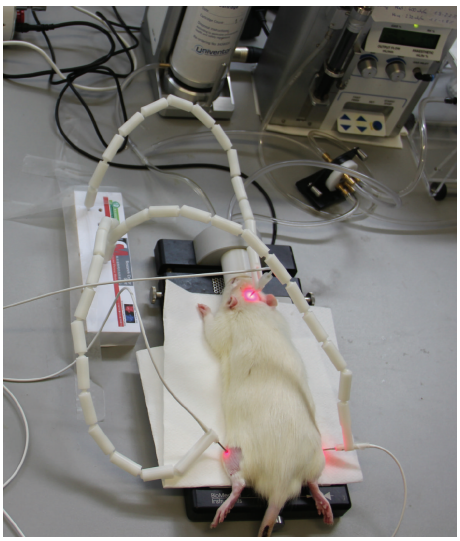

Figure 1: Anesthetic procedure and laser stimulation.

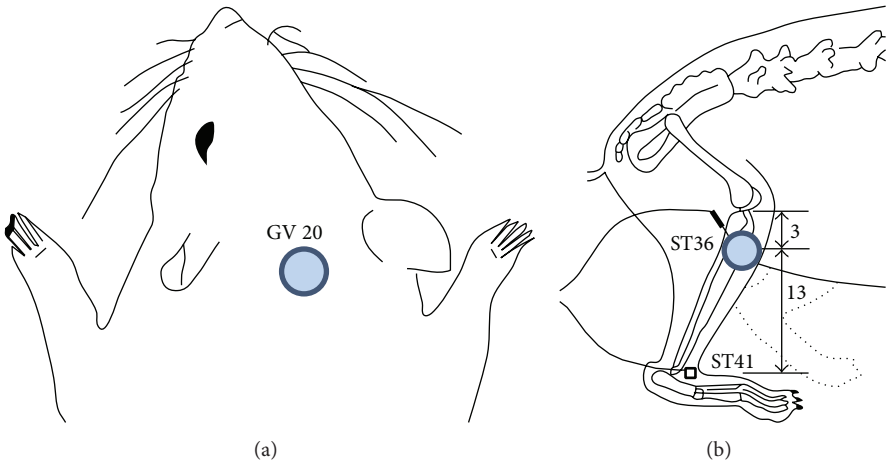

Figure 2: Schematic diagram of the stimulated acupoints.

Netherlands) and allowed to habituate to the new housing conditions for two weeks. The rats were housed in groups of 4 in standard Macrolon cages (Eurostandard Type IV) in an environmentally controlled colony room with a 12/12 h light-dark cycle. The lights were switched on at 8:00 AM. Food and water were available *ad libitum*. All experiments were performed during the light phase. Before the experiment started, the rats were shaved in the areas around the acupoints and the control point. All noninvasive measurements were conducted in accordance with the ethical guidelines for the care and use of laboratory animals.

2.2. Anesthesia. The rats were anesthetized throughout the laser acupuncture session. For the induction of anesthesia, the animals were placed into a plexiglass chamber flooded with 4% isoflurane for 90–120 seconds. Subsequently, the rats were removed and placed prone while anesthesia was maintained through a nose cone with 2% isoflurane (see Figure 1).

2.3. Laser Acupuncture and Acupoints. Red laser stimulation was performed in 6 rats at the acupuncture points Baihui (GV20) and Zusanli (ST36, bilateral) (see Figure 2). In addition, a nonstimulated control point (on the right forelimb) was also used for temperature evaluation. Placebo control (deactivated laser) was also performed in 6 rats. During this procedure, the laser needles were placed on GV20 and bilateral ST36, but the laser remained switched off. The laserneedle stimulation system (Laserneedle GmbH, Berlin, Germany) allows for the continuous stimulation of one or more acupuncture points on the body of the animal or human being. In order to reduce the time span during which the rat is anesthetized, we investigated the different acupoints simultaneously. In this investigation, laser irradiation of 658 nm and 55 mW laser diodes was coupled into an optical fiber, and the laserneedle was arranged at the distal end of this fiber. Due to coupling losses, the output power of the laserneedles was reduced to 40 mW. The fiber core used in this study was about 500 μm in diameter. A continuous

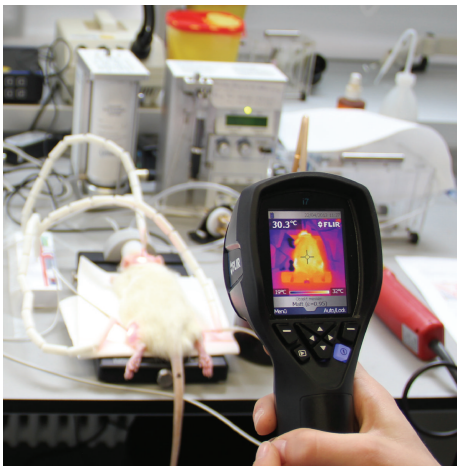

Figure 3: Temperature measurements using a Flir i7 infrared thermocamera.

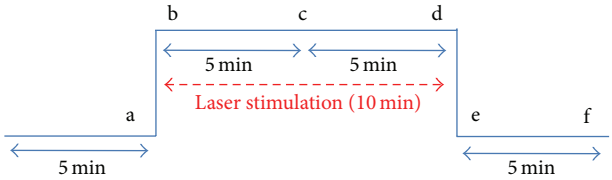

Figure 4: Experimental protocol.

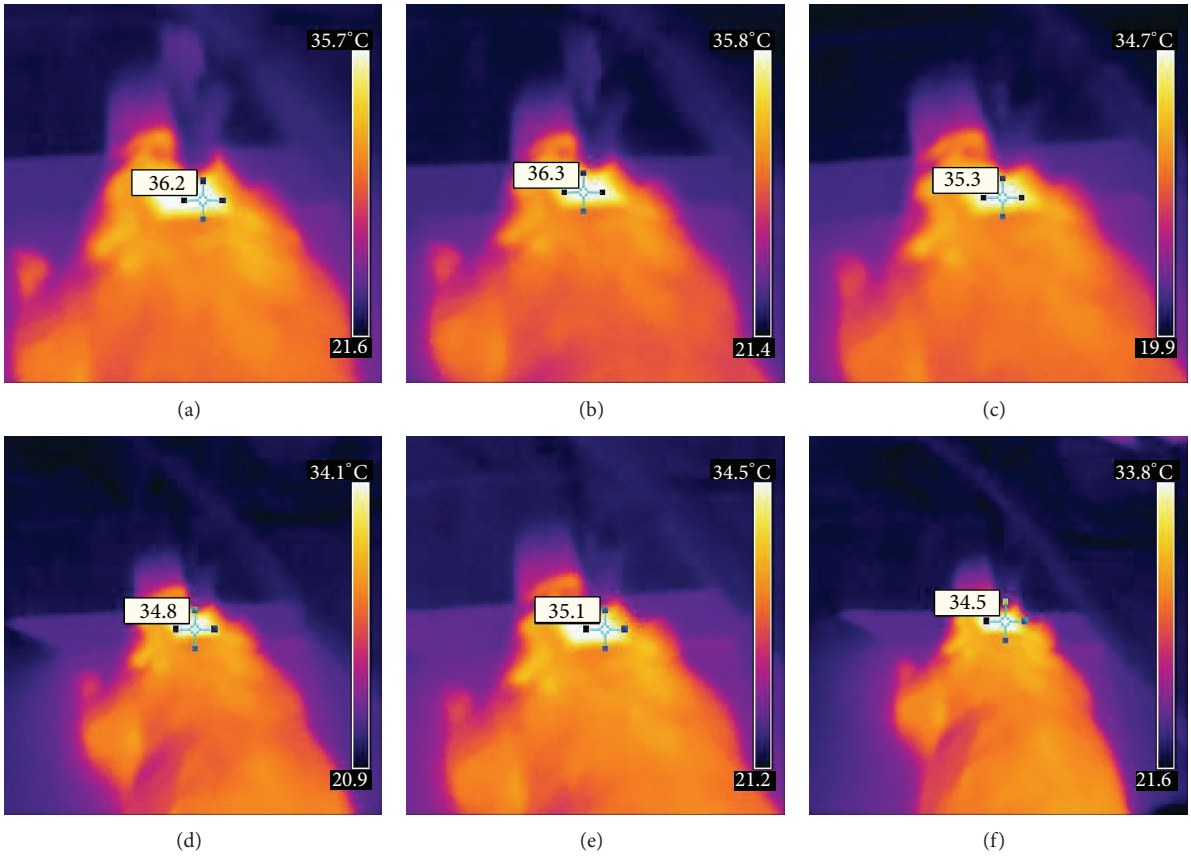

Figure 5: Six thermal images from an anesthetized rat before (a), during ((b), (c), and (d)), and after ((e) and (f)) red light stimulation at the Baihui acupoint. Note the decrease of the temperature during and after red light irradiation.

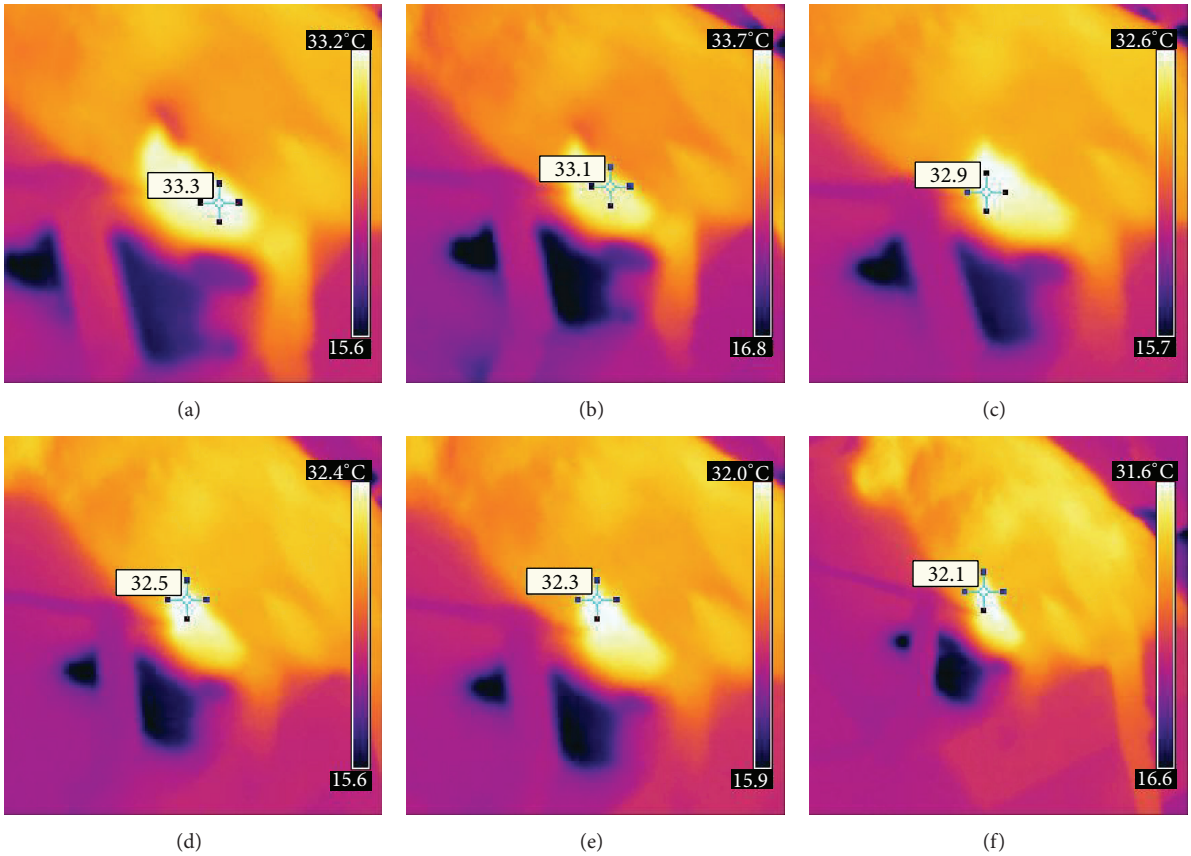

Figure 6: Six thermal images from an anesthetized rat before (a), during ((b)–(d)), and after ((e) and (f)) red light stimulation at the left Zusanli acupoint. Note the decrease of the temperature during and after red light irradiation.

wave mode was used, and the duration of the stimulation was 10 min. The method is described in detail in previous publications [1].

2.4. Temperature Measurements. The measurements of the skin temperature were performed using a Flir i7 (Flir Systems, Wilsonville, USA) infrared camera which operates at a wavelength range from 7.5 to 13 μm. The focal distance of the infrared lens is $f = 6.8$ mm. The temperature measurement range is between −20°C and +250°C. Its accuracy lies at ±2% of the reading. Sensitivity is <0.1°C at 30°C, and the infrared resolution is 140 × 140 pixels. The system is ready for use in 15–20 seconds (see Figure 3).

2.5. Procedure. All experiments took place during daytime (between 09:00 and 14:00; room temperature: ~21°C). Every rat completed the investigation. The rats were anesthetized throughout the laser acupuncture session with isoflurane (see Section 2.2). At the end of a five-minute resting phase which served as baseline condition, the first thermal image (a) was taken (see Figure 4). Immediately after stimulation onset, the next thermogram was stored (b). After 5 min of laser irradiation the next picture (c) was analyzed. At the end of the following 5 min of laser irradiation (d) and immediately after the laser had been switched off (e), two images were taken. At the end of the entire 20 min of experimental procedure, the last picture (f) was taken. After reversal of anesthesia, all rats were replaced into their cages.

2.6. Statistical Analysis. Data were analyzed using SigmaPlot 12.0 software (Systat Software Inc., Chicago, USA). Testing was performed with one-way repeated measures ANOVA. Post hoc analysis was performed with Tukey's test. The data are graphically presented as mean ± SE (standard error). The criterion for significance was $P < 0.05$.

3. Results

Typical examples of the results of thermal imaging are shown in Figures 5, 6, and 7. The examples demonstrate anesthetized rats. The room temperature was ~21°C. After red light stimulation the temperature at the Baihui acupoint (marker in Figure 5) decreased from 36.2°C to 34.5°C during the experimental procedure (compare Figure 4). Also at the Zusanli acupoint (left and right) the temperature declined during and after red light irradiation, but not to the same extent as at the Baihui acupoint. At the left Zusanli acupoint the temperature decreased from 33.3°C to 32.1°C, and at the right Zusanli acupoint the temperature dropped from 34.2°C to 33.4°C.

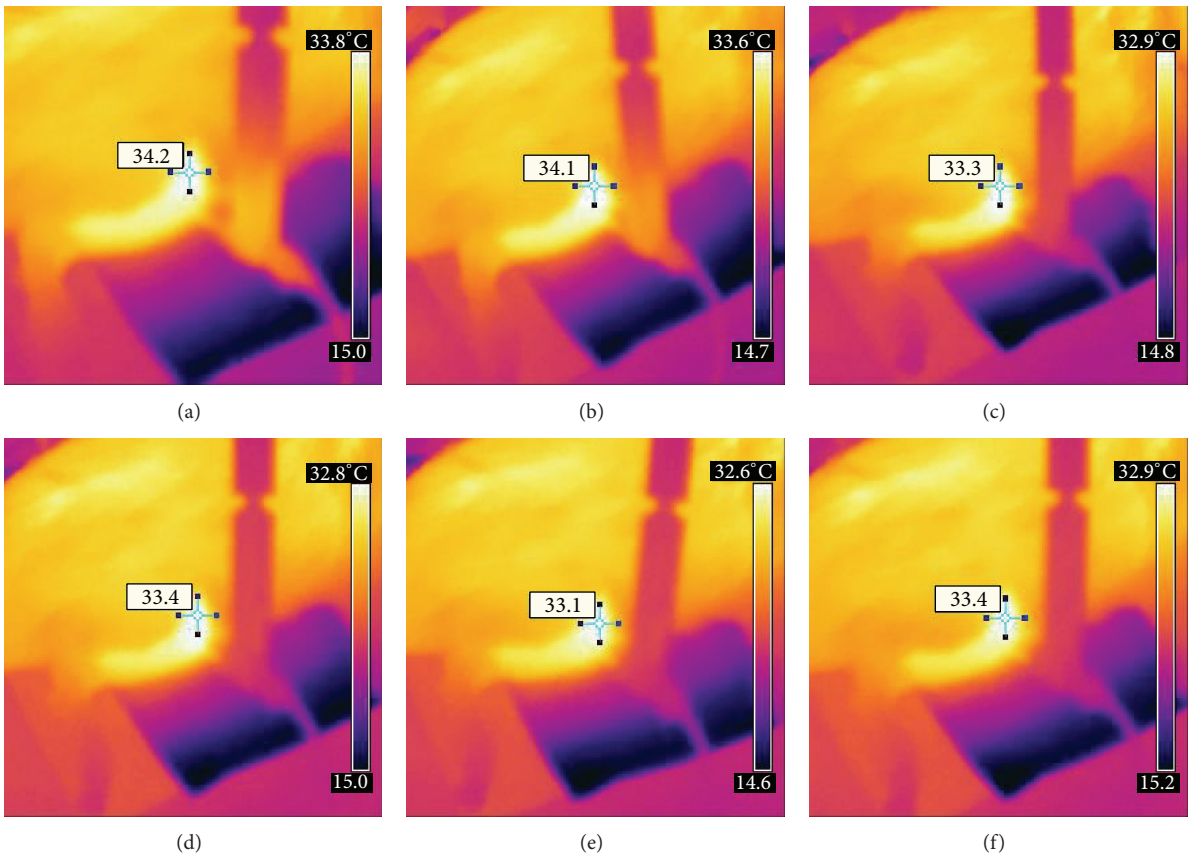

Figure 7: Six thermal images from an anesthetized rat before (a), during ((b)–(d)), and after ((e) and (f)) red light stimulation at the right Zusanli acupoint. Note the decrease of the temperature during and after red light irradiation.

Figure 8 summarizes the data extracted from thermal images of all 6 rats with red laser light stimulation. The skin temperature decreased at all acupoints, with a maximum at the Baihui point ($P < 0.003$). At the control point on the forelimb (non-meridian point), the decrease was not significant.

Figure 9 shows data extracted from the thermal images without laser stimulation (placebo control). No significant changes were found at the control point.

4. Discussion

The present study examined the effects of laser irradiation on two acupuncture points on skin temperature in laboratory rats.

Already in 1895, Pembrey [5] stated that the normal rectal temperature of adult rats is about 37.5°C. MacLeod [6] also used a mercury thermometer for measuring the temperature in rats, and he found out that the mean value is about 37.9°C. In further investigations, Gudjonsson [7] reported that the body temperature of adult rats is rather unstable, but that normally it lies midway between 37 and 38°C. Since rat models are an important tool in research on different diseases and modern infrared thermal imaging methods open up new insights into the changes of superficial temperature, several investigations on this topic were performed [8]. Scientific literature concerning temperature investigations during laser acupuncture in animal experimental studies is very rare. In 2010 the research team at the Medical University of Graz investigated the effects of red laser light stimulation in pigs [9]. Partly placebo-controlled, randomized, crossover animal experimental pilot studies showed that needling, as well as laser stimulation of the Yintang acupoint and other points, can induce effects on parameters of bioelectrical brain activity, whereas the stimulation of control points did not yield significant changes. This is in accordance with studies carried out in humans [10, 11]. In humans, the Yintang acupoint is located between the medial ends of the two eyebrows at the root of the nose.

In our present study in rats we also stimulated an acupuncture point located on the head (Baihui, GV20) and two points on the hind limbs (Zusanli, ST 36, bilateral). This stimulation scheme was found to decrease skin temperature in the local areas around the acupoints. At a control point no significant temperature effects could be found. During placebo stimulation (deactivated laser), some significant changes could be detected. The reason for these changes could be the mechanical stimulus applied via the laserneedle. At the moment the mechanisms underlying these interesting results are still unknown, but brain stimulation and central mechanisms may be involved in the local and peripheral

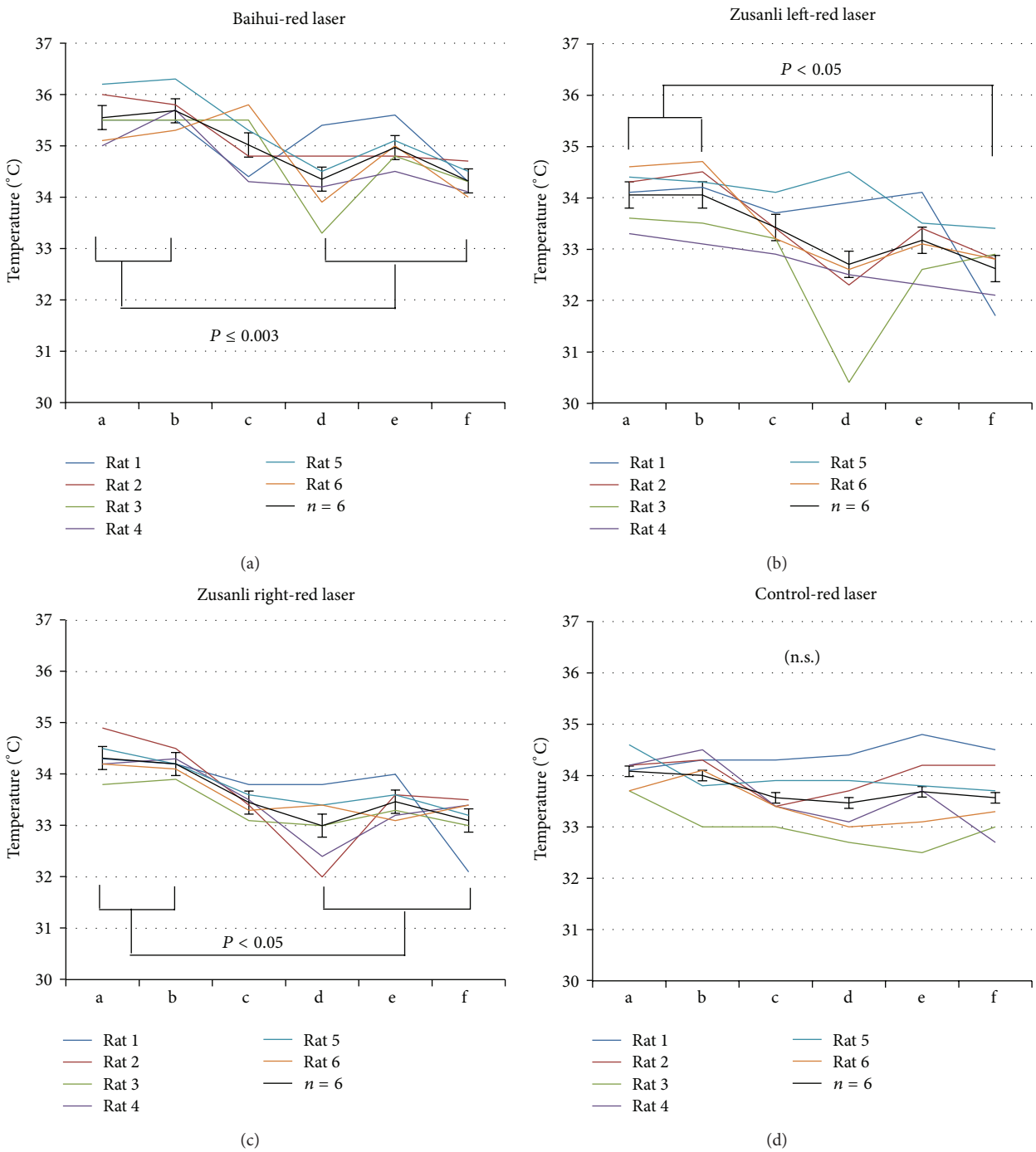

FIGURE 8: Temperature values of all rats during the different phases (a–f, see Figure 4) of red laser stimulation. Significant changes were found for all acupoints, but not for the control point.

temperature decrease observed in our experiments. As we could demonstrate in a previous study, red laser light with a wavelength of 658 nm is able to penetrate the human skull with a transmission factor of about 0.02 [12], indicating that about 2% of the laser light is able to penetrate the human skull. Using the same wavelength, diameter (500 μm), and a similar output power (~40 mW) we can be sure that a sufficient amount of laser energy stimulates the rat brain. These stimulations (laser light and mechanical pressure) obviously have a direct effect on the temperature at the stimulated skin areas. Further investigations using only one acupoint or direct brain stimulation will be necessary to confirm or refute our present preliminary results.

Surface temperature investigations and changes elicited by laser were also reported by Yen et al. in 1994 [13]; however, these authors used single laser pulse irradiation, and therefore

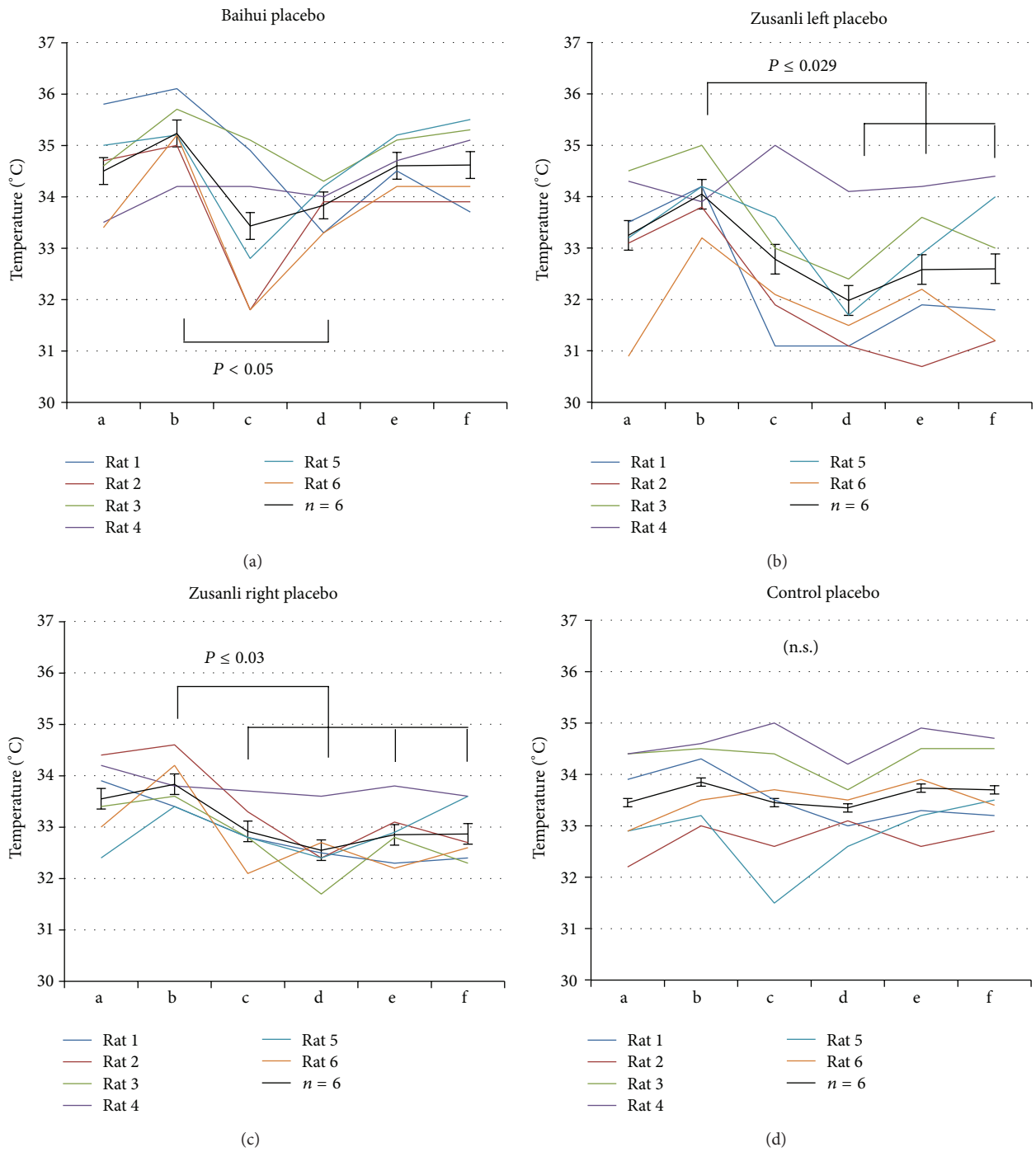

Figure 9: Temperature values of all rats during the different phases (a–f, see Figure 4) of placebo stimulation.

the results cannot be compared with our continuous wave mode. In addition, not only surface stimulation on the rat skull but also direct brain infrared neural stimulation was recently (in 2013) performed by Liljemalm et al. [14]. Due to the different technical parameters in their study, the results also cannot be compared to our present study.

Our study has several limitations. Depending on the type of equipment used, the shaving of the rats might lead to changes in microcirculation and, in consequence, also temperature alterations. To avoid this possible influence in future investigations, fuzzy (nude) rats could be used for whole body skin temperature investigations because this type of rat is essentially hairless and does not require shaving in the regions of interest [15]. In addition, the number of the investigated rats was very small. Nevertheless it is noteworthy that the results reached the level of statistical significance. Furthermore, investigations using other wavelengths, for example, violet laser (405 nm), should be performed and

are already in progress. The preliminary results obtained in three rats show similar trends (decrease in temperature at the acupoints).

On the other hand, standardization of the evaluated parameters in rats has not yet been performed, so results from different studies and different rats cannot be compared. Because so many parameters play a role, the thermal effect should be investigated for each specific application separately [16], as the authors of a paper published in the Journal of Biomedical Optics in 2014 stated.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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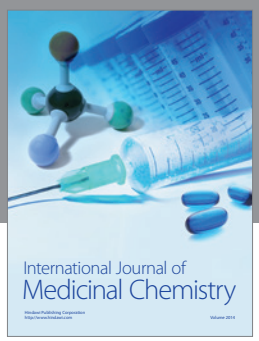

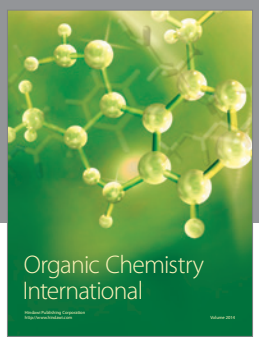

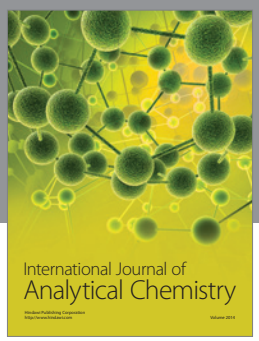

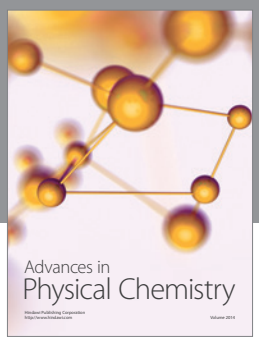

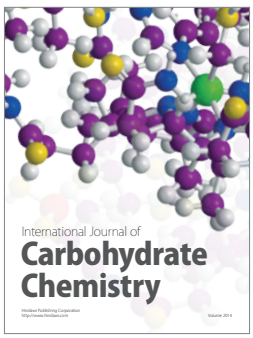

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