Diploma Thesis

NONINVASIVE, LASER-INDUCED ACUPOINT STIMULATION

Continuous and Systematic Investigation of Ear Acupuncture in Addition to Body Acupuncture Using Modern Quantification Methods

Submitted by

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Date of birth: 19.10.1985

In partial fulfillment of the requirements for the degree of

Doktor der gesamten Heilkunde (Dr. med. univ.)

At the

Medical University of Graz, Austria

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February 2013

Declaration in Lieu of an Oath

I herewith declare in lieu of an oath that I have produced the aforementioned thesis independently and without using any other than the aids listed. Any thoughts directly or indirectly taken from somebody else's sources are made discernible as such.

Graz, the 1st of February 2013

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Graz, den 01. Februar 2013

For my parents

Acknowledgments

First of all, I would like to thank Univ.-Prof. Dipl.-Ing. Dr.scient.med. Dr.techn. Gerhard Litscher for making this Diploma Thesis possible and giving me the opportunity to be part of his research team and conduct this study under his supervision. His guidance and helpful advices have helped me throughout the creation of this work. As part of his research team, Mag. Ingrid Gaischek and Dr. Lu Wang have to be mentioned especially, both for supporting my practical examinations and helping me with the realization of the written thesis. Without them some parts of the thesis wouldn't be possible. Thank you.

My sincere gratitude belongs to my parents Heike and Werner and my brother Benjamin, who have always supported me to the fullest and guided me throughout all these years regardless if for better or worse. Without your love my life's journey would not have been possible as it was.

A very special thanks is addressed to my grandmother Irene, my aunt and uncle Brigitte and Uwe and my godmother and her husband Biggi and Christian. You supported me with many donations over all these years and, not less important, with a whole lot of encouragement.

Thank you my love, Bettina, for bearing with me.

Last but not least, I want to thank my colleagues in Graz, for the spontaneous participation in this study and the fun in the last years and my good old friends back home and all over the world, for keeping in touch.

Zusammenfassung

Hintergrund: Hauptziel dieser Diplomarbeit ist die kontinuierliche, systematische Untersuchung von Laserakupunktur an Ohrakupunkten in Ergänzung zu Körperakupunkturpunkten. Lasernadelakupunktur erlaubt die optische Stimulation individueller Akupunkturpunktkombinationen und ermöglicht dadurch Variationen der Ohr- und Körperakupunktur. Effekte auf kardiovaskuläre Parameter wurden spezifiziert.

Methoden: 13 gesunde Probanden wurden mittels eines nicht-invasiven Lasermicroneedle® Systems am Ohrakupunkt Shenmen und an den Körperakupunkten Neiguan und Baihui stimuliert. Es wurden zwei Durchgänge mit jeweils 30 Minuten und Laserstimulation mit 2 Hz und 100 Hz durchgeführt. Zur Quantifizierung der biologischen Parameter wurden Messungen mit dem Arteriographen durchgeführt, die Herzratenvariabilität gemessen und Temperaturunterschiede mittels Thermokamera erfasst und statistisch ausgewertet.

Ergebnisse: Während der 2 Hz Laserstimulation konnte eine signifikante Senkung der Herzfrequenz nachgewiesen werden. Der gleiche Effekt trat gegen Ende des Messzeitraums während der 100 Hz Laserstimulation und gegen Ende der 100 Hz Kontrollphase ($p<0.05$) auf. Das LF (low frequency)/HF (high frequency) Verhältnis zeigt einen signifikanten Unterschied während der 2 Hz Stimulation ($p=0.023$, bzw. $p<0.019$) und einen hoch-signifikanten Unterschied während und nach der 100 Hz Stimulation ($p<0.001$). Es kommt zu einer signifikanten Steigerung der Temperatur am Ohrakupunkt Shenmen während der 100 Hz Stimulation ($p<0.05$). Die Auswertungen des Arteriographen, der Herzratenvariabilität und der Temperaturmessung ergaben keine signifikanten Ergebnisse.

Konklusion: Laserakupunktur mittels verschiedener Frequenzen kann die Herzfrequenz senken und zu einer Änderung des LF/HF Verhältnisses führen. Quan-

tifizierungstechniken stellen eine geeignete Methode dar, um Veränderungen der biologischen Parameter während Laserakupunktur aufzuzeigen.

Abstract

Background: The main goal of this diploma thesis is the continuous and systematic examination of laser acupuncture at ear acupoints in addition to body acupoints. Laser needle acupuncture allows the optical stimulation of individualized acupoint combinations and thereby enables variations of ear and body acupuncture. Effects on cardiovascular parameters were specified.

Methods: 13 healthy volunteers were stimulated with a noninvasive Lasermicroneedle® system at the ear acupoint Shenmen and at the body acupoints Neiguan and Baihui. Two stages with 30 minutes each and laserstimulation with 2 Hz and 100 Hz were implemented. To quantify the biological parameters, measurements with the arteriograph were executed, the heart rate variability was measured and temperature differences were covered and subsequently statistically evaluated.

Results: During the 2 Hz laser stimulation a significant decrease of the heart rate could be verified. The same effect occurred at the end of the period of measurement during the 100 Hz stimulation and at the end of the control phase ($p<0.05$). The LF (low frequency)/HF (high frequency) ratio showed a significant difference during the stimulation with 2 Hz ($p=0.023$ and $p<0.019$, respectively) and a highly significant difference during and after stimulation with 100 Hz ($p<0.001$). A significant increase of the temperature at the ear acupoint Shenmen during stimulation with 100 Hz ($p<0.05$) occurred. The evaluation of the arteriograph data, the data of the heart rate variability and the temperature measurement showed no significant results.

Conclusion: Laser acupuncture with different frequencies is able to decrease the heart rate and can lead to an alteration of the LF/HF ratio. Quantification technics constitute an appropriate method to display verifiable changes of the biological parameters during laser acupuncture.

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List of Abbreviations

AI radial	Augmentation index from radial artery
AIx brachial	Augmentation index from the brachial artery
AP	Pressure augmentation
BP	Blood pressure
BPM	Beats per minute
CA-MRSA	Community-acquired methicillin-resistant staphylococcus aureus
cm	Centimeter
° **C**	Degree centigrade
EA	Electro acupuncture
ECG	Electrocardiogram
e.g.	exempli gratia
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FBF	Finger blood flow
Fig.	Figure
HA-MRSA	Hospital-acquired methicillin-resistant staphylococcus aureus
Hb	Hemoglobin
HRV	Heart rate variability
Hz	Hertz
LASER	Light Amplification by Stimulated Emission of Radiation
LF/HF ratio	Low-frequency/high-frequency ratio

μm	Mikrometer
mW	Milliwatt
nm	Nanometer
NIH	United States National Institute of Health
NO	Nitric oxide
PVT	Paravertebral temperature
PWVao	Aortic pulse wave velocity
SDDVP index	Index of second derivate of digital volume pulse
TCM	Traditional Chinese Medicine
TENS	Transcutaneous electrical nerve stimulation

1 Introduction

1.1 Acupuncture

The traditional practice of acupuncture is based on the hypothesis that all the body's physiological functions are modulated by 12 bilaterally distributed channels (six Yin and six Yang channels), supplemented by two midline channels (one on the front and the other one on the back of the body). Yin and Yang are in polarity to each other which leads to a certain tension. Based on this tension, the hypothetical "Qi", which is the universal vital energy and therefore regulates the body functions, begins to flow. This is one of the fundamental laws of Chinese medicine and Chinese philosophy. Yin and Yang must never be static but related to each other and are therefore supplementary principles. The following aspects are essential for a continuous flow of Qi: Yin and Yang have to be in opposition (which is relative and never absolute), they are mutually dependent (no winter without summer, no day without night, no birth without death), and a shift towards either Yin or Yang leads to a stronger tension between these levels. Furthermore, they can consume each other (water extinguishes fire, day ends night) and they can convert into each other. The ideal state is reached when the mentioned aspects are in a harmonic and dynamic equilibrium (see **Figure 1.1**) [Focks 2010].

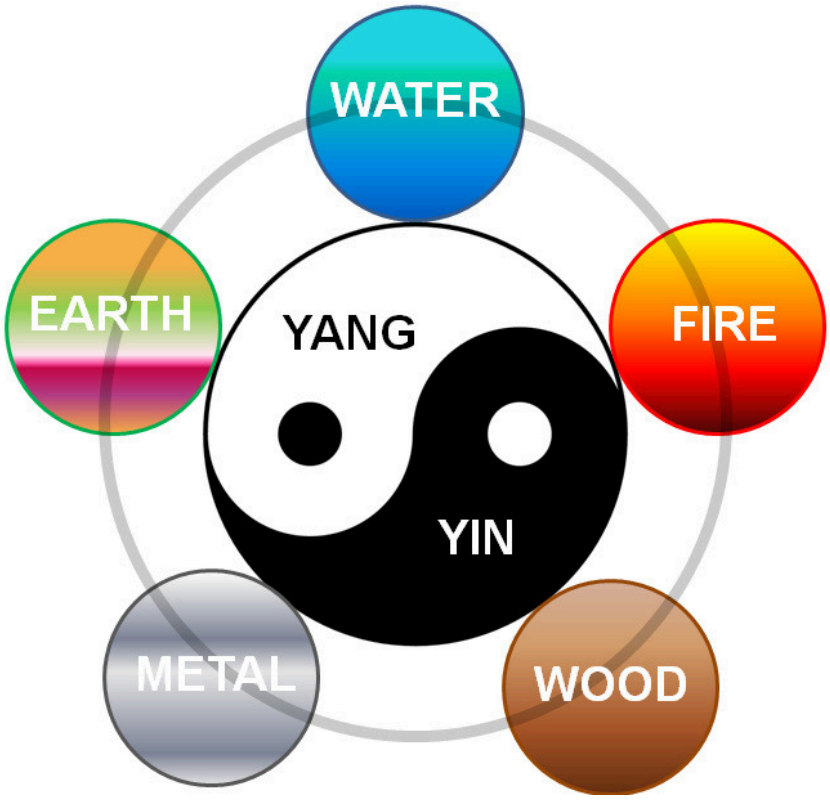


Figure 1.1: Yin and Yang/Five elements - Principles of Chinese Medicine

When the flow of Qi is blocked however, pain and illness occur [Focks 2010]. Through inserting a needle at a known body acupoint, followed by its appropriate manipulation (e.g. twisting, squeezing), the channel is supposed to unblock. Thereby one can re-establish the free flow of Qi and ultimately relieve pain, for example. These hypotheses, however, have not yet been validated by modern medical science and technology comprehensively. A major difference between Western Medicine and Traditional Chinese Medicine (TCM) is the narrow and quantitative definition for a disease by Western medicine, whereas TCM holds a holistic concept, viewing the disease as a disturbance of the equilibrium among body functions and between the subject and the environment [Han 2011]. Based on these principles, acupuncture is an effective and non-medicinal form of treatment and growing in popularity worldwide due to the increasing desire of living a natural and balanced life [Litscher and Schikora 2005]. This popularity is dating back to 1972, when a reporter, who ac-

companied an official visit of the former US President Nixon to the People's Republic of China, had to undertake surgery and analgesia was solely achieved through acupuncture [Hempen 2005].

With the growing demand for alternative forms of treatment, medical science had to take notice of acupuncture. The World Health Organization (WHO) started dealing with acupuncture in 1979 and created lists of medical indications. More than 40 disorders have been endorsed by the WHO so far, as conditions benefiting from acupuncture treatment [Han 2011]. The United States National Institute of Health (NIH) recommends acupuncture as an alternative or complementary therapy approach since its Consensus Conference in 1998. The effectiveness in symptoms such as post operative nausea and vomiting, nausea and vomiting after chemotherapy, but also for addictive illnesses, menstrual pain, headaches, tennis elbow, fibromyalgia, back pain, carpal tunnel syndrome and bronchial asthma was addressed [NIH 1998].

To be of the greatest utility, however, acupuncture should always be applied following certain rules: A treatment begins with a complete diagnosis in accordance with Traditional Chinese Medicine. Hence follows the therapeutic intention and strategy which is based not only on ideal acupuncture, which is individually adjusted, but on dietetics, Qi-Gong (Chinese kinetics) and Tuina (Chinese massage). The patient's attention should be inward-facing, the treatment room has to be quiet and the time of needling should last 20 to 30 minutes at least. Furthermore, the quantity of treatment sessions and the individual responsiveness of the patient have to be considered [Stux 2007].

Modern acupuncture researchers usually respect the traditional acupuncturist's decision on where to insert needles. These rationales of selection, based on theories of TCM, usually cannot be completely understood and explained by modern medical science. Many studies concerning the applicability exist though, particular in the

field of analgesia and pain reduction through acupuncture treatment [Stux 2007]. For years now, neurophysiologists try to find biological pathways which could lead to a scientific foundation of acupuncture. Some results could be observed so far: Stimulation of acupoints leads to a temporary inhibition of algesia induced by the mechanism of ”diffuse noxious inhibitory control” (DNIC) for mechanical, chemical and thermal stimuli [Le Bars 2002]. Opiates, which are produced naturally by the body, are released during acupuncture. This happens via activation of descending inhibitory mechanisms in the cerebrospinal fluid and the substantia gelatinosa and further on in the central plane in the substantia grisea centralis, the raphe nuclei, the caudate nucleus, the thalamus and in the limbic system. The analgesic effect is mainly mediated through endorphins and dynorphins [Irnich and Beyer 2002].

1.1.1 Ear Acupuncture

One of the oldest varieties of classical body acupuncture is ear acupuncture. It dates back to ancient Egypt (Egyptian seafarers attempted to improve their vision through piercing the earlobe), Greece (Hippocrates tried to cure acratia with a hemorrhage at the outer ear) and Rome. The concept of somatotopy (derived from the Greek words soma = body and topos = location) is the underlying basic concept. Somatotopy implies the differentiated image of the human body on a certain area, in this case the ear. This representation reminds someone of a bottom-up embryo. Compared to body and traditional acupuncture, respectively, the most important principle of ear acupuncture is that only existing disorders lead to irritated and therefore to identifiable ear acupoints. A so called ”active” ear acupoint is characterized by a pressure pain or can show visible alterations, for example redness or ectasia [Thiedemann 2004]. Nonetheless, it lasted till the end of the 1950s that ear acupuncture was

established and further developed in Europe by French neurologist Paul Nogier. Through a series of investigations he systematically demonstrated that specific internal organs and different regions of the ear have a functional relationship. These results were published by Nogier in 1957 [Gori and Firenzuoli 2007]. Not until 1959 could ear acupuncture be found in Chinese literature, however. In further consequence Chinese ear acupuncture developed, containing its own nomenclature and cartography. Due to the fact that this study is based on Chinese acupuncture, its system will be used and examined. It can be assumed that laser acupuncture stimulation of ear acupoints can be performed to obtain an impact on specific organs [Hecker and Steveling and Peuker 2002].

1.1.2 The de Qi Sensation

When using needle acupuncture, or in this study laser acupuncture, the de Qi sensation has to be considered as a validity for acupunctural effects. The de Qi feeling can be characterized as a sensation occurring during needling acupoints, although it does not always occur and in every proband or patient. Probands with a de Qi sensation often describe it as a dragging, dull or tingling feeling, with a spreading or gathering of heat [Hempen 2005, Langevin 2001]. Today, only a few studies concerning the investigation of the source of the de Qi exist. Furthermore, no specific, placebo-controlled studies could be found which examined the sensation during laser acupuncture. The influence of de Qi on designated symptoms is not yet clearly validated and some authors even raise questions if de Qi has any influence on the outcome of acupuncture at all [White and Prescott and Lewith 2010].

1.2 Laser Acupuncture

The word LASER is an english acronym meaning ”Light Amplification by Stimulated Emission of Radiation”. To generate laser light, an active medium needs to be stimulated by an external energy source. The used medium can thereby be either solid (e.g. crystal) or fluid (e.g. dye) or gaseous (e.g. helium-neon). The implemented stimulation leads to an energy-rich state of the atoms of the medium. This again leads to occupancy in which the atoms are in an induced condition. The requirement for stimulated emission of radiation is fulfilled therewith. The stored energy of the induced atoms is spontaneously emitted as a light quantum. The intensity of the light ray is enhanced through repeated total internal reflection in the optical resonator, which consists of two mirrors. One of these mirrors is partly transparent and light quanta can be passed out (see **Figure 1.2**) [Pöntinen and Pothmann 2005].

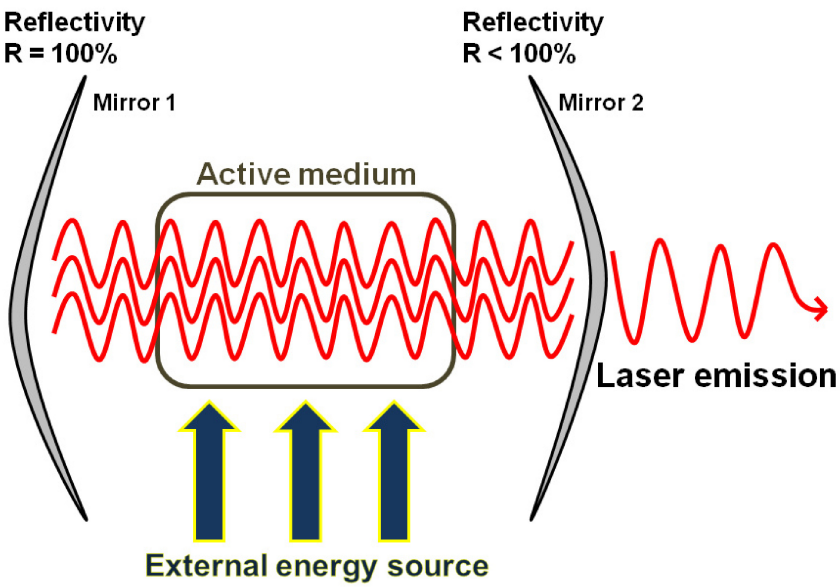


Figure 1.2: Light Amplification by Stimulated Emission of Radiation

In physical terms laser light can be distinguished from optical light. Laser light is monochrome, it has a defined wavelength determined by the laser medium, it is coherent which means the temporal and local vibrations of photons are in phase to each other and the photons vibrate in one common plane, which is described as polarization (see **Figure 1.3**). Furthermore, laser beams have a very low beam divergence. Even at a great distance they are still strongly collimated and the intensity hardly decreases. The power density is calculated from the emitted power (Watt) divided by the irradiation area (cm^2) and is greater the thinner the laser beam is [Salih 2008].

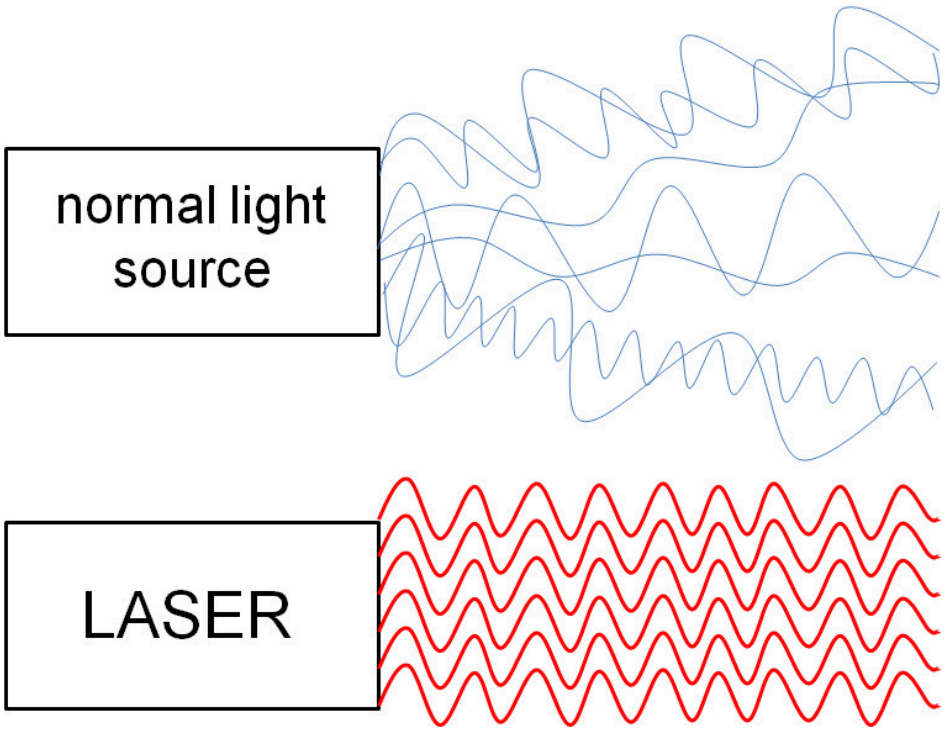


Figure 1.3: Characteristics of laser light compared to a normal light source: monochrome, coherent, polarized [modified from Salih 2008]

Acupuncture is a word of Latin origin (acus = needle, punctio = twinge). Together, laser and acupuncture result in the most spectacular technical development in modern complementary medicine: The introduction of laser acupuncture, defined as the

stimulation of traditional acupuncture points with low-intensity, nonthermal laser irradiation [Whittaker 2004]. Within this new technology development the goal has been to maintain the aspects of therapeutic procedures and effects of traditional acupuncture: the simultaneous procedure, the simple handling and needle equivalent stimulation effects and characteristics. The result is a complex diagnostic system on the one hand and the simple therapeutic procedure on the other [Schikora 2005]. Initially only one acupoint could be stimulated at a time which resulted in a practical disadvantage of laser acupuncture due to the fact that classical Chinese acupuncture uses treatment schemes [Whittaker 2004]. The development of a 12 channel device by Weber and Schikora marked the turn [Weber 2005]. Laser acupuncture has been clinically applied since the 1970s when the research group around Mester from Hungary dealt with wound healing [Mester et al. 1971]. Although the pioneering nature of such work cannot be discounted, it was not until the late 1980s and early 1990s that increased numbers of well-designed experiments appeared [Snyder-Mackler et al. 1986, Snyder-Mackler et al. 1989, King et al. 1990, Beckerman et al. 1992, Gam and Thorsen and Lønnberg 1993, Whittaker 2004]. Today, laser acupuncture is administered in a variety of pathologies and conditions of pain, e.g. when having myofascial, postoperative and traumatic pain, headache, neuralgia, back pain, rheumatism, tumor pain or muscular pain [Pöntinen and Pothmann 2005]. The mechanisms of action, especially the physiological parameters, are not utterly resolved however. Quantitative documented proof of the equivalence between laser acupuncture and classical needles is necessary. The effects of placebo (so-called sham laser acupuncture) treatment should be considered. Not generating any acupuncture effect, having an identical shape and size compared to verum needles, an identical application procedure, an application procedure not dependent on the individual experience of the treating physician and the perception of acupoints

are the main requirements for future placebo laser acupuncture studies [Schikora 2005]. This study aims to contribute to still open questions.

1.2.1 Violet Laser Acupuncture

Within this study, modern violet laser acupuncture technology was used. Violet laser acupuncture can stimulate different acupoints continuously and simultaneously [Litscher 2012]. Nakamura et al. [2000] developed these small and convenient blue and violet lasers which had not been available before. The most frequently used lasers in experimental studies are violet lasers with a wavelength of 405 nm, an output power of 100 mW and a diameter of 500 µm. Furthermore, each single needle in modern devices can emit a different wavelength. As another important fact the painless application of violet laser has to be mentioned [Litscher 2012]. One major difference of violet laser compared to red and infrared laser is the evoked de Qi sensation. Nearly 90 percent of Chinese volunteers distinguished stimulation by violet laser and stated to have a de Qi sensation [Litscher et al. 2010]. This sensation occurs although violet laser does not have the same penetration depth in human skin (violet: 2 mm versus red/infrared: 2-3 cm). A de Qi sensation, which in TCM is a prerequisite for effective acupuncture stimulation, could justify the scientific investigation of violet laser stimulation in acupuncture [Anderson and Parrish 1981, Litscher 2009, Litscher et al. 2009]. The patients normally do not notice if the laser is started, when red (685 nm) or infrared (785 nm) lasers are used . It is important to notice that both blue and violet laser are mentioned parallel in current literature. The wavelength of 405 nm is not in fact blue but appears violet. The human eye can process an electromagnetic spectrum from about 390 to 750 nm, therefore it has a limited sensitivity to a color with a wavelength of 405 nm (see **Figure 1.4**).

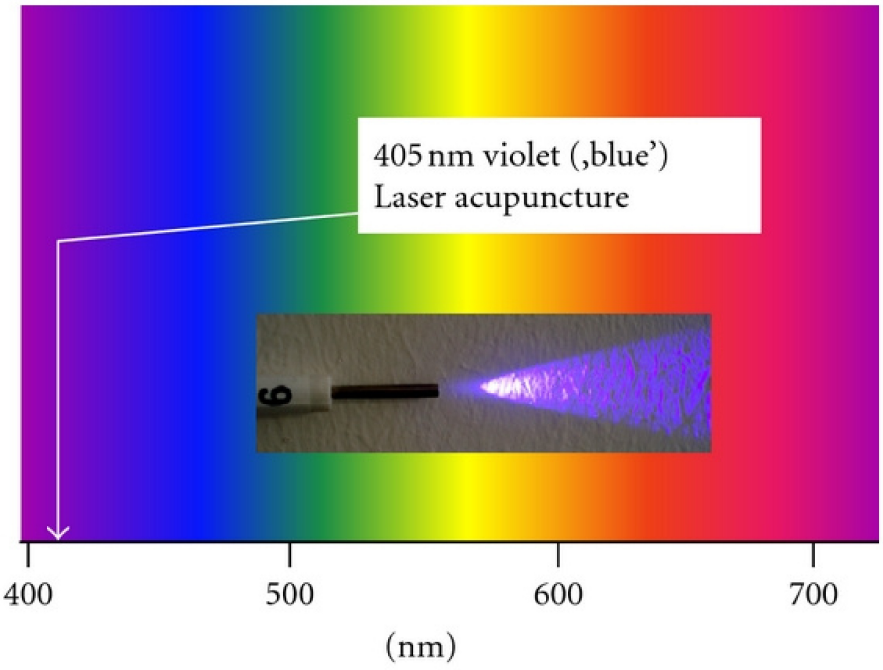


Figure 1.4: Array of the human eye and wavelength of violet laser [modified from Litscher 2012]

In modern medicine, violet lasers are mostly used in special areas. Mittermayr et al. [2007], for example, demonstrated that violet laser irradiation improves local tissue perfusion through stimulating NO (Nitric oxide) release from NO-Hemoglobin complexes. Enwemeka et al. [2008] established destruction of HA-MRSA (Hospital-acquired Methicillin-Resistant Staphylococcus aureus) and CA-MRSA (Community-acquired Methicillin-Resistant Staphylococcus aureus) in vitro with blue light at low doses. Yet in acupuncture research only a few scientific investigations were published. Litscher et al. [2009] detected a significant decrease of the heart rate when stimulating the acupoint Neiguan (Pe.6). Reproducible effects on brain circulation (Doppler sonography), peripheral microcirculation (laser Doppler flowmetry), temperature distribution (thermal infrared imaging), human arterial stiffness and wave reflection (Arteriograph^{TM}) and heart rate variability were presented in the Journal of Acupuncture and Meridian Studies by the research group of Prof. Litscher in Graz

[Litscher et al. 2010, Wang et al. 2011a, Litscher et al. 2011a, Litscher et al. 2011b, Litscher et al. 2012a].

1.3 Modern Quantification Methods

1.3.1 The Arteriograph

Palpating the pulse, and therefore diagnosing the health state of a person, is one of the earliest procedures a physician could perform in ancient China. In modern medicine, several technical devices exist to evaluate the pulse wave. The pulse wave is an index for arterial stiffness and can be used to predict cardiovascular mortality [The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) 2007].

The Arteriograph^{TM} (TensioMed, Budapest, Hungary) is a relatively new non-invasive device and compared to established tonometric and piezo-electronic methods easy to use and time-effective [Baulmann et al. 2008]. The Arteriograph^{TM} indicates the direct and indirect parameters for measuring the arterial stiffness: the aortal pulse wave velocity (PWV), augmentation index (AIx), peripheral blood pressure and the pulse pressure amplification [Baulmann et al. 2010].

The pulse wave velocity is widely used in clinical settings to indicate the risk of arteriosclerosis, coronary heart disease and stroke in healthy subjects. In aging people the arterial stiffness becomes elevated. As a consequence systolic blood pressure increases, causing a rise in left ventricular workload and subsequent hypertrophy, and diastolic blood pressure decreases, leading to an impaired coronary perfusion. Stiffer arteries lead to a higher PWV, a higher PWV indicates stiffer arteries, respectively [Mattace-Raso et al. 2006]. Furthermore, with the PWV one can easily

evaluate the cardiovascular risk due to simple handling, accuracy and reproducibility [Mattace-Raso et al. 2006, Inoue et al. 2009].

The cardiac after-load is determined by the systolic blood pressure, the diastolic blood pressure is of relevance for the coronary perfusion, and the pulse pressure (PP) is an important parameter for the physiological age and degenerative changes within the great arteries, e.g. hypertension or diabetes. The arrival of the reflected pulse wave in the ascending aorta during the diastole can be described as the optimal healthy condition. This condition is denoted as the "'inflection point"'. The pressure increase in the aorta after the inflection point until the maximum pressure, or systolic blood pressure, is called the pressure augmentation (AP). The augmentation index (AIx) is part of this pressure augmentation, the amplitude difference between the first (P1) and the second (P2) wave is correlated with the augmentation index (AIx) and the pulse pressure. The AIx is calculated with the formula AIx% =[(P2-P1)/PP]x100 (seen in **Fig. 1.5**) [Baulmann et al. 2008]. Age, body height, heart rate, gender, heart function, blood pressure, cardiovascular risk factors and medication are essential clinical determining factors of the AIx [Weber et al. 2008].

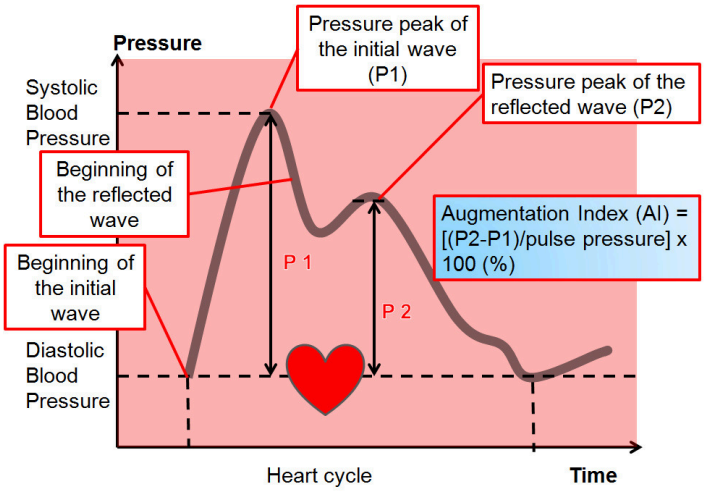


Figure 1.5: Parameters for non-invasive assessment of arterial stiffness and wave reflection [modified from Litscher et al. 2011b]

The arteriograph measurement is based on plethysmography, which is a measuring procedure for volume fluctuation and therefore pressure changes in arteries. Beneath the inflated pressure cuff, which is applied to the upper arm, fluctuations in pulsatile pressure in the artery lead to periodic pressure changes. These oscillations are measured by the arteriograph indirectly through the cuff. The pressure recorded is dependent on three factors: the amplitude and duration of ventricular ejection, the amplitude of the reflected wave, and the velocity of the reflected wave from the periphery [Nelson et al. 2010].

Initially, the blood pressure is measured oscillometrically. A cuff pressure, that is 35mmHg above the systolic blood pressure measured, is inflated. Pressure fluctuations can now be detected and analyzed on the computer as pulse waves (can be seen in **Figure 1.5**) [Baulmann et al. 2008].

Previous studies could verify that the pulse wave velocity (PWV), measured by the non-invasive arteriograph, is very close to the true and invasively determined one [Horváth et al. 2010]. The pulse wave velocity in m/s is shown through the difference in time between the beginning of the first wave and the beginning of the second or reflected wave. This time difference is described as the distance from the jugulum to the symphysis. The early and late systolic and diastolic waves are analyzed by the arteriograph and the onset and the peaks of the waves are determined with first and second derivatives. It is important to know that the arteriograph only records and analyzes pulse waves when a supra-systolic pressure of 35mmHg is achieved [Baulmann et al. 2008].

In summary it can be said that the quantification of arterial stiffness and pulse wave reflexion, with the aid of PWV and AIx and blood pressure, makes it easy to evaluate the damage to end organs when having arterial hypertension and furthermore to make forward looking statements for healthy subjects directly and non-invasively [Weber et al. 2008].

1.3.2 Heart Rate Variability

Today, the appearance of lethal arrhythmias in patients and the demand to constitute either increased sympathetic or reduced vagal activity has led to enhanced efforts in the development of quantitative markers of autonomic activity. Heart rate variability (HRV) represents such a promising and popular marker. In clinical medicine HRV has become the accepted term to describe variations of the oscillation in the interval between consecutive heartbeats and therefore the percentage change in the RR interval (sequential chamber complexes) as well as the oscillation between the consecutive instantaneous heart rates [Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996]. The importance of HRV became obvious in the late 1980s, when HRV was associated with the mortality following an acute myocardial infarction [Bigger et al. 1992]. Furthermore, HRV indicates parameters of the neuro-vegetative activity, or the autonomic function of the heart, respectively [Löllgen 1999].

The parasympathetic influence on the heart and the circulation is based on the release of acetylcholine by the vagus nerve. Stimulation of muscarinic receptors leads to an increase of the potassium conductivity in the cellular membrane. As a result, stimulation of the slow diastolic depolarisation is evoked. The vagal and sympathetic activity interact. The sympathetic stimulation is based on the release of epinephrine and norepinephrine. These neurotransmitters activate β-adrenergic receptors which then phosphorylate membrane proteins via cyclic AMP. This results in the acceleration of the slow diastolic depolarization. The vagal stimulation outweighs the sympathetic stimulation under resting condition and the RR interval variations depend on changes of the vagal modulation. Spectral analysis of HRV has led to an understanding of autonomic effects of neural mechanisms on the sinus node. The so-called high frequency (HF, between 0.15 and 0.40 Hz) is associated with the parasympathetic nervous system, as can be seen in clinical observations

of autonomic maneuvers such as muscarinic receptor blockade and vagotomy [Akselrod et al. 1981, HRV Manual]. The low frequency (LF, between 0.04 and 0.25 Hz) is usually taken as a marker for the sympathetic modulation, although this is controversial. Consequently, the LF/HF ratio is considered by some investigators to mirror sympathovagal balance or to reflect the sympathetic modulations [Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Löllgen 1999].

Important influence quantities on HRV are age, gender, body position, time of day and medication [Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Jung et al. 1996, Umetami et al. 1998]. In a healthy person the heart rate depends on the breathing mechanism and normally alternates more than 15 beats per minute. Values between 11 and 14 beats per minute are marginal, values under 10 beats per minute are pathological [Löllgen 1999].

The ”Fire of Life” software, which was used in this study, analyzes the HRV. The acquired data can be shown in a spectrogram and help to judge the function of the autonomic nervous system. The reaction of the human body to either stress or recovery, and therefore to acupuncture also, is recognized and evaluated by the “Fire of Life” software (for exemplification see **Figure 1.6**).

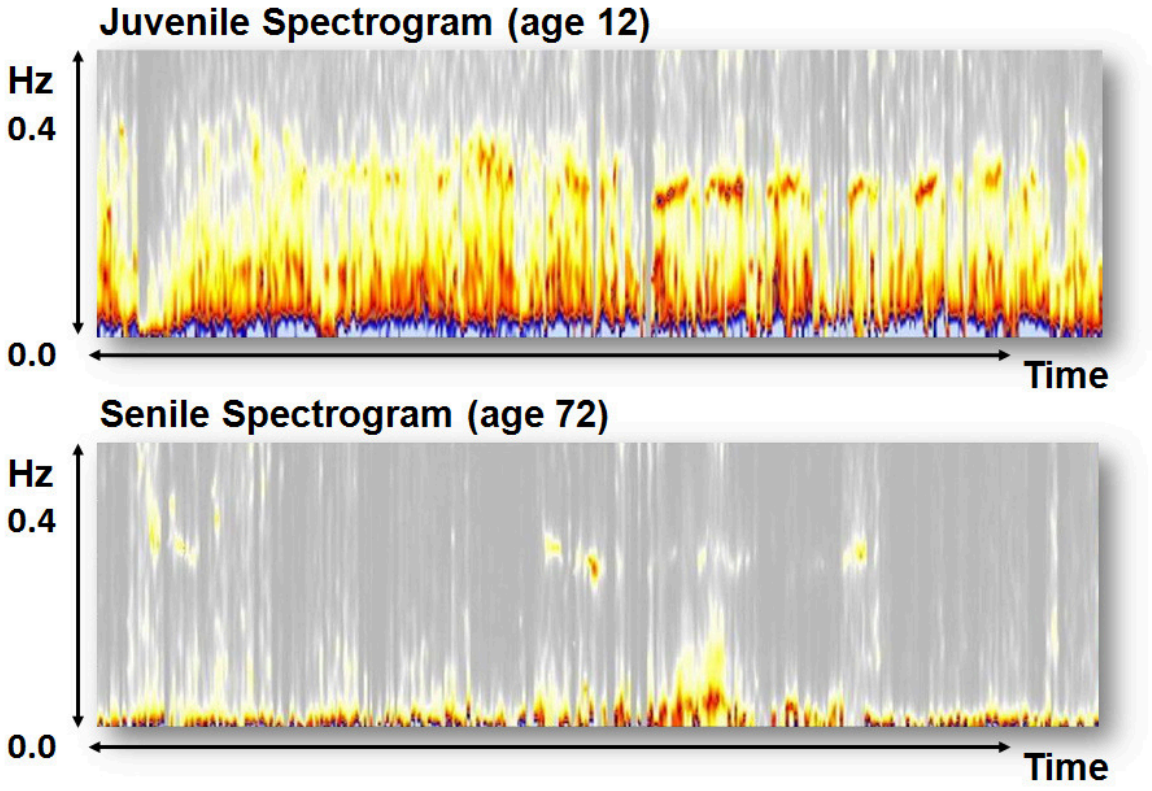


Figure 1.6: Fire of Life Spectrogram showing the difference between measurements of a juvenile and a senile proband [modified from http://www.primedica.de/en/fire-of-life-2, 16.10.12]

Repeated measurements can indicate if therapeutic or interventional actions lead to a reduction of stress and to a general improvement of the body functions [Schiller Medilog 2012].

1.3.3 Thermography

The body temperature is one of the most important measurement parameters in daily medical routine. An inflammation leading to an increase in temperature is known to be a symptom and a follow-up parameter of many immunological diseases. An induced hypothermia in anesthesia, on the other hand, is important for operations at the heart or brain, because better tolerance towards hypoxia will be achieved. In

the normal processes of physiological thermoregulation the response of the human skin blood flow is essential [Gekle and Singer and Jessen 2005]. An increase in body temperature is always associated with a peripheral vasodilatation which is regulated by the autonomic nervous system. Deactivation of the sympathetic nervous system leads to dilatation in the vessels, to an increase in peripheral skin blood flow and therefore to increasing temperature while activation of the sympathetic nervous system generates the opposite. This provides the necessary augmentation of convective heat loss during heat exposure, while on the other hand cutaneous vasoconstriction prevents heat loss during cold exposure [Gekle and Singer and Jessen 2005, Charkoudian 2010].

Today, a significant number of clinical studies investigating the benefit of thermography as a diagnostical tool exist. Researchers could prove that infrared thermography is an excellent non-invasive tool in the follow-up of hemangiomas, vascular malformations and burns in pediatric patients and particularly indicate the value for diagnosis of extremity thrombosis, inflammation, abscesses, gangrene and wound infections, just to mention some [Saxena and Willital 2008]. Another field of infrared thermography is cancer research. Thermographic imaging can detect temperature changes as small as 0.1° C on the skin surface at an early stage of tumor development. Therefore, it is suggested that thermographic imaging has a potential in monitoring human tumor xenografts and their response to anticancer drugs and should be used as standard in clinical routine [Song et al. 2007].

In conclusion, infrared thermography is non-invasive, without side effects, requires no sedation and can be repeated as often as useful with objective results [Saxena and Willital 2008]. Furthermore, heat radiation is emitted by every human body in the form of energy. Energy is also called infrared radiation and cannot be seen by the human eye [Lange 2008]. Consequently, infrared thermography is an important method to evaluate the patients' or healthy volunteers' skin surface temperature

without influences caused by direct contact to the skin. Peripheral effects of laser-acupuncture can be assessed [Litscher 2012].

2 Methods

2.1 Volunteers

This study was performed at the Stronach Research Unit for Complementary and Integrative Laser Medicine at the Department of Anesthesiology and Intensive Care Medicine Graz.

Within this study, the non-invasive parameters of thirteen healthy volunteers (M/F, 8/5; mean age $\pm$ standard deviation, 23.9 $\pm$ 1.7 [range 22 - 27] years; mean height $\pm$ standard deviation, 175.2 $\pm$ 7.2 cm; mean weight $\pm$ standard deviation, 69.4 $\pm$ 9.8 kg) were evaluated during violet laser acupuncture. In **Figure 2.1** (Page 25) the measurement procedure and the measurement times (a-f; thermographic imaging and arteriograph measurement) are illustrated schematically before, during and after violet laser stimulation.

None of the volunteers was taking medication. The study was approved by the local ethics committee at the Medical University Graz. The volunteers were, as far as the study design allowed, personally informed about the nature of the investigation and all volunteers gave their written informed consent.

The volunteers were lying on a cot in the lab. The room temperature was kept constant at 24℃. For recording the ECG, three electrodes (Skintact Premier F-55, Leonhard Lang, Innsbruck, Austria) were attached on the thorax as standard.

2.2 Acupuncture Points

The laser needles were attached to the following acupuncture points:

- Nei Guan - *"Inner Pass"* (Pericardium 6)
  Localization: Two cun [1] proximal to the transverse crease of the wrist, on the palmar side of the hand, between the tendons of the Mm. palmaris longus and flexor carpi radialis.
- Bai Hui - *"Hundred Convergences"* (Governing vessel 20)
  Localization: Crossing point on an imaginary line between the apexes of both ears and the midpoint of the calvaria, seven cun above the posterior and five cun above the anterior hairline.
- Shen Men - *"Spiritual Gate"* (Ear Point 55)
  Localization: In the bifurcating point between the superior and inferior crura of the antihelix, close to the crus superior at the lateral third of the triangular fossa [Focks 2010].

2.3 Violet Laser Acupuncture

Within this study a non-invasive Lasermicroneedle® system with integrated red and blue/violet emission semiconductor diodes was used. For the first time, the laser system includes violet (5) as well as red (5) laser needles, and each single needle can emit light with a different wavelength. The wavelength accounted for 405 nm, with an output power of 110 mW and a laser needle spot diameter of 500 µm. The output power at the needle tip can be estimated with 100 mW based on coupling losses [Litscher et al. 2010b]. The contact-laser needles were applied onto the skin

[1] Individual measurement unit defined as the width of the distal inter-phalangeal joint of the thumb.

through a patch, and two different (2 Hz and 100 Hz) frequency modes were used. The irradiation lasted for 10 minutes each (600 seconds) which led to a very high optical power density (range: kJ/cm^2)[He et al. 2012].

2.4 Signal Analysis and Data Detection of Arterial Stiffness and Pulse Wave

In this study an oscillometric non-invasive device, the Arteriograph^{TM}, was used. The parameters in **Table 2.1** were calculated. Signals were detected from an upper arm cuff. The cuff was attached following the rules of an ideal blood pressure measurement:

- Volunteers were lying comfortable on a cot to keep down changes in blood pressure. A consistent blood pressure results in consistent vessel stiffness parameters and therefore to constant data.
- Secondly, the detection and accurate recording of the pressure alteration during a pulse beat requires different time scales depending on the device. Therefore a pleasurable environment has to be created for the test person to prevent unwanted recording abortions [Baulmann et al. 2010].

Abbreviation	Unit	Parameter	Description
HR	BPM (1/min)	Heart rate	Heart rate.
MAP	mmHg	Mean arterial blood pressure	Calculated from systolic and diastolic blood pressure values.

Abbreviation	Unit	Parameter	Description
PP	mmHg	Pulse pressure or blood pressure amplitude	Difference of systolic and diastolic blood pressure values. PP values over 60 are considered independent risk factors, especially if diastolic pressure is normal or low. Increased values are an indirect indicator of increased arterial stiffness.
AIx brachial	%	Brachial augmentation index	Describes influence of reflected pulse wave on systolic pressure. AIx is closely linked to functional disturbances of the vasculature.
AIx aortic	%	Aortic augmentation index	Aortic augmentation.
ED	ms	Ejection duration of left ventricle	Time between opening and closing of the aortic valve.
RT	ms	Reflection time	Time the pulse wave needs from the aortic root to the bifurcation and back. The stiffer the aortic walls, the shorter the reflection time.
PWVao	m/s	Aortic pulse wave velocity	Describes the stiffness of the aortic vascular wall; considered a direct measure of aortic stiffness.
PPao	mmHg	Aortic pulse pressure or blood pressure amplitude	Superior prognostically compared with conventional , peripheral PP measured at the upper arm.
SBPao	mmHg	Central systolic blood pressure, close to the aortic root	Values are those ”seen” by the heart (after load); they represent stress to which coronary and cerebral arteries are exposed; thus are closely correlated to circulatory disorders and prognosis.

Table 2.1: Parameters Calculated by the Arteriograph^{TM}, modified from Litscher et al. 2011b

2.5 Measuring HRV (heart rate variability)

For monitoring the cardiac setting an HRV medilog® AR12 (Huntleigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria), with a sampling rate of 4096 Hz, was used. An extremely accurate R-wave detection could be conducted with this device. Raw data were saved on memory cards which can be read by a suitable card reader connected to a computer.

To visualize how the body reacts to acupuncture, the new “Fire of Life” software for analyzing the HRV was used. Furthermore, the low frequency (LF) and high frequency (HF) ratio has been calculated.

2.6 Monitoring Temperature Distribution with Infrared Thermography

In this study a Flir i5 infrared camera (Flir Systems Inc., Portland, USA), with a wavelength ranging from 7.5 - 13 µm and a temperature distribution measurement ranging between 0 - 250°C, was used. The camera uses an array of infrared sensors to measure the temperature on the surface area of the human body. The body surface emits infrared beams which will then be registered by the camera. The camera works contact-less, without feeding energy to the human body or manipulating it in another way. It can detect differences in temperature as low as 0.1° C. Thermal images of the right-handed acupuncture points “Shenmen” and “Neiguan” were taken. The collected data was transmitted to a notebook computer using the ThermaCAM Researchers Pro 2.8 software (Flir Systems Inc., Portland, USA).

2.7 Measurement Procedure

Two measurement stages per volunteer were implemented. One stage lasted 30 minutes subdivided in three times ten minutes. After a ten-minute resting phase (a,b; comp. **Fig.2.1**) during which baseline values were monitored, the laser was turned on for ten minutes (c,d; **Fig.2.1**). Stimulation was conducted in 2 Hz frequency mode and in 100 Hz frequency mode, respectively, on the same day and in randomized order (c,d: **Fig.2.1**). A ten-minute control phase followed the active stimulation (e,f; **Fig.2.1**).

After eight, 18 and 28 minutes the arteriograph measurement was executed and after three (no laser application), 18 (laser application) and 28 (no laser application) minutes of the experiment stage thermal images were taken.

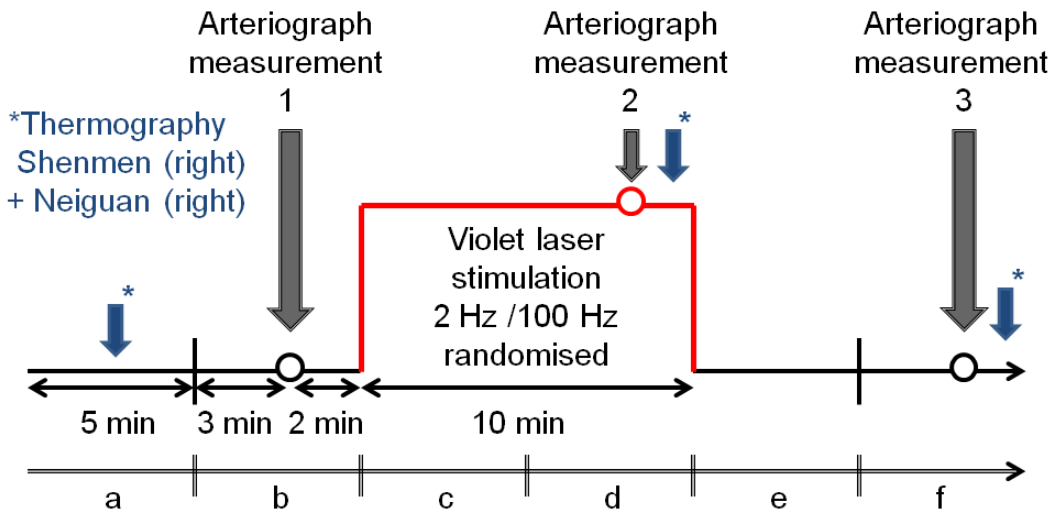


Figure 2.1: Measurement Procedure

2.8 Statistical Analysis

The data were analyzed by one-way repeated measurements ANOVA on ranks (SigmaPlot 11.0, Systat Software, Chicago, USA) and the Holm-Sidak method for post hoc analysis. The level of significance was defined as $p<0.05$.

3 Results

During stimulation with two different laser frequencies the probands were asked to describe any deviant sensation around the stimulation area. The following terms were used for description of the sensation: Feeling of warmth/heat, formication, painful sensation, tiredness during stimulation or any feeling of pressure. Of all probands, 69 percent or 9/13 named one or more of the above sensations, 31 percent or 4/13 felt nothing at all (**Fig. 3.1**). The prevalence of the different sensations can be seen in **Fig. 3.2**

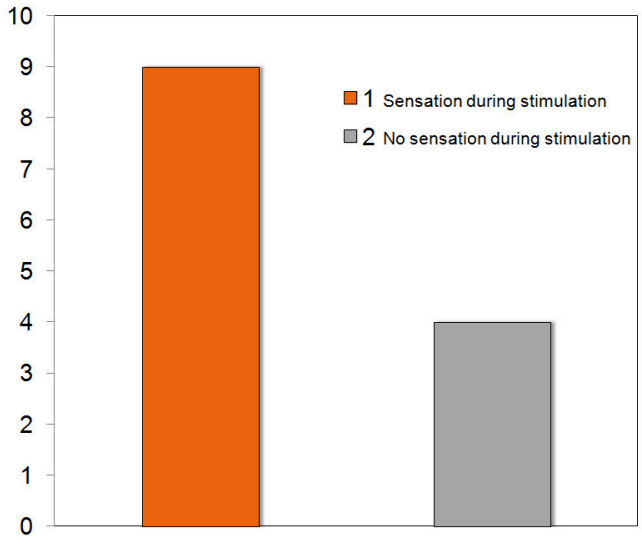


Figure 3.1: Sensation felt during stimulation.

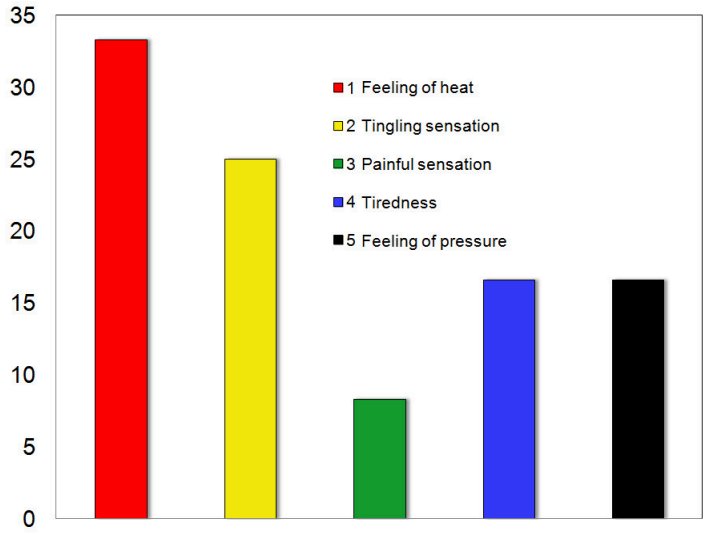


Figure 3.2: Prevalence (in percentage terms) of different de Qi sensations.

No significant changes were found during arteriograph measurement. The use of a figure was refrained.

No significant changes were found for the total heart rate variability (HRV, **Figure 3.3**).

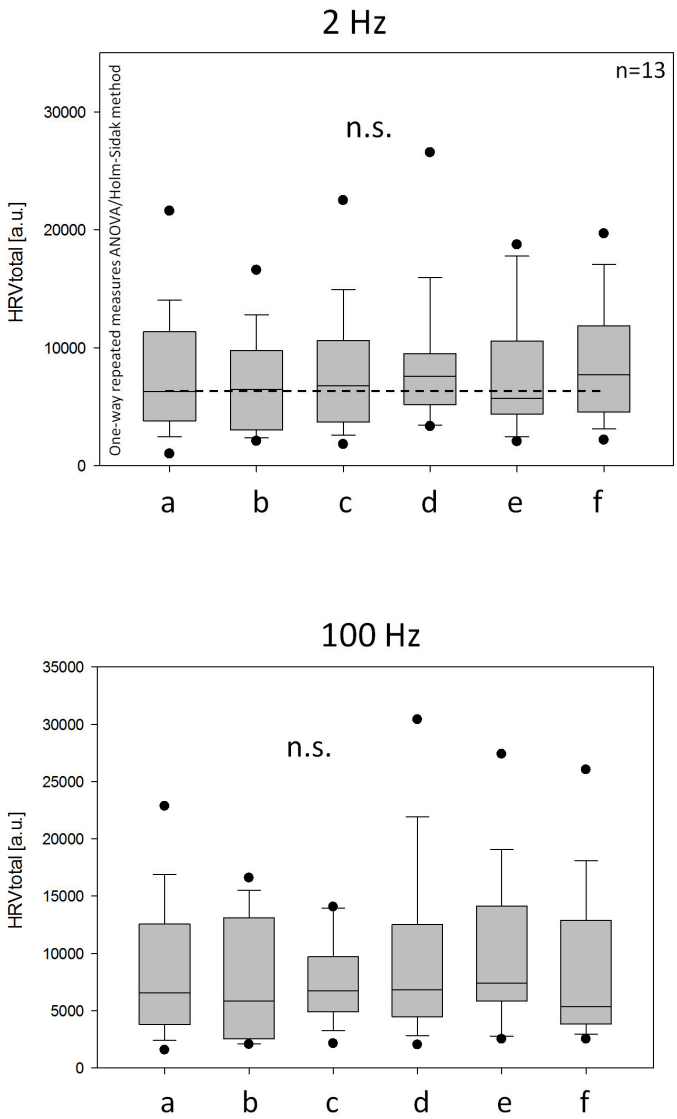


Figure 3.3: Changes in the total heart rate variability. No significant alterations were found. The ends of the boxes define the 25th and the 75th percentiles, with a central line at the median and error bars defining the 10th and 90th percentile.

The changes in the mean heart rate before, during and after stimulation with the violet laser at 2 Hz (left) and 100 Hz (right) are shown in **Figure 3.4**.

At the beginning of the 2 Hz violet laser recording session (a; comp. **Fig.2.1**), the mean heart rate was about 70 beats/min. During the first 15 minutes (a-c) the mean HR decreased with a significance of p=0.031. Overall the mean heart rate decreased significantly to about 64 beats/min at the end of the control phase (f; p=0.007).

During stimulation with 100 Hz, the decrease in HR was significant ($p<0.05$) towards the end of the stimulation phase (d) and at the end of the control phase (f). The mean HR decreased from about 68 beats/min to about 65 beats/min.

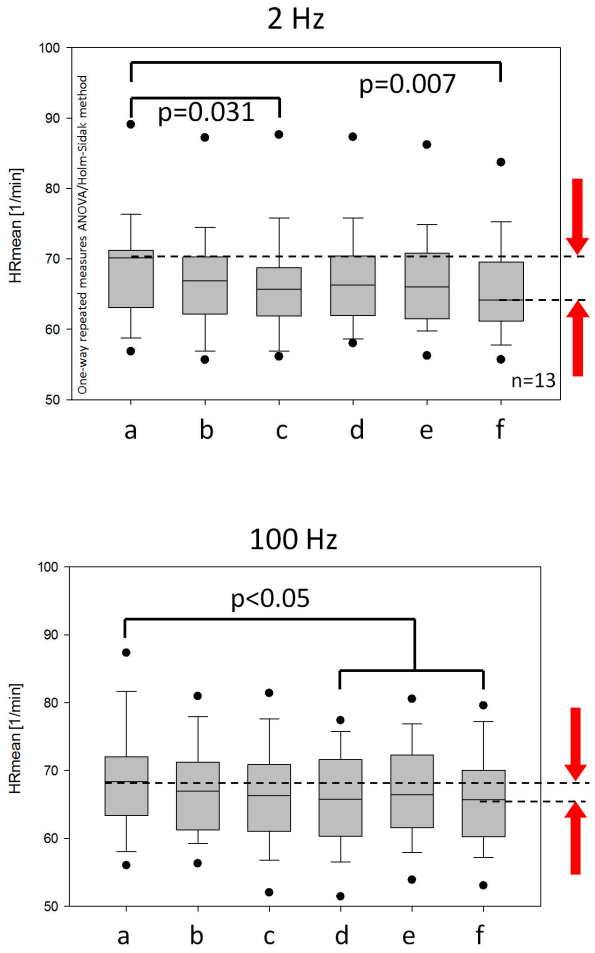


Figure 3.4: Box plot illustration of the mean heart rate before (a-b), during (c-d) and after (e-f) stimulation with violet laser at 2 Hz (left) and 100 Hz (right) of the 13 volunteers. For further explanation, see **Fig.3.1**.

In **Figure 3.5** the changes in the LF/HF ratio before, during and after 2 Hz and 100 Hz laser needle stimulation are shown. Note the highly significant increase in the LF/HF ratio during and after the 100 Hz measurement.

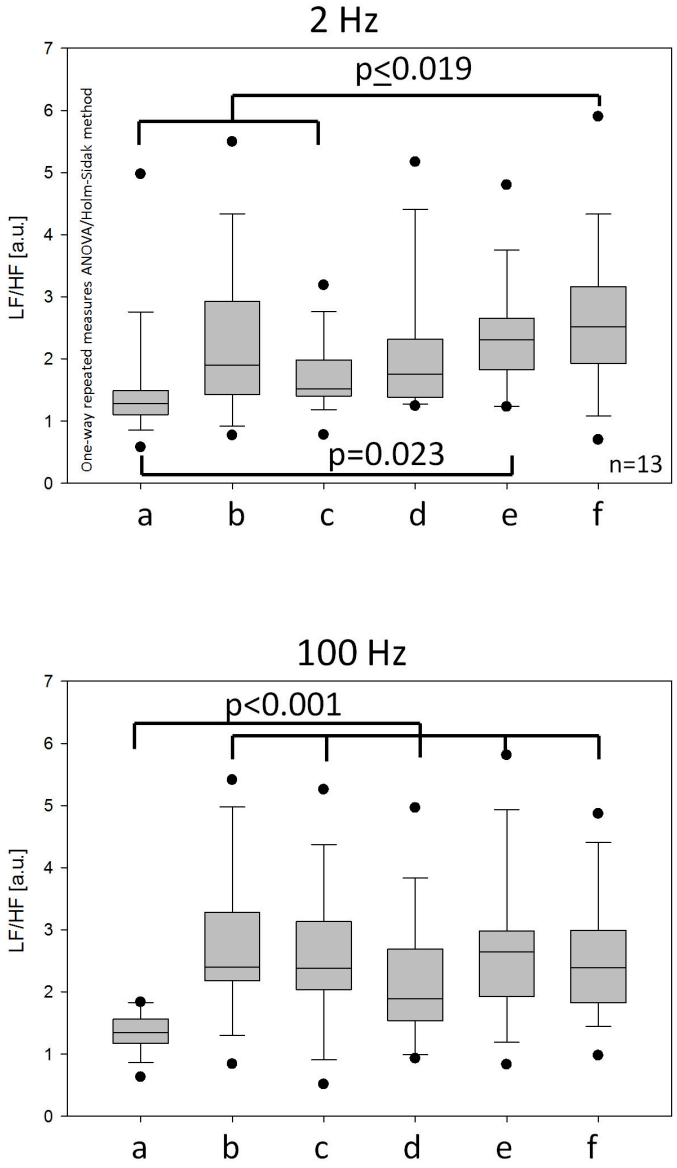


Figure 3.5: LF (low-frequency and HF(high frequency) ratio in the 13 volunteers before, during and after 2 Hz and 100 Hz laser needle stimulation. For further explanation, see **Fig.3.1**.

Figure 3.6 and **3.7** summarize the results of temperature analysis of the thirteen healthy volunteers. Skin temperature measured by infrared thermography changed highly significantly ($p<0.05$) only during 100 Hz stimulation of the ear acupoint Shenmen (**Fig. 3.5**). Nonsignificant changes were found during stimulation with either 2 Hz at the ear acupoint Shenmen (**Fig. 3.4**) nor 2 Hz or 100 Hz at the acupoint Neiguan.

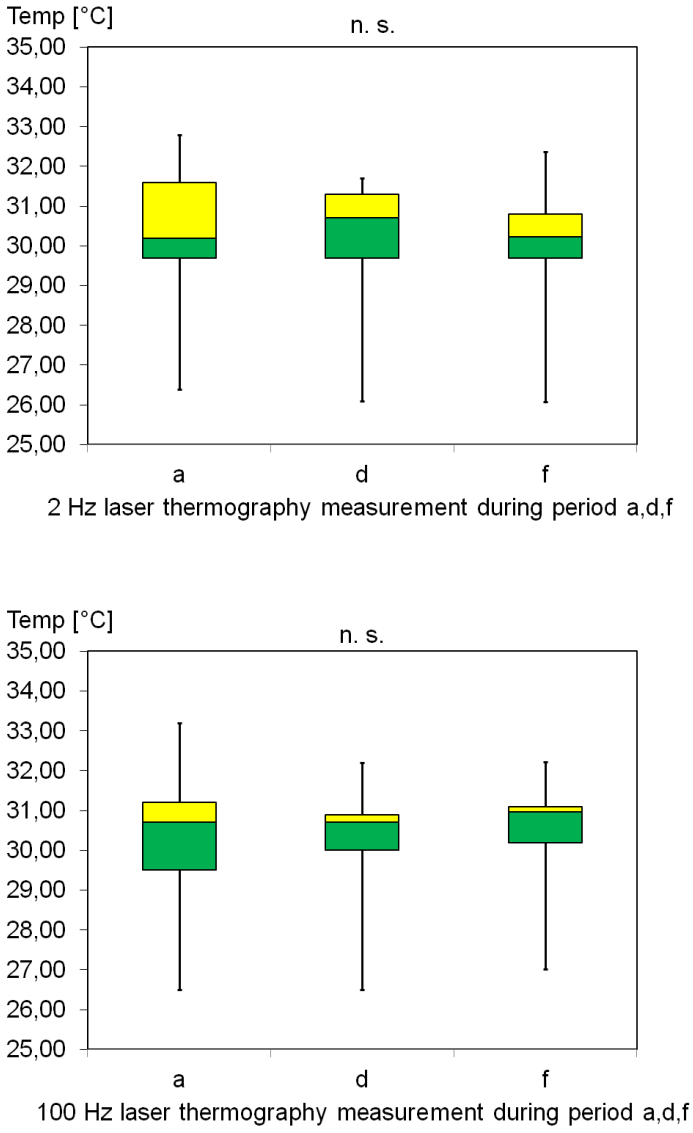
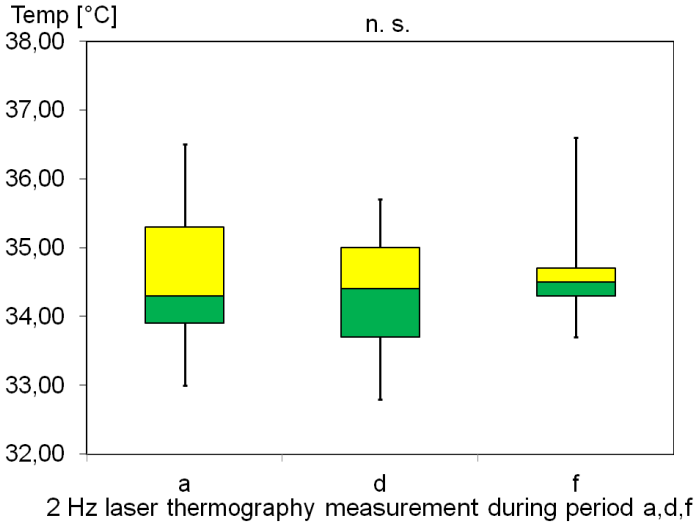


Figure 3.6: Changes in skin surface temperature registered by thermal monitoring at the acupoint Neiguan with 2 Hz and 100 Hz. No significant changes were found. For further explanation, see **Fig.3.1**.



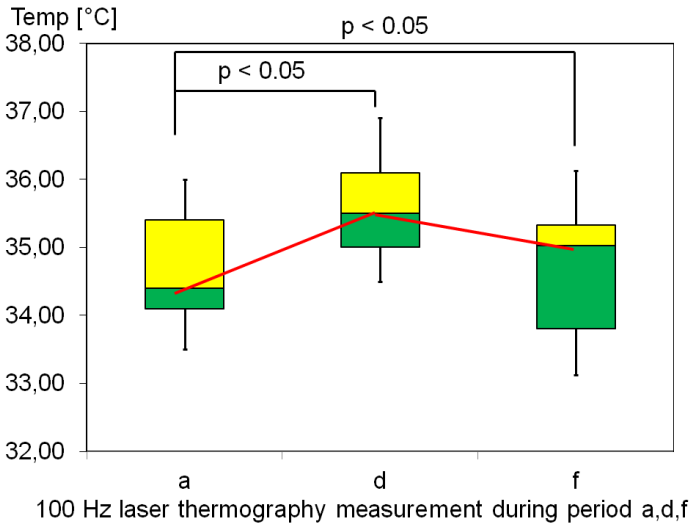


Figure 3.7: Changes in skin surface temperature registered by thermal monitoring at the ear acupoint Shenmen with 2 Hz and 100 Hz. Note the significant increase during 100 Hz violet laser stimulation (d) and after stimulation (f). For further explanation, see **Fig.3.1**.

4 Discussion

The aim of this study was to evaluate the influence of violet laser acupuncture on the body's autonomous systems, especially the cardiovascular system and the temperature regulation. Two different frequencies (2 Hz and 100 Hz) were applied. Body and ear acupuncture were used and three well-known acupoints, Neiguan, Baihui, and the ear acupoint Shenmen.

When searching the scientific database PubMed (www.pubmed.gov) several acupuncture studies using the acupoint Neiguan (PC6) can be found. In Traditional Chinese Medicine, the acupoint Neiguan is known to regulate and strengthen the heart function, especially palpitations and chest distress and the Qi-circulation [Focks 2010]. Investigations led to new treatment indications for stimulating the acupoint Neiguan. Acupressure with Sea Bands® [1] was used to control migraine-associated nausea [Allais et al. 2012]. Self-administration, which was evaluated even better than administration by the doctor, with simple battery-operated TENS caused a decrease in nausea in patients undergoing chemotherapy [Dundee et al. 1991]. 40 Hz laser stimulation at Neiguan reduced the severity and incidence of hypotension after epidural anesthesia. The authors stated that frequency is an important variable on the outcome [Arai et al. 2012].

Stimulating the acupoint Baihui enhances the mood and is mainly used in headache and vertigo therapy [Focks 2010]. Sun et al. examined the effects of high-frequency

[1] Elastic wristbands with a 1 cm protruding round plastic button; these devices apply continual pressure.

(100 Hz) laser acupuncture on a rat model. They could prove a significant decrease of abnormally elevated glutamate (Glu) and acetylcholine (ACh) levels in the striatum [Sun et al. 2012]. This could initiate further studies concerning Parkinson's disease, for example. Laser acupuncture could also improve motor recovery in rats with cerebral ischemia which would suggest an improvement for patients in post-stroke rehabilitation [Kim et al. 2012].

The ear acupoint Shenmen is used to reduce pain, stress, anxiety, and is known to have an anti-inflammatory effect [Focks 2010]. Kao et al. [2012] significantly tapered medication (benzodiazepines) in postmenopausal women with anxiety through auricular acupressure at the ear acupoint Shenmen. In the treatment of primary insomnia the implementation of acupuncture at the ear acupoint Shenmen, along others, could lead to improvement of sleep [Yeung et al. 2009]. The ear acupoint Shenmen is also used to slow down the heart rate and activate the parasympathomimetic nervous system [Hsu et al. 2007]. Clinical studies could demonstrate that acupuncture stimulation on the mentioned points is equally effective or even superior to conventional medical treatment [Pfab et al. 2011]. In most cases, however, it is suggested to perform further and larger controlled studies to validate the results. As can be seen above, several previously published research articles exist, examining the effectiveness of stimulating Neiguan, Baihui and Shenmen, together or independently. Based on these, the present study tried to investigate the effectiveness of violet laser acupuncture.

When searching for the key words "violet laser acupuncture AND frequency" in the scientific databases PubMed (www.pubmed.gov) and Ovid (www.ovidsp.ovid.com), there is no publication listed until September 28th, 2012. Four preliminary scientific studies have been performed by the TCM Research Center Graz, using violet laser acupuncture [Litscher et al. 2010a, Wang et al. 2011a, Litscher et al. 2011a, Litscher et al. 2011b]. Yet, in these studies continuos violet laser simulation was

applied. Evidence could be found that violet laser acupuncture stimulation at the acupuncture point ”Dazhui” increased the brain circulation, peripheral circulation and the local temperature [Litscher et al. 2010a, Litscher et al. 2011a]. In parameters like heart rate and mean arterial blood pressure however, no significant changes could be found when stimulating the acupuncture point ”Baihui” with continuous violet laser stimulation [Litscher et al. 2011b].
For the first time, stimulation of the acupoints Neiguan, Baihui and the ear acupoint Shenmen were evaluated simultaneously with two different violet laser frequencies and stimulation was performed at body and ear acupuncture points. To measure and identify effects on central and peripheral cardiovascular parameters, modern quantification devices (see above) were used.

When using electro acupuncture (EA), different parameters including frequency, intensity and duration have to be considered. Among these parameters, the frequency seems to be the most critical element. Usually 2 Hz and 100 Hz are utilized as standard configurations for low- and high- frequency electro acupuncture [Han 2003, Zhang et al. 2003, Xing et al. 2007, Li et al. 2008]. The efficacy of 2 Hz or 100 Hz seems to be of importance when treating different diseases [Han 2011]. In rats with neuropathic pain, for example, acupuncture analgesia with electro acupuncture of 2 Hz had a greater and more prolonged pain relief on mechanical allodynia and thermal hyperalgesia than electro acupuncture with 100 Hz [Xing et al. 2007]. Neurochemical studies revealed that stimulation with 2 Hz electro acupuncture accelerates the release of enkephalin, beta-endorphin and endomorphin, while, in comparison with 2 Hz, stimulation with 100 Hz electro acupuncture selectively increases the release of dynorphin in acupuncture analgesia [Li 2008, Han 2004, Han et al. 1991]. Silva, Silva and Prado [2012] and Zhang et al. [2003] suggested that different frequencies of electro acupuncture cause different therapeutic effects and are therefore mediated by different neural pathways. Arai et al. [2012] concluded in their investi-

gation that different frequencies have a different outcome on severity and incidence of hypotension after epidural anesthesia. 2 Hz and 100 Hz frequency lasers seem to be the most important ones in prospective studies. Wang et al. [2012] have found that more genes related to neurogenesis were differentially regulated by 2 Hz EA than 100 Hz EA. To do so, they used cDNA [2] micro arrays to investigate gene expressions in the region of the arcuate nucleus (Arc) in rats. The Arc is a region of the hypothalamus and responsible for the effect of EA stimulation and the regulation of pathophysiological processes, including autonomic activity.

The decreasing of the HR induced by violet laser acupuncture in the present study is earlier, stronger and more prolonged when using 2 Hz compared to 100 Hz. However, there are still many different results when using either 2 Hz or 100 Hz violet laser acupuncture. The aim of prospective examinations has to be the consideration of application and indication of different laser frequencies. Furthermore, the correlation between the HR changes stimulated by violet laser acupuncture and acupuncture analgesia needs to be investigated.

No relevant results could be determined by analyzing the arteriograph data.

Likewise, Litscher et al. [2011] could not prove significance, that violet laser acupuncture leads to an increase in the brachial augmentation index (AIx) and an decrease in the aortic pulse wave velocity (PWV). However, minor fluctuations were observed. Reasons could be the limited number of volunteers (n=10) and the missing comparison to a control group with sham laser acupuncture. In this study, similar problems can be stated. Only in one previous study, Satoh [2009] could detect reproducible physiological alterations of human arterial stiffness and wave reflection using needle acupuncture. PWV and the AIx are important parameters which provide enormous information on the arterial vascular system and arterial stiffness. They are direct indicators for cardiovascular risk [Baulmann 2008]. When searching the scientific

[2] complementary DNA, synthesized from a messenger RNA

database PubMed (www.pubmed.gov) with the following catchwords “arterial stiffness AND acupuncture”,“wave reflection AND acupuncture”and “pulse wave velocity AND acupuncture”, only six articles could be found (May 28th, 2012). A summary of the literature is listed in **Table 4.1**.

Author(s)	Method	Investigations in	Results	Acupuncture
Satoh 2009	Arterial stiffness, wave reflection, augmentation index	25 Male volunteers	AI radial ↑ BPdia ↑	Manual needle (Baihui GV20)
Rivas-Vilchis et al. 2008	Digital volume pulse	70 smokers and non-smokers	SDDVP index ↑	Manual needle (Neiguan PC6)
Rivas-Vilchis et al. 2007	Digital volume pulse	65 healthy and hypertensive subjects	SDDVP index ↑	Manual needle (Neiguan PC6)
Tan et al. 2006	F-wave	56 patients after cervical operations	Amplitude ↓	Manual needle (traditional points)
Lin et al. 2003	Finger blood flow	14 healthy volunteers	FBF ↓ BP ↓ PVT ↑	

Table 4.1: Summary of Research on Human Arterial Stiffness and Wave Reflection during Acupuncture, modified from Litscher et al. 2011b

No evidence could be found in a reproducible manner. In future studies, differences between laser and needle acupuncture should be considered. The number of volunteers is another important impact factor and should be considered much higher in numbers. Sham laser acupuncture should be performed to draw comparisons to laser acupuncture. Nevertheless, the Arteriograph is an easy-to-use and time effective oscillometric device for measuring the arterial stiffness during acupuncture treatment. The measurement of the “pulse wave velocity” (PWV) is very important for risk evaluation of arterial hypertension and the damage to end organs. It is recommended by the The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) [2007].

The effect on the parameters heart rate (HR), heart rate variability (HRV) and low frequency/high frequency (LF/HF) was measured. No significance could be found in the difference of the HRV before, during and after 2 Hz or 100 Hz violet laser stimulation. The difference of the mean HR before, during and after 2 Hz or 100 Hz violet laser stimulation is significant. In addition to the mentioned results, there is a significant difference of the LF/HF ratio before, during and after 2 Hz or 100 Hz laser stimulation. Maybe the high significance of the altered HR and LF/HF ratio results out of the stimulation of three acupoints, Baihui, Shenmen (Extra, at both ears) and Neiguan (at both arms) simultaneously.

In recent years, several studies were performed by Litscher et al. [Litscher 2009, Litscher 2010, Gao et al. 2012a, Litscher et al. 2012a], which identified specific brain-modulated autonomic influences through computer analysis of heart rate (HR) and heart rate variability (HRV). The significant decrease of the heart rates in the present study is in accordance to previous trials with continuous violet laser acupuncture [Litscher et al. 2012c, Litscher et al. 2009]. Gao et al. [2012] stimulated anesthetized rats with violet laser acupuncture and found a significant change of the HR during and after stimulation at the acupoint Baihui. It is questionable if these results are comparable to results in humans and should be investigated further. In two other trials the total HRV increased significantly after acupuncture stimulation of the ear acupoint Shenmen and acupoint Tongli (HT 5, which was not used in the present study), but did not last long in one case [Litscher et al. 2012a, Wang et al. 2011b]. Although a significant difference of LF/HF ratio occurred in this study and likewise in previous results [Litscher et al. 2009], Jones et al. [2011] could not see significant changes during postural changes in healthy volunteers who had to lie -10° head-down and then change their position. Nevertheless, they suppose that electro acupuncture might reduce the blood pressure.

Nearly all of the above mentioned HRV studies were performed in China, while

the data was analyzed in Graz, Austria. Chinese volunteers/probands were enlisted. Cultural differences, especially the probably deep belief in Traditional Chinese Medicine, have to be considered. Further on, almost only metal needles and repeated manipulation were used [Litscher et al. 2012a, Wang et al. 2011b, Litscher et al. 2012b]. In two studies, only patients were needled [Wang et al. 2011b, Litscher et al. 2012b]. It is inexplicable if acupuncture stimulation in patients differs from young and healthy volunteers. It could be of importance in following studies to evaluate the appropriate use of acupoints in different study designs.

Within this study, the aim was to visualize temperature changes around the laser acupuncture stimulation point (Neiguan and Shenmen). When searching the scientific database PubMed (www.pubmed.gov), no reviewed publication of temperature effects when stimulating one of the acupuncture points, or both, could be found.
The skin temperature measured by infrared thermography changed highly significantly ($p<0.05$) only during 100 Hz stimulation of the ear acupoint Shenmen. Non-significant changes were found during stimulation with either 2 Hz at the ear acupoint Shenmen nor 2 Hz or 100 Hz at the acupoint Neiguan.
As can be seen in the previous sections and results as well, study results of thermography or thermal images and acupuncture vary considerably. Litscher et al. [2011a] investigated the temperature distribution at the acupoint Dazhui (GV14) during violet (405 nm) laser needle stimulation and could not only demonstrate a significant increase in temperature ($p<0.05$) but also a temperature change at the so-called “far field” area Zhiyang (GV9) which is located proximal at the same meridian. Limitations of the study were the missing stimulation of a non-acupuncture (sham acupuncture) point however. Some studies investigated the temperature distribution of meridian like structures. Schlebusch, Maric-Oehler and Popp [2005] provided information that moxibustion (mugwort placed and burned on an acupoint) of the body leads to the appearance of “light channels”, which can be compared to meridian like

structures as illustrated in textbooks of Traditional Chinese Medicine. On contrast, Litscher [2005] tried to visualize structures which could be connected to meridians, but failed to do so. No biological correlation could be found. Technical reflection artifacts and equipment dependent measurement errors could be objectified and quantified, however [Litscher and Ammer 2007]. In 2009 Agarwal-Kozlowski, Lange and Beck [2009] could show that a significant increase in surface temperature occurred within 2 min after needling the acupuncture point Hegu (Li 4), while needling of a cutaneous or muscular point (sham acupuncture) or no needling manipulation at all, resulted in a decrease of temperature.

Many authors come to similar conclusions concerning thermography and acupuncture. Infrared thermography represents a commonly used and accurate procedure in acupuncture studies to distinguish the effects, especially in studies with placebo needling as a control. Main advantages are the easy to handle and contact-free data acquisition and the realtime visualization, the superior sensitivity, contrast and resolution [Bahr et al. 2007, Agarwal-Kozlowski and Lange and Beck 2009].

The limitations of infrared thermography and acupuncture, and one major critical point, is the missing validity of the underlying mechanisms that lead to surface temperature effects [Agarwal-Kozlowski and Lange and Beck 2009]. In future studies, influence quantities such as skin type, the use of skin creams and most importantly the measurement of placebo or sham acupuncture points as a control, have to be considered.

In conclusion, it can be said that thermographic methods such as infrared cameras at wavelength ranges of 2-5 µm and 7.5-13 µm and other High-Tech methods are effective complementary methods in acupuncture research, which support demystification of this treatment method.

Today, there is still no detailed scientific foundation or evidence of the psychological effects of acupuncture on volunteers or patients. Furthermore, to evaluate the phys-

iological basis, the sympathetic activity (e.g. hormone release, micro neurography) and the endogenous opioid release should be investigated further. It is inevitable to investigate the multimodal way Chinese medicine focuses on. In China, acupuncture is usually combined with tuina, dietetics, herbal medicine and qi gong while in western acupuncture trials only specific symptoms undergo examination [Agarwal-Kozlowski and Lange and Beck 2009]. In 1984 Feinstein suggested that double-blind placebo-controlled clinical trials are gold standard for demonstrating specific effects over placebo. Yet in acupuncture studies double-blinding is hardly achievable. The acupuncturist has to be aware of the applied method [Vincent and Lewith 1995]. The main focus in future laser acupuncture studies should be on the study design.

5 Conclusion

For the first time, different quantification methods were used within this study to evaluate physiological alterations and effects on the human body when using two different laser acupuncture frequencies. The Arteriograph™, the HRV medilog® AR12, and the infrared thermography seem to be devices for a proper approach towards effects like blood pressure, pulse wave velocity and temperature effects around stimulation areas during acupuncture. They offer good reliability and reproducibility and are non-invasive. In the future, further and larger studies have to be conducted to consolidate results in laser acupuncture though. There are still too many variables concerning the study design and open questions concerning the physiological aspects of acupuncture and its effect on pathological changes.

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