

Dissertation / Thesis

**PREVENTION AND EARLY INTERVENTION
OF LIFESTYLE-RELATED DISEASES
BY SPECIAL EVIDENCE-BASED
COMPLEMENTARY MEDICINE METHODS**

submitted by

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Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig angefertigt und abgefasst, und jene Personen und Institutionen, die am Zustandekommen der Forschungsdaten beteiligt waren, namentlich genannt habe. Andere als die angegebenen Quellen habe ich nicht verwendet und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen habe ich als solche kenntlich gemacht. Die Arbeit an der Dissertation und daraus entstandener Publikationen wurde gemäß der Regeln der „Good Scientific Practice“ durchgeführt.

Graz, am 20.04.2015

Unterschrift: _____

Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of “Good Scientific Practice”.

Graz, April 20th, 2015

Signature: _____

Foreword

The Doctoral programme in Medical Sciences at the Medical University of Graz offers us as students many different topics in the areas of biomedical and clinical research. The Doctoral School for Lifestyle-Related Diseases, which I have chosen, is under the supervision of Assoz.Prof. Priv.-Doz. Mag. Dr.rer.nat. Sandra Wallner-Liebmann and offers a wide range of exciting fields of research.

In the present thesis six scientific publications (six chapters) related to the topic “Prevention and early intervention of lifestyle-related diseases by special evidence-based complementary medicine methods“ are introduced, all of which have already been published [Ref. 1-6].

Research on this topic is very important, because lifestyle-related diseases are a big problem worldwide. For example stroke is one of the leading causes of death and also dementia is a big problem for our population, because of the movement in the age pyramid. One of the studies (chapter 1), which is part of my dissertation, deals with the topic hypertonic blood pressure, which is related to many of those lifestyle-related diseases. In this first trial worldwide using yellow laser acupuncture, we investigated the effects of yellow laser stimulation on blood pressure in volunteers.

During the preparation of my thesis I had the opportunity to give many international speeches about that topic for example in Athens (Greece), Beverungen (Germany), Hangzhou (China), Nanjing (China), Beijing (China), or Toronto (Canada). A poster presentation in Porto (Portugal) was also part of my dissertation.

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Table of Contents

STATUTORY DECLARATION	I
FOREWORD	II
ACKNOWLEDGEMENTS	III
TABLE OF CONTENTS	IV
ABBREVIATIONS AND DEFINITIONS	VII
ABSTRACT IN GERMAN	IX
ABSTRACT IN ENGLISH	XI
<u>GENERAL INTRODUCTION TO THE CHAPTERS 1 TO 6 [REF. 1-6]</u>	1
<u>1 YELLOW LASER ACUPUNCTURE – A NEW OPTION FOR PREVENTION AND EARLY INTERVENTION OF LIFESTYLE-RELATED DISEASES: A RANDOMIZED, PLACEBO-CONTROLLED TRIAL IN VOLUNTEERS</u>	5
1.1 INTRODUCTION	5
1.2 SUBJECTS AND METHODS	7
1.2.1 SUBJECTS	7
1.2.2 YELLOW LASER STIMULATION AND ACUPOINTS	7
1.2.3 TEMPERATURE MEASUREMENTS	9
1.2.4 ELECTROCARDIOGRAPHIC MEASUREMENTS	10
1.2.5 PROCEDURE	10
1.2.6 STATISTICAL ANALYSIS	11
1.3 RESULTS	11
1.4 DISCUSSION	18
1.5 CONCLUSION	21
1.6 REFERENCES	22
<u>2 LASER THERAPY AND STROKE: QUANTIFICATION OF METHODOLOGICAL REQUIREMENTS IN CONSIDERATION OF YELLOW LASER</u>	25
2.1 INTRODUCTION	25
2.2 MATERIALS AND METHODS	26
2.2.1 STATISTICAL ANALYSIS	27
2.3 RESULTS	27
2.4 DISCUSSION	28
2.5 CONCLUSION	30
2.6 REFERENCES	30

<u>3</u>	<u>LASER THERAPY AND DEMENTIA: A DATABASE ANALYSIS AND FUTURE ASPECTS ON LED-BASED SYSTEMS</u>	34
3.1	INTRODUCTION	34
3.2	CURRENT SCIENTIFIC RESEARCH	35
3.3	FUTURE ASPECTS—LED-BASED SYSTEMS	39
3.4	CONCLUSION	42
3.5	REFERENCES	43
<u>4</u>	<u>INHIBITORY EFFECTS OF 658 NM LASER IRRADIATION ON SKIN TEMPERATURE IN ANESTHETIZED RATS: PRELIMINARY RESULTS FROM A CONTROLLED STUDY</u>	46
4.1	INTRODUCTION	46
4.2	MATERIALS AND METHODS	46
4.2.1	ANIMALS	46
4.2.2	ANESTHESIA	47
4.2.3	LASER ACUPUNCTURE AND ACUPOINTS	48
4.2.4	TEMPERATURE MEASUREMENTS	48
4.2.5	PROCEDURE	49
4.2.6	STATISTICAL ANALYSIS	50
4.3	RESULTS	50
4.4	DISCUSSION	54
4.5	REFERENCES	57
<u>5</u>	<u>THE INFLUENCE OF NEW COLORED LIGHT STIMULATION METHODS ON HEART RATE VARIABILITY, TEMPERATURE, AND WELL-BEING: RESULTS OF A PILOT STUDY IN HUMANS</u>	59
5.1	INTRODUCTION	59
5.2	MATERIALS AND METHODS	59
5.2.1	SUBJECTS	59
5.2.2	NEW COLORED LIGHT STIMULATION METHODS	60
5.2.3	TEMPERATURE MEASUREMENTS	61
5.2.4	ELECTROCARDIOGRAPHIC MEASUREMENTS	62
5.2.5	PROCEDURE	62
5.2.6	STATISTICAL ANALYSIS	63
5.3	RESULTS	63
5.4	DISCUSSION	67
5.5	REFERENCES	70
<u>6</u>	<u>SPECTROSCOPIC MEASUREMENTS IN PATIENTS WITH COMPLETELY RUPTURED ANTERIOR CRUCIATE LIGAMENT BEFORE AND AFTER REGENTK AND PHYSIOTHERAPY</u>	72

6.1	INTRODUCTION	72
6.2	MATERIALS AND METHODS	73
6.2.1	REGENTK	73
6.2.2	PHYSIOTHERAPY	73
6.2.3	PATIENTS	74
6.2.4	EVALUATION PARAMETERS	75
6.2.5	PROCEDURE	75
6.2.6	STATISTICAL ANALYSIS	76
6.3	RESULTS	76
6.4	DISCUSSION	79
6.5	REFERENCES	81
7	<u>LIST OF REFERENCES</u>	84

Abbreviations and Definitions

°C	degree Celsius
%	percent
\$	dollar
µm	micrometre
a.u.	arbitrary units
Aβ	amyloid-beta
ACL	anterior cruciate ligament
AD	Alzheimer's disease
AM	ante meridiem
ANOVA	analysis of variance
APP	amyloid precursor protein
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BP	blood pressure
bpm	beats per minute
CA	corpora amylacea
cm	centimetre
CREB	CRE-binding protein
cun	a traditional Chinese unit of length
DW	dominant wavelength
e.g.	exempli gratia (for example)
ECG	electrocardiogram
EEG	electroencephalogram
EGCG	epigallocatechin gallate
EIT	electromagnetically induced transparency
et. al.	et alii (Maskulinum) / et aliae (Femininum)
etc.	et cetera
Fig.	figure
g	gram
G6PD	glucose-6-phosphate dehydrogenase
GmbH	Gesellschaft mit beschränkter Haftung (company with limited liability)

h	hour
HR	heart rate
HRV	heart rate variability
Hz	Hertz
i.e.	id est
kg	kilogram
LED	light-emitting diode
LF/HF	low frequency / high frequency
LLLT	low-level laser therapy
LPLI	low-power laser irradiation
min	minutes
mmHG	millimetre of mercury
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
mW	milliwatt
n.s.	not significant
NIRS	near infrared spectroscopy
nm	nanometre
p-value	level of significance
PDT	photodynamic therapy
QIT	quantum optical induced transparency
RegentK	regeneration therapy by Khalifa
ROI	region of interest
rSO ₂	regional oxygen saturation
s	significant
SD	standard deviation
SE	standard error
Tab.	table
TCM	Traditional Chinese Medicine
TF	transmission factor
USA	United States of America
vs.	versus
YFP	yellow fluorescent protein

Abstract in German

Zivilisationskrankheiten sind weltweit ein großes Problem. Schlaganfall ist beispielsweise einer der Haupttodesursachen. Diese Dissertation beschäftigt sich mit der Grundlagenforschung über die Prävention und Frühintervention von Zivilisationskrankheiten mithilfe spezieller evidenzbasierter komplementärmedizinischer Methoden.

Das erste Kapitel handelt vom Einfluss gelber Laserstimulation auf Bluthochdruck. Dafür wurden an 26 gesunden ProbandInnen an der Medizinischen Universität Graz unter anderem Parameter wie Blutdruck (BP), Herzfrequenz (HR), Herzfrequenzvariabilität (HRV) und Temperatureffekte vor, während und nach der Stimulation verschiedener Akupunkturpunkte (Baihui, Neiguan, Taichong, Placebo-Punkt) mit gelbem Laser untersucht. Es resultierte eine signifikante Abnahme des systolischen Blutdrucks nach der Laserstimulation, wohingegen man eine Erhöhung der HRV wahrnehmen konnte. Die Temperatur sank während der Stimulation an allen Punkten signifikant. Danach war wieder ein signifikanter Anstieg erkennbar.

Auch Lasertherapie bei Schlaganfall ist ein wichtiges Thema. Niemand kennt derzeit im Detail die Laserparameter, die am intakten Schädel ausgewählt werden müssen, sodass das Laserlicht seine potentielle Wirkung im menschlichen Schädel entfalten kann. In der vorliegenden Studie in Kapitel 2 wird gezeigt, dass die Werte für die Wellenlängen 810 nm und 658 nm gut mit früheren Studien korrelieren, jedoch wurde die Information über den gelben Laser von anderen AutorInnen um einen Faktor von zehn falsch geschätzt.

Kapitel 3 beinhaltet eine Datenbankanalyse (PubMed, Cochrane Library), um das Profil der Publikationstätigkeit zum Thema Demenz zu untersuchen. Es wurden über 600 Arbeiten zu "Demenz und Laser" gefunden. In diesem Kapitel werden auch erste Ergebnisse zur Messung des Durchlässigkeitsfaktors durch den menschlichen Schädel mit einem neuen LED- (lichtemittierende Dioden-) basierten System vorgestellt. Die Messungen zeigen, dass das LED-System

(unter Verwendung des QIT (quantenoptisch induzierte Transparenz) Effekts) bei PatientInnen mit Demenz eingesetzt werden kann.

Im folgenden Kapitel 4 geht es um eine tierexperimentelle Studie an Ratten. Ziel war es, zu untersuchen, wie rote Laserstimulation die Temperatur von 12 narkotisierten Ratten (6 bei ausgeschaltetem Laser) an verschiedenen Akupunkturpunkten (Baihui, Zusanli, Kontrollpunkt) beeinflusst. Es kam zur signifikanten Abnahme der Temperatur an Baihui und Zusanli (beidseitig). Am Kontrollpunkt trat kein signifikanter Temperatureffekt auf.

Kapitel 5 dieser Dissertation handelt von neuartiger Farblichtstimulation. Das Ziel dieser Pilotstudie war es, an sieben gesunden ProbandInnen akute Temperatur, HR, HRV und Gemütszustand nach der Bestrahlung mit rotem und blauem Licht zu untersuchen. Signifikante Rückgänge waren nach 10 min Blaulichtbestrahlung an der Nasentemperatur, HR und HRV zusammen mit einem signifikant besseren Gemütszustand zu verzeichnen. Die Stimulation mit rotem Licht an den gleichen ProbandInnen bewirkte nicht dieselben Effekte.

In der Publikation aus Kapitel 6 wurden die akuten Wirkungen einer speziellen manuellen Therapie (RegentK[®]) mit jenen der Standard-Physiotherapie auf die regionale Sauerstoffsättigung (rSO₂) im Kniegewebe bei PatientInnen mit vollständig gerissenem vorderen Kreuzband verglichen. Zwanzig PatientInnen wurden in die Studie eingeschlossen, randomisiert und erhielten entweder RegentK[®] oder Physiotherapie. rSO₂ war nach RegentK[®] auf beiden Seiten des verletzten Knies (anterolateral, anteromedial) signifikant erhöht, während nach der Physiotherapie nur die anterolaterale Seite eine signifikante Erhöhung zeigte.

Die sechs Kapitel dieser Dissertation heben die vielfältigen Möglichkeiten der komplementären und integrativen Medizin deutlich hervor. Im Einzelnen wurde wichtige Grundlagenforschung in den Bereichen Laser-Akupunktur, Laser- und Lichttherapie sowie manuelle und Physiotherapie durchgeführt.

Abstract in English

Lifestyle-related diseases are a big problem all over the world. For example stroke is one of the leading causes of death worldwide. This thesis is about basic research concerning prevention and early intervention of lifestyle-related diseases by special evidence-based complementary medicine methods.

The first chapter deals with yellow laser stimulation and the influence on high blood pressure. Among other parameters blood pressure (BP), heart rate (HR), heart rate variability (HRV), and temperature effects before, during, and after stimulation of different acupoints (Baihui, Neiguan, Taichong, placebo point) with yellow laser were investigated in 26 healthy volunteers at the Medical University of Graz. There were significant decreases after yellow laser acupuncture in the systolic BP, whereas HRV increased. The temperature during the yellow laser stimulation decreased significantly at all measured points. After the stimulation it increased again significantly.

Also laser therapy in stroke is a very important topic. No one currently knows in detail the laser parameters that have to be chosen on the intact skull, so that the laser light can develop its possible efficacy inside the human skull. The present study in chapter 2 demonstrates that the values for the wavelengths 810 nm and 658 nm correlate well with previous experimental findings however the information on the yellow laser was estimated incorrectly by a factor of ten by other authors.

A database analysis (PubMed, Cochrane Library) to examine the profile of publication activity related to dementia is included in chapter 3 in this thesis. About 600 papers related to the research area “dementia and laser” were found. First results concerning the measurement of the transmission factor through the human skull of a new LED- (light emitting diode-) based system are presented. The measurements show that this LED system (using the QIT (quantum optical induced transparency) effect) might be used in patients with dementia.

The following chapter 4 is about an animal experimental trial in rats. The goal of this study was to investigate how red laser stimulation influences the temperature of 12 anesthetized rats (6 with deactivated laser) at different acupuncture points and non acupoints (Baihui, Zusanli, control point). 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.

Chapter 5 of this doctoral thesis is about new colored light stimulation. The aim of this pilot study was to investigate acute temperature, HR, HRV, and state of mind reactivities after illumination with red and blue light in seven healthy volunteers. Significant decreases were found only after 10 min blue light stimulation in nose temperature, HR, and total HRV, in association with a significant alteration of the emotional state. However, red light stimulation of the same persons did not induce the same effects in these parameters.

In the publication of chapter 6, acute effects of a special kind of manual therapy (RegentK[®]) on regional oxygen saturation (rSO₂) of knee tissues in patients with completely ruptured anterior cruciate ligament (ACL) were compared to standard physiotherapy. Twenty patients were enrolled in this study and randomly assigned to receive either RegentK[®] or physiotherapy. RSO₂ increased significantly after RegentK[®] on both registration sites of the injured knee (anterolaterally, anteromedially), whereas after physiotherapy only the anterolateral side showed significant increases.

The six chapters of the thesis clearly show the manifold possibilities of complementary and integrative medicine methods. In detail important basic research was performed in areas of laser acupuncture, laser and light therapy and manual and physiotherapy.

General Introduction to the chapters 1 to 6 [Ref. 1-6]

This present doctoral thesis deals with so called lifestyle-related diseases and their possible prevention and early intervention with complementary medicine methods. Six scientific papers are presented in chapters 1-6 [Ref 1-6].

The single parts contain many different aspects concerning lifestyle-related diseases. The first chapter deals with hypertension and the influence of yellow laser. The yellow laser reflects a totally new option in the field of laser acupuncture, in addition to the already existing red, near infrared, green and violet lasers. This is the first study worldwide on the topic yellow laser and acupuncture. Especially for so called lifestyle-related diseases, this could open up new methods of integrative therapy. The goal of the study, described in chapter 1, was to investigate among other parameters blood pressure (BP), heart rate (HR), heart rate variability (HRV), and temperature effects before, during, and after stimulation of different acupoints with yellow laser. We recruited 26 healthy volunteers (13 female, 13 male; mean age \pm SD: 24.1 \pm 3.3 years) at the Medical University of Graz. The acupoints Baihui (GV 20), Neiguan (PC 6), Taichong (LR 3) and in addition a placebo point were stimulated with a 589 nm (50 mW, 500 μ m; 5 min) yellow laser. Blood pressure was measured noninvasively at the wrist; for the registration of the electrocardiogram a medilog AR12 HRV system partially developed in Graz was used. Effects on temperature were measured with a Flir i7 infrared camera. There were significant decreases after yellow laser acupuncture in the systolic BP, diastolic BP also decreased (n.s.). HRV in both (men and women) increased. The temperature during the yellow laser stimulation decreased significantly in all measured points. After the stimulation it increased again significantly. Based on a questionnaire volunteers reported a significantly decreased level of stress after yellow laser stimulation. Significant positive effects on BP and well-being were found after yellow laser stimulation. The results are very promising and can be very important especially for the treatment of lifestyle related diseases [Ref. 1].

Not only high blood pressure is associated with lifestyle-related diseases, also stroke is a very important topic. In chapter 2 different new aspects concerning this

area of research are discussed. Every year more than 600,000 Europeans suffer a stroke. This burdens the health system and it can be assumed that the incidence of stroke will increase in the coming decades. In addition to conventional methods of drug therapy, stroke is sometimes treated with highly invasive methods. The non-invasive laser procedures on the other hand operate through the bony skull. Large-scale laser studies are being conducted with varying degrees of success around the world, and without the certainty that the corresponding laser beam even penetrates the bone. No one currently knows in detail the laser parameters that have to be chosen on the intact skull, so that the laser light can develop its possible efficacy inside the human skull. The present study in chapter 2 demonstrates that the values for the wavelengths 810 nm and 658 nm correlate well with previous experimental findings however the information on the yellow laser was estimated incorrectly by other authors by a factor of ten. Further research on the topic is important so that one can be sure to apply the correct wavelength and parameters. This can open up new dimensions in transcranial laser therapy, not only in stroke patients [Ref. 2].

The first two chapters of this doctoral thesis are about basic research. In chapter 3 a database analysis to examine the profile of publication activities related to dementia is demonstrated. Mainly because of the movement in the age pyramid, one can assume that the incidence of Alzheimer's disease or dementia in general will increase in the coming decades. Within this chapter 3, two databases were searched: PubMed and Cochrane Library. About 600 papers related to the research area "dementia and laser" and about 450 papers related to the search terms "Alzheimer and laser" were found in these two most commonly used databases. Ten plus one papers are described in detail and are discussed in the context of the integrative laser research performed at the Medical University of Graz. First results concerning the measurement of the transmission factor (TF) through the human skull of a new LED- (light emitting diode-) based system are presented ($TF = 0.0434 \pm 0.0104$ (SD)). The measurements show that this LED system (using the QIT (quantum optical induced transparency) effect) might be used in future studies in the treatment of dementia [Ref. 3].

The following chapter 4 is an animal experimental trial in rats. 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 non acupoints. 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 μ m, 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 [Ref. 4].

An important parameter of well-being and therefore for prevention of lifestyle-related diseases is light. Chapter 5 of this doctoral thesis is a question of new colored light stimulation. Changes of light intensity of different colors can shift many physiological parameters and conditions like melatonin, alertness, body temperature, HR, and HRV. The aim of this pilot study was to investigate acute temperature, HR, HRV, and state of mind reactivities after illumination with red (631 nm) and blue (456 nm) light (illuminance 140 lux for both). Seven healthy volunteers (5 females, 2 males; mean age \pm SD: 34.1 \pm 11.9 years) were investigated at the Medical University of Graz, using new color light panels. Significant decreases were found only after 10 min blue light stimulation in nose temperature ($p=0.046$), HR ($p<0.05$), and total HRV ($p=0.029$), in association with a significant alteration of the emotional state (stress level score, $p=0.006$). However, red light stimulation of the same persons did not induce the same effects in these parameters. The effect of blue light as environmental stimulation on human health is still not clarified in detail and needs further investigations [Ref. 5].

The last chapter 6 of this doctoral thesis also deals with a very interesting topic which can also be related to lifestyle diseases. A complete rupture of the anterior cruciate ligament (ACL) occurs among other reasons very often during alpine skiing or playing football. Therefore it's also a big problem worldwide and needs further attention. The goal of the study described in the last chapter was to investigate acute effects of a special kind of manual therapy developed by Mohamed Khalifa (RegentK[®]), compared to standard physiotherapy, on regional oxygen saturation (rSO₂) of knee tissues in patients with completely ruptured ACL. Twenty patients were enrolled in this study and randomly assigned to receive either RegentK[®] (group A; n = 10, 8 female, 2 male, mean age ± SD: 31.3 ± 8.5 years) or physiotherapy (group B; n = 10, 6 female, 4 male, mean age 34.8 ± 10.2 years). The values of rSO₂, assessed using an INVOS 5100c near-infrared spectroscopic equipment, were registered anterolaterally and anteromedially on both the injured and the healthy (control) knee. The results showed that rSO₂ increased significantly (p<0.001) after RegentK[®] on both registration sites of the injured knee, whereas after physiotherapy only the anterolateral side showed significant increases. Interestingly, we found significant increases in rSO₂ also on the control knee after RegentK[®]; in group B, these results were insignificant. In conclusion it can be stated that manual therapy influences the hemodynamics of muscles and deeper structures. The results will also serve to quantify the effects of nonpharmacological and nonsurgical interventions (e.g. RegentK[®]) on the microvascular circulation in deep tissue after a complete rupture of the ACL [Ref. 6].

1 Yellow Laser Acupuncture – A New Option for Prevention and Early Intervention of Lifestyle-Related Diseases: A Randomized, Placebo-Controlled Trial in Volunteers

[Ref. 1; D. Litscher et al.]

1.1 Introduction

In many areas, laser is nowadays a very important instrument. It has become almost irreplaceable in data storage, laser printers, signal processing and amplification in optical fiber networks, biomaterial processing or medical measurement technology. The city of Graz in Austria, Europe, is a very special place when lasers are concerned. The oldest still existing, functional laser of Austria was built almost exactly 50 years ago by Franz Aussenegg, who at that time was a young, dynamic physicist and developed this laser together with a Swiss company. At first, the ruby laser was used in research for five years in Graz. Afterwards the laser was used in student trainings (Fig. 1.1) [1].

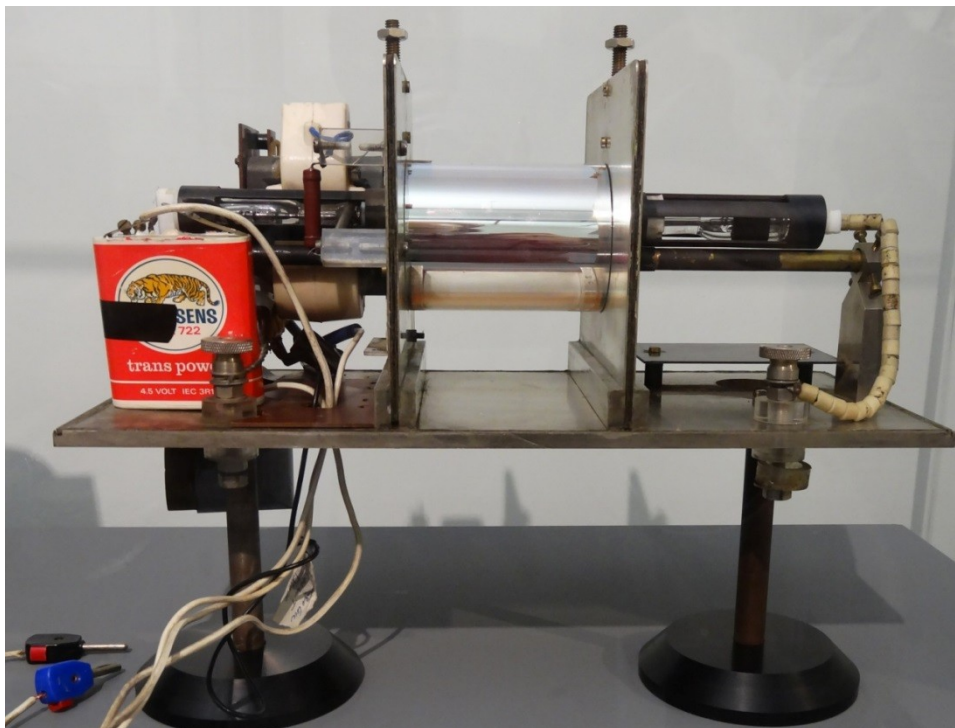


Fig. 1.1: First laser in Graz developed by F. Aussenegg (December 1964).

After several years of research and development, the first yellow laser for medical purposes was constructed in Germany in 2014. This system is available for non-invasive and/or interstitial acupuncture treatment at the Medical University of Graz, where it is being used for evidence-based medical research at the Research Center for Traditional Chinese Medicine (Fig. 1.2) [2]. Yellow laser constitutes a new option in the field of laser acupuncture, in addition to the already existing red, near infrared, green and violet lasers. First evidence suggests that the yellow laser may be able to stimulate the mitochondrial respiratory chain at complex III (cytochrome) [3].

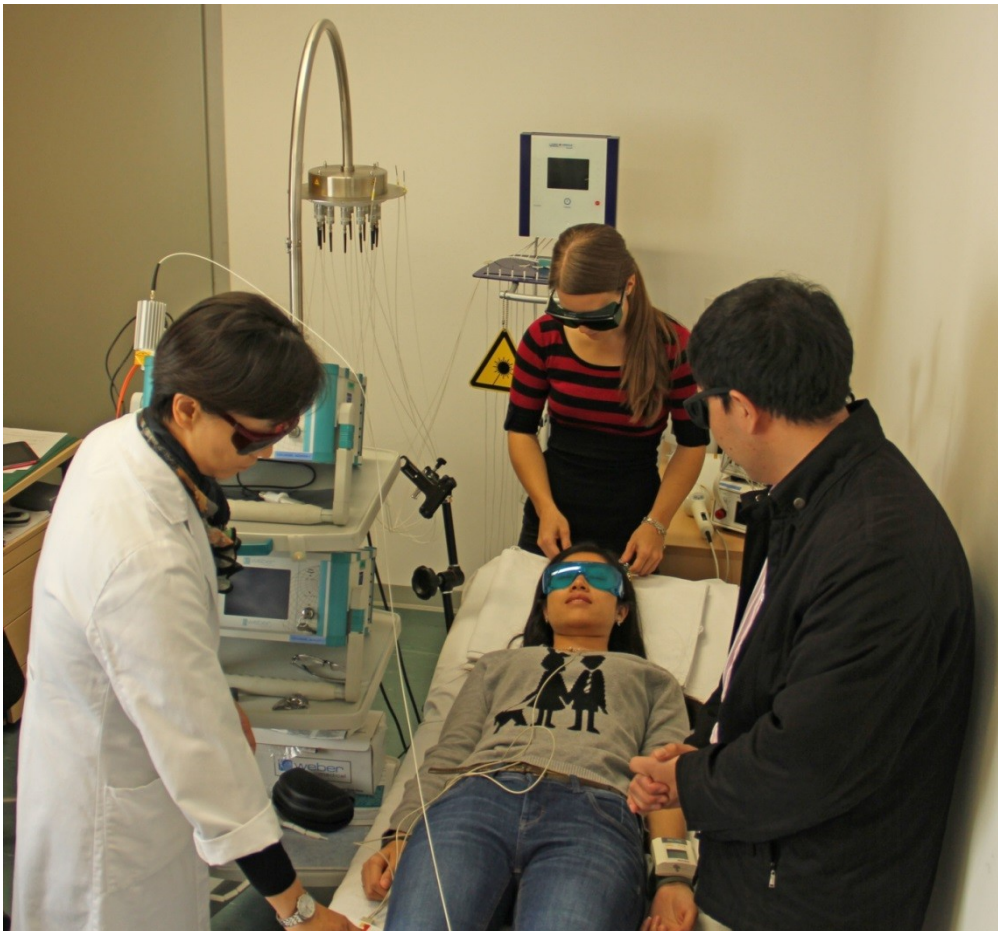


Fig. 1.2: New system for yellow laser needle acupuncture at the Medical University of Graz.

The goal of the present study was to investigate for the first time blood pressure (BP), heart rate (HR), heart rate variability (HRV), and temperature effects before, during, and after stimulation of different acupoints with yellow laser. It should be

mentioned explicitly that this is the first acupuncture study worldwide using yellow laser acupuncture.

1.2 Subjects and Methods

1.2.1 Subjects

We investigated 26 volunteers (13 female, 13 male) with a mean age \pm SD of 24.1 ± 3.3 years, range 20 – 36 years, a height of 175.6 ± 10.6 cm, and a weight of 68.0 ± 10.0 kg at the Medical University of Graz. The 13 female volunteers had a mean age \pm SD of 23.8 ± 1.7 years (range 21 – 28 years), and the 13 male subjects had a mean age of 24.4 ± 4.4 years (range 20 – 36 years), so women and men were age-matched. None of the volunteers had a history of heart or cerebrovascular disease or was under the influence of centrally active medication, and all gave written informed consent. The investigations were approved by the local Ethics Committee and carried out in compliance with the Declaration of Helsinki.

1.2.2 Yellow laser stimulation and acupoints

The yellow laser used for the investigations works with a wavelength of 589 nm and an output power of 50 mW, and the fiber diameter is 500 μ m. A modified Weberneedle Endolaser system (Weber Medical, Lauenförde, Germany) was used. From the technical point of view, it is not easy to produce yellow laser light. Usually, a laser consists of an infrared laser diode and a so-called combo crystal. This pair of crystals produces the visible laser light. In this process, the combo crystal receives the necessary energy from the infrared diode. For a green laser, one crystal produces laser light of 1064 nm and the other one doubles the frequency, which means that the wavelength is divided in half, resulting in 532 nm, i.e. green light. For yellow laser light to be produced, 1340 nm are necessary. If these were simply frequency-doubled, the emitted laser light would be red, but if red and green light is mixed, the result is yellow light. It is, however, a disadvantage that at 1340 nm only very little light is emitted, so the infrared diode needs to have a large power. Moreover, special filters have to be used in order for the correct ratio of 1064 and 1340 nm to permeate. This makes the production

expensive and complex, which is why yellow lasers are very expensive and accordingly rare [1].

Using a worldwide new, highly focussed yellow laser system (589 nm, 50 mW, 500 μ m) we investigated acute effects of laser stimulation at the acupoints Baihui (GV20), Neiguan (PC6), and Taichong (LR3; see Fig. 1.3 a-c) on blood pressure (BP) and heart rate variability (HRV) within a controlled, experimental, randomized biomedical human volunteer study.

Baihui is located at electroencephalographic (EEG) electrode position C_z , on the continuation of the line connecting the lowest and highest points of the ear, on the median line of the head, and it is thought to be a very effective point, with a general sedative and harmonizing effect [4,5]. Neiguan is located at the wrist, between the tendons of m. palmaris longus and m. flexor carpi radialis, 2 cun proximal to the transverse crease of the wrist, and it is a very important point in disorders of the upper abdomen and for heart disorders [4,5]. Taichong is located on the dorsum of the foot, between the first and second metatarsal bones, 2 cun proximal to the margin of the web; it should be a very important point in the treatment of hypertension [4,5].

All volunteers also received active laser stimulation at a placebo point (located lateral from the radius, 6 cun above the horizontal fold of the wrist exactly on the radial ledge, lateral from the pulmonary meridian; Fig. 1.3 d) [6], which makes this a controlled study.

The stimulation of the acupoints was carried out in a randomized order. The hypothesis was that the invisible (special protective goggles) and non perceptible yellow laser stimulation might affect BP, HR and/or HRV.

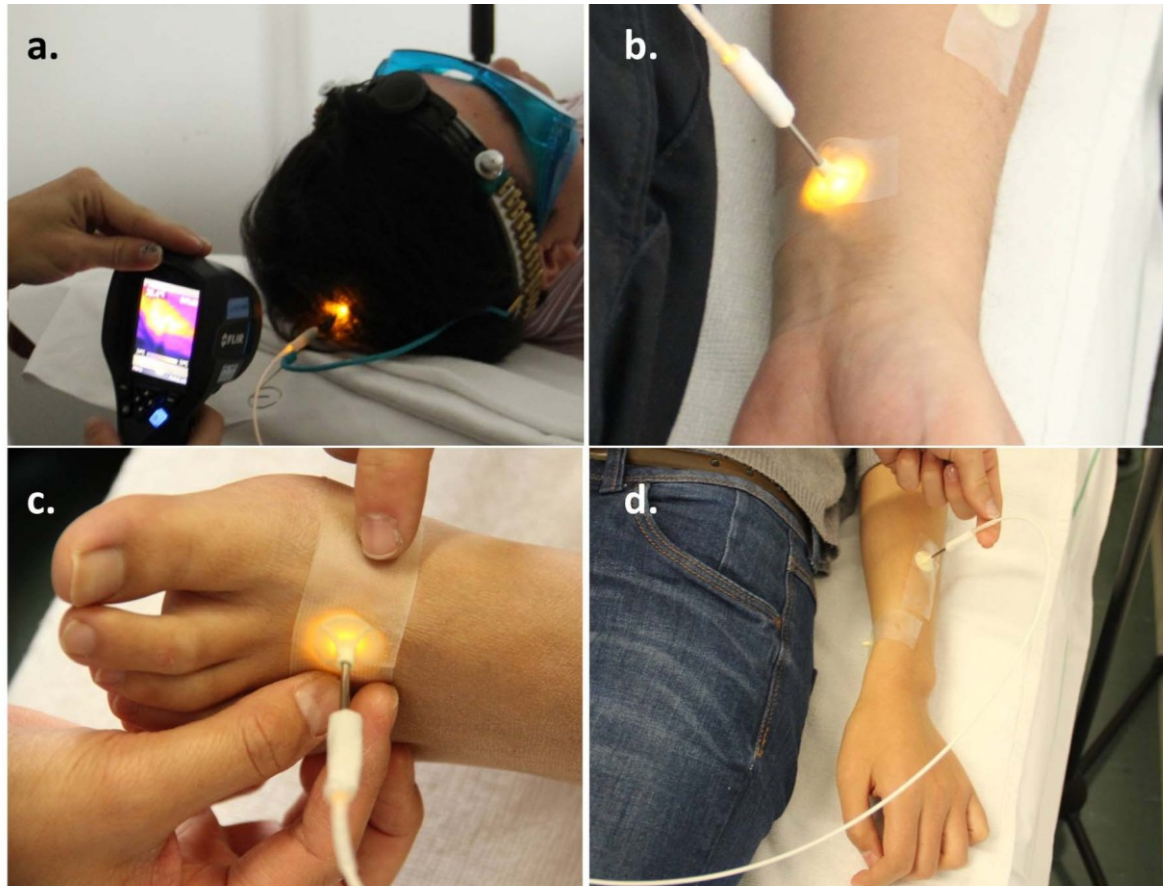


Fig. 1.3: Yellow laser stimulation at the acupoints Baihui (a), Neiguan (b), Taichong (c) and a placebo point (d).

1.2.3 Temperature measurements

For the temperature measurements on the skin surface around the acupoints we used a Flir i7 (Flir Systems, Wilsonville, USA) infrared camera. This thermal imaging camera operates at a wavelength range from 7.5 - 13 μm . The focal distance of the infrared lens is $f=6.8$ mm. The temperature measurement range is between -20 $^{\circ}\text{C}$ and $+250$ $^{\circ}\text{C}$ and the sensitivity is determined <0.1 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C}$. The accuracy of the Flir i7 infrared camera lies at $\pm 2\%$ of the reading and the infrared resolution is 140×140 pixel. The system is ready for use in 15 - 20 seconds after pressing the power-on button. The locations for the thermographic measurements were the areas around the acupoints Baihui, Neiguan, Taichong and the placebo point. In this area, the highest temperature value within the ROI (region of interest) was included in the analysis. All areas were measured before, during, and after stimulation with the yellow laser. The procedure of a thermographic measurement at the point Baihui can be seen in Fig. 1.3 a.

1.2.4 Electrocardiographic measurements

For the electrocardiographic measurements three adhesive electrodes (Skintact Premier F-55; Leonhard Lang GmbH, Innsbruck, Austria) which are applied to the chest, were used. The duration of RR-intervals is measured during time periods of 5 min, and on spectral analysis basis HRV is determined.

For the registration of the electrocardiogram (ECG) a medilog AR12 HRV (Huntleigh Healthcare, Cardiff, United Kingdom) system is used. The system has a sampling rate of 4096 Hz, [7] and the raw data are stored on a memory card. Mean HR, total HRV, and the LF (low frequency)/HF (high frequency) ratio of HRV were chosen as preliminary electrocardiographic evaluation parameters, as such being recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [8].

1.2.5 Procedure

The volunteers were asked to lie down on a bed in one of our labs. Before starting the laser stimulation, BP was measured at the left wrist (blood pressure monitor HGN, Medisana AG, Neuss, Germany; range 0 – 300 mmHg), and a questionnaire about the current stress level was completed by the volunteers. After this procedure, a resting phase of at least 5 min was kept in order to obtain steady-state conditions. After this 5 min period the electrocardiographic recording started (see Fig. 1.4, Resting state). During the last 2 minutes of this phase, thermographic pictures of all points were taken. The first registered 5 min-phase of the ECG thus represents the baseline values. Then, the acupuncture points and the placebo point were stimulated for 5 min each in randomized order; during the stimulation, thermal images were taken of the respective point.

The last 5 min of the ECG recording served as control and resting phase. Again, during the last 2 minutes, thermographic pictures were taken. After a total of 30 minutes, the electrocardiographic recording was stopped. Immediately after stopping the recording, the BP was measured again, and the questionnaire about the stress level was also completed again. The exact procedure can be seen in Fig. 1.4.

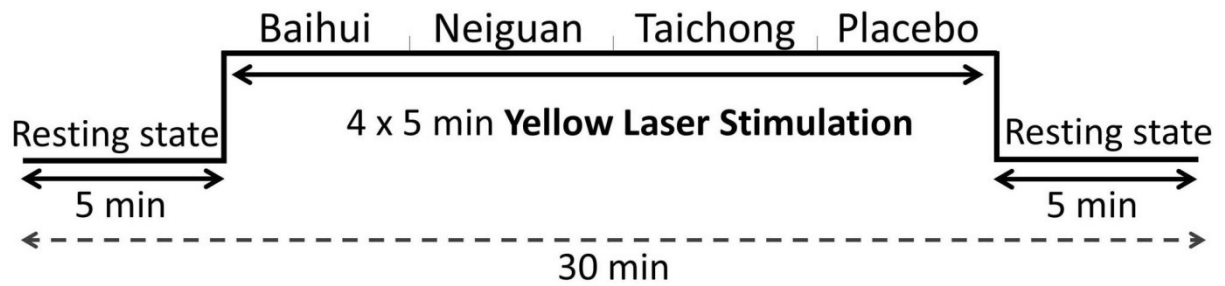


Fig. 1.4: Measurement procedure.

1.2.6 Statistical analysis

Data were analyzed using one-way repeated measures ANOVA or paired t-test. As post hoc analysis, Tukey test was used (SigmaPlot 12.0, Systat , Chicago, USA). The level of significance was defined as $p < 0.05$.

1.3 Results

Figures 1.5 and 1.6 show the results of the BP measurements before and after yellow laser acupuncture at the three acupoints. It can be seen that the systolic BP decreased significantly (Fig. 1.5). The mean diastolic BP also decreased, but not significantly (Fig. 1.6).

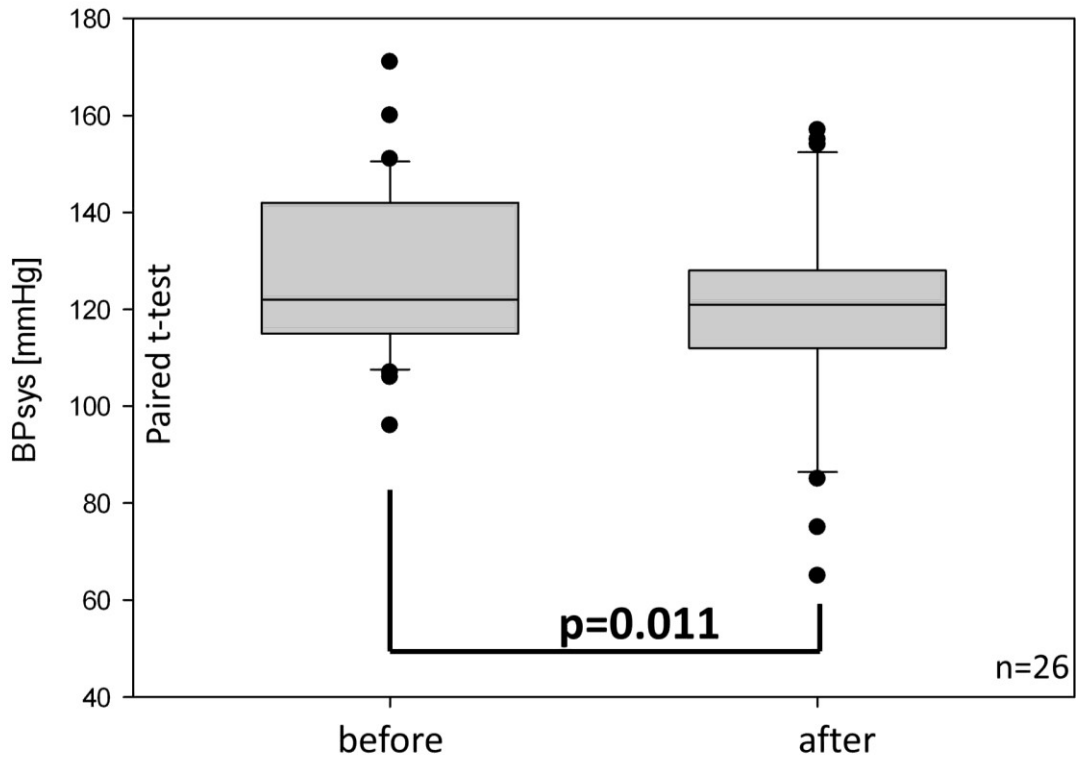


Fig. 1.5: Changes of systolic BP in all 26 volunteers before and after yellow laser acupuncture. The line in the box represents the median, the ends of the boxes the 25th and 75th percentile, and the error bars the 10th and 90th percentile; dots represent outliers.

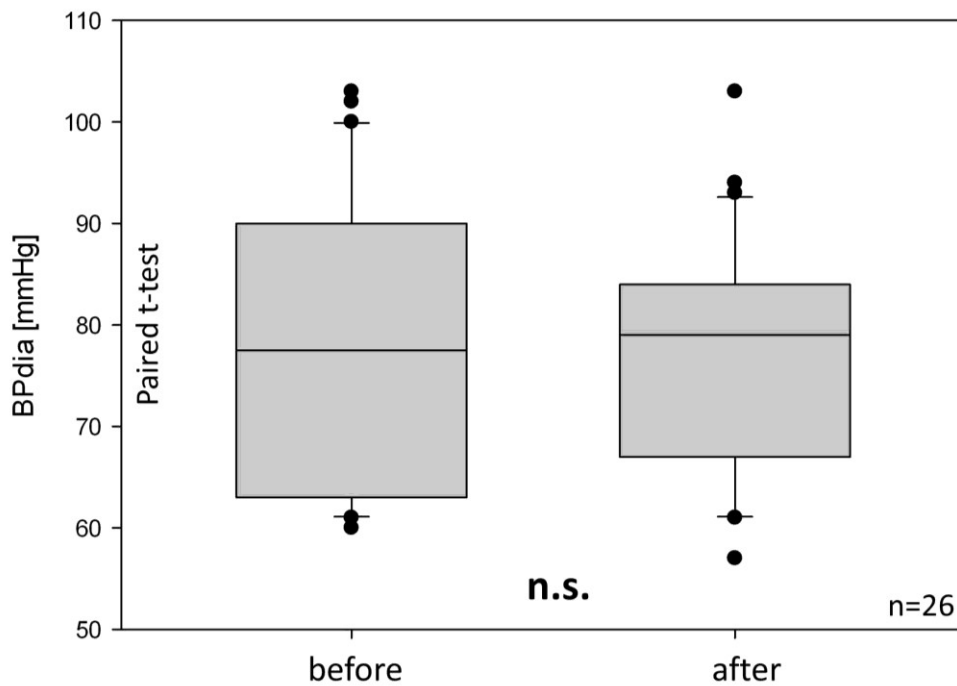


Fig. 1.6: Changes of diastolic BP in all 26 volunteers before and after yellow laser acupuncture. For further explanations, see Fig. 1.5.

In addition to the BP measurements, we also evaluated the mean HR during the whole measurement session. This parameter remained almost constant, although a small decrease from 65.7 bpm (beats per minute) to 64.5 bpm could be seen (see Table 1.1).

We detected HRV during the whole measurement session of 30 min. Table 1.1 shows the decrease (n.s.) of the HRV from the beginning to the end of the measurement. The comparison of female and male related to HR and HRV is also shown in table 1.1.

Table 1.1: HR and HRV values are demonstrated. Note the decrease of HR and the increase of HRV.

	all (n=26)	women (n=13)	men (n=13)
HR start [bpm]	65.7±10.9	64.5±10.8	66.8±11.2
HR Baihui [bpm]	65.0±10.4	63.3±9.5	66.7±11.4
HR Neiguan [bpm]	64.6±10.5	62.9±9.3	66.4±11.6
HR Taichong [bpm]	64.1±9.3	62.4±8.4	65.8±10.2
HR Placebo [bpm]	64.6±10.3	62.9±9.5	66.2±11.1
HR end [bpm]	64.5±10.0	63.0±8.7	66.1±11.2
total HRV start [a.u.]	4085.8±2938.0	3834.1±2696.2	4337.5±3252.2
total HRV Baihui [a.u.]	3899.0±2972.9	3311.9±2383.3	4486.2±3462.1
total HRV Neiguan [a.u.]	4236.2±2972.8	3467.4±2335.6	5005.0±3416.9
total HRV Taichong [a.u.]	4258.6±3613.4	3814.9±3155.4	4702.3±4100.9
total HRV Placebo [a.u.]	4039.2±3077.8	3255.5±2795.3	4822.9±3254.3
total HRV end [a.u.]	4439.6±3367.4	3600.2±2532.4	5279.1±3960.3

Values are given as mean ± standard deviation (SD).

bpm ... beats per minute; a.u. ... arbitrary units

In line with the HRV measurement we also investigated the LF/HF value. The analysis showed that this parameter increased significantly after yellow laser acupuncture (see Fig. 1.7).

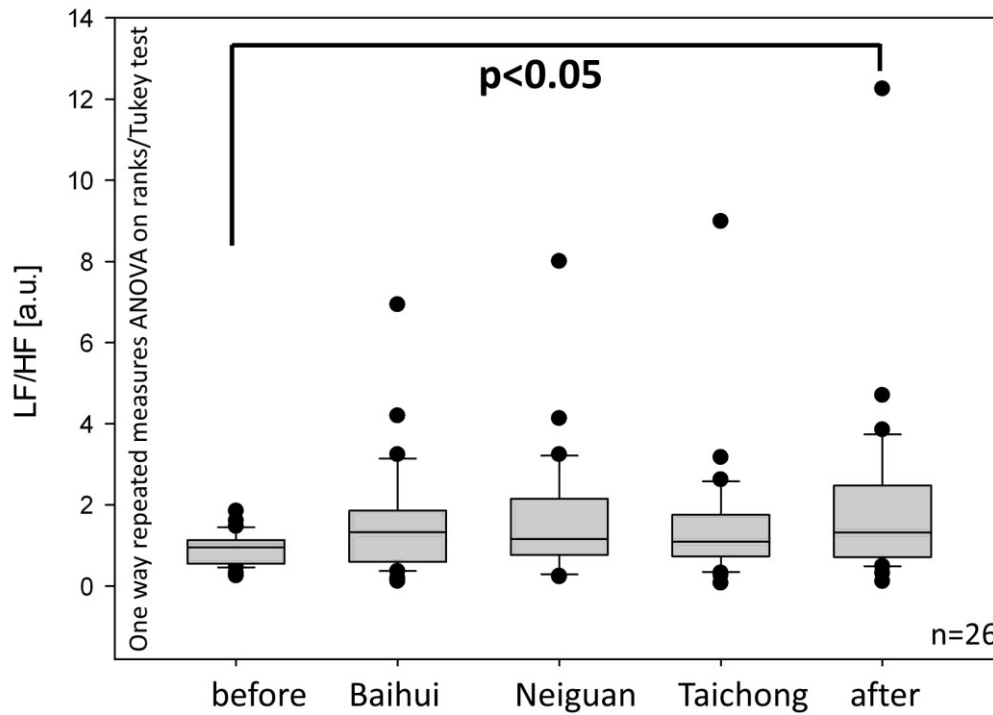


Fig. 1.7: Results of the LF/HF value analysis before and after yellow laser acupuncture. Further explanations: see Fig. 1.5.

Another parameter detected within this study was the skin surface temperature of the subjects. Every volunteer was measured three times at the given acupoints as described already in the methods. The room temperature was nearly constant all over the measurement periods (24 °C).

The summarized data of the thermal imaging can be seen in table 1.2.

Table 1.2: Values of temperature measurements are demonstrated.

Temp. [°C]	Baihui	Neiguan	Taichong	Placebo
before	31.9	33.8	31.5	33.3
during	30.6	31.1	29.0	30.7
after	31.4	33.5	30.7	33.1

Analysis shows a statistical significance in almost every case. There is a statistically significant decrease of temperature during the stimulation (“during” in Table 1.2) in comparison to the beginning (“before”) for every single point. Furthermore, the following increase of the temperature (“after”) is also significant for every point, except Baihui. Here we also detected an increase of temperature after the stimulation, but it was not significant anymore (from 30.6 to 31.4 °C). There is no significance related to the temperature at the starting time vs. after the stimulation.

A typical example of the results of thermal imaging is shown in Fig. 1.8. It shows the temperature decrease (31.4 °C) during the stimulation in comparison to the starting time (34.9 °C) for the ROI of the acupoint Neiguan. The renewed increase (34.1 °C) of the temperature during the resting phase after the stimulation can also be seen. The next figure (Fig. 1.9) shows similar results for the acupoint Taichong. There was also a notable temperature decrease (from 33.4 °C to 30.9 °C) during the stimulation phase and a renewed increase (33.4 °C) of the temperature after the stimulation. The data from Baihui and the placebo point also show similar results (Figs. 1.10 and 1.11).

Neiguan Volunteer 12

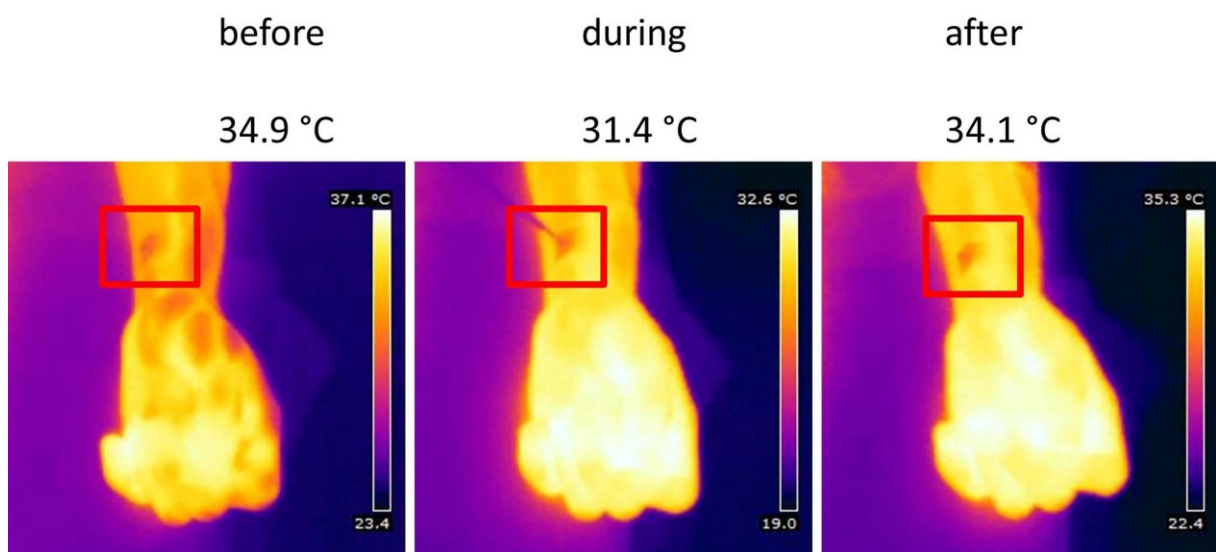


Fig. 1.8: Three thermal images from a 21-year-old male volunteer before, during and after yellow laser stimulation at the acupoint Neiguan.

Taichong Volunteer 10

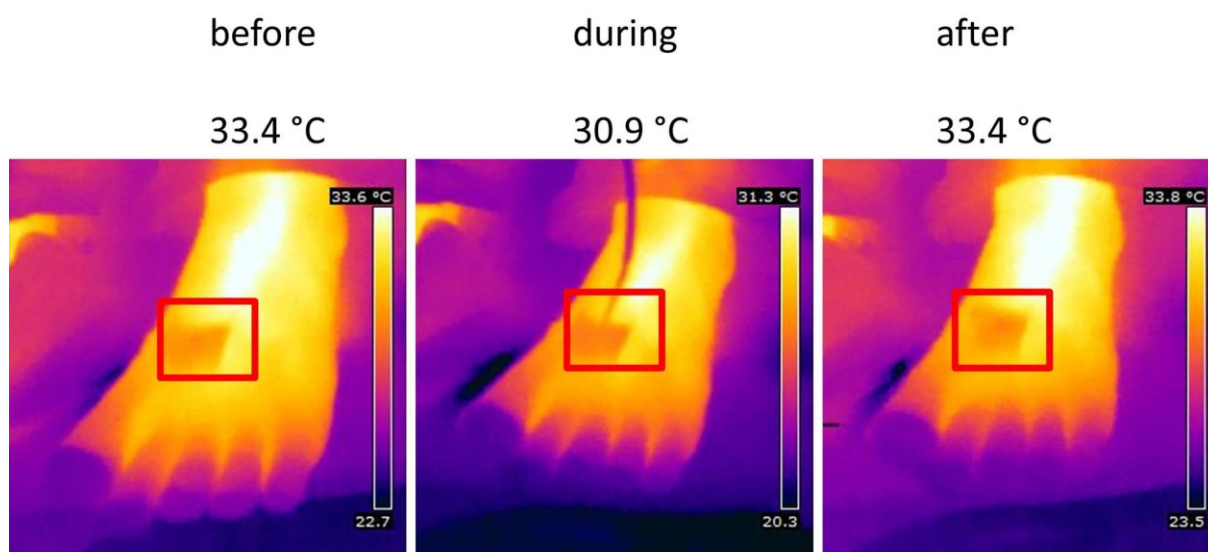


Fig. 1.9: Three thermal images from a 25-year-old male volunteer before, during and after yellow laser stimulation at the acupoint Taichong.

Baihui Volunteer 12

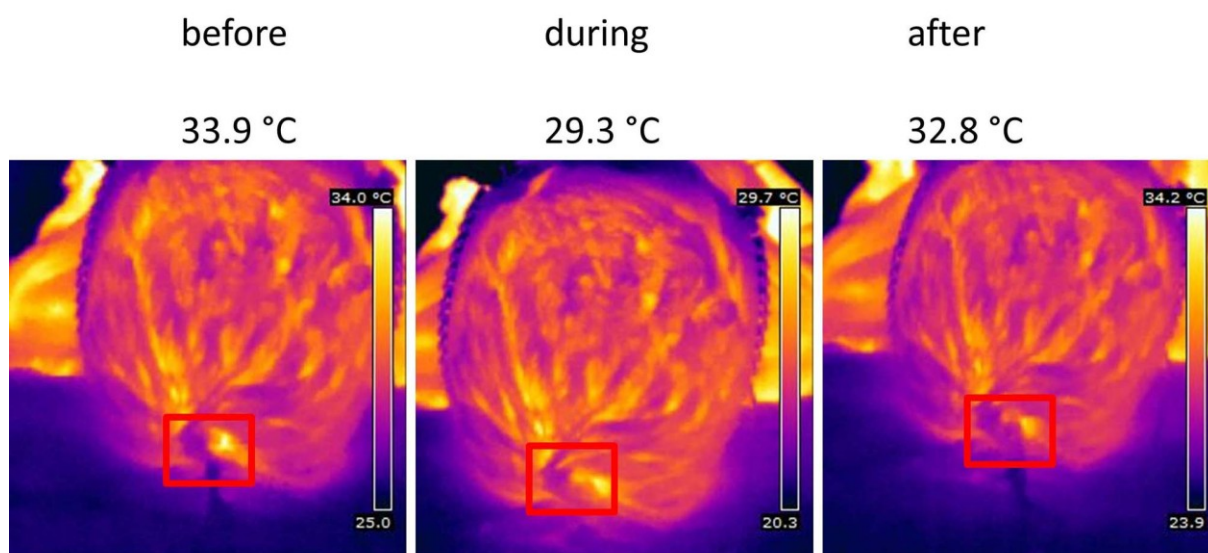


Fig. 1.10: Three thermal images from a 21-year-old male volunteer before, during and after yellow laser stimulation at the acupoint Baihui.

Placebo Volunteer 12

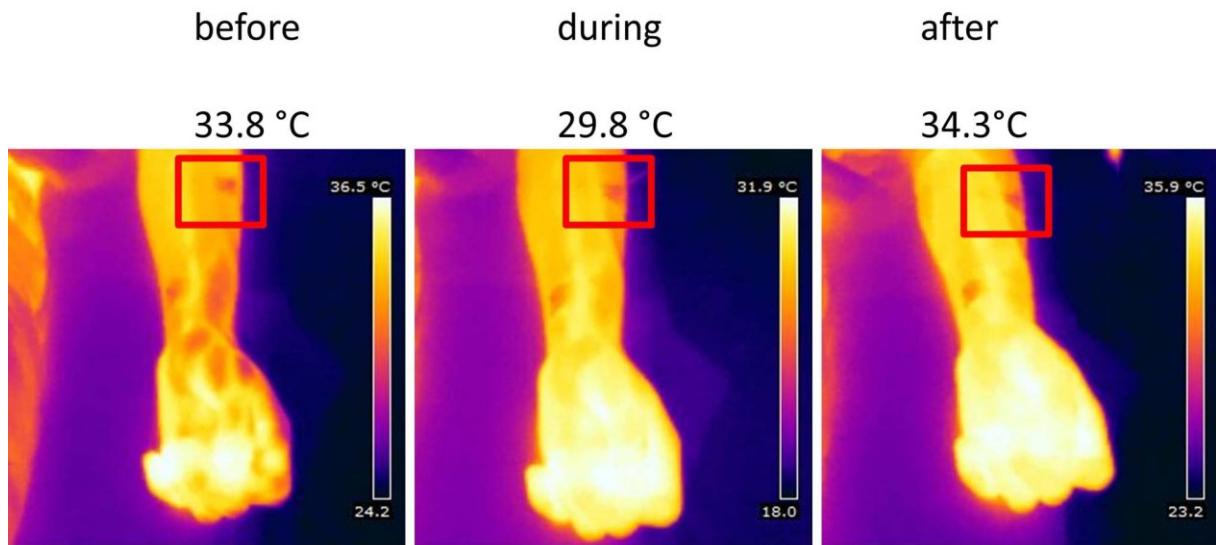


Fig. 1.11: Three thermal images from a 21-year-old male volunteer before, during and after yellow laser stimulation at a placebo point.

In addition to these parameters we also asked the volunteers to fill in a questionnaire about their current stress level. These reports were categorized at a scale from 0 to 10 (0: positive, comfortable, and relaxed; 10: negative, uncomfortable, and anxious). Figure 1.12 shows the results of this questionnaire. As a result it can be stated that the stress level of the volunteers decreased significantly. In addition to the analysis of all 26 volunteers, we also compared the stress level of women and men. For both cases there was a statistical significance related to the decrease of stress shown (f: $p=0.031$; m: $p=0.008$).

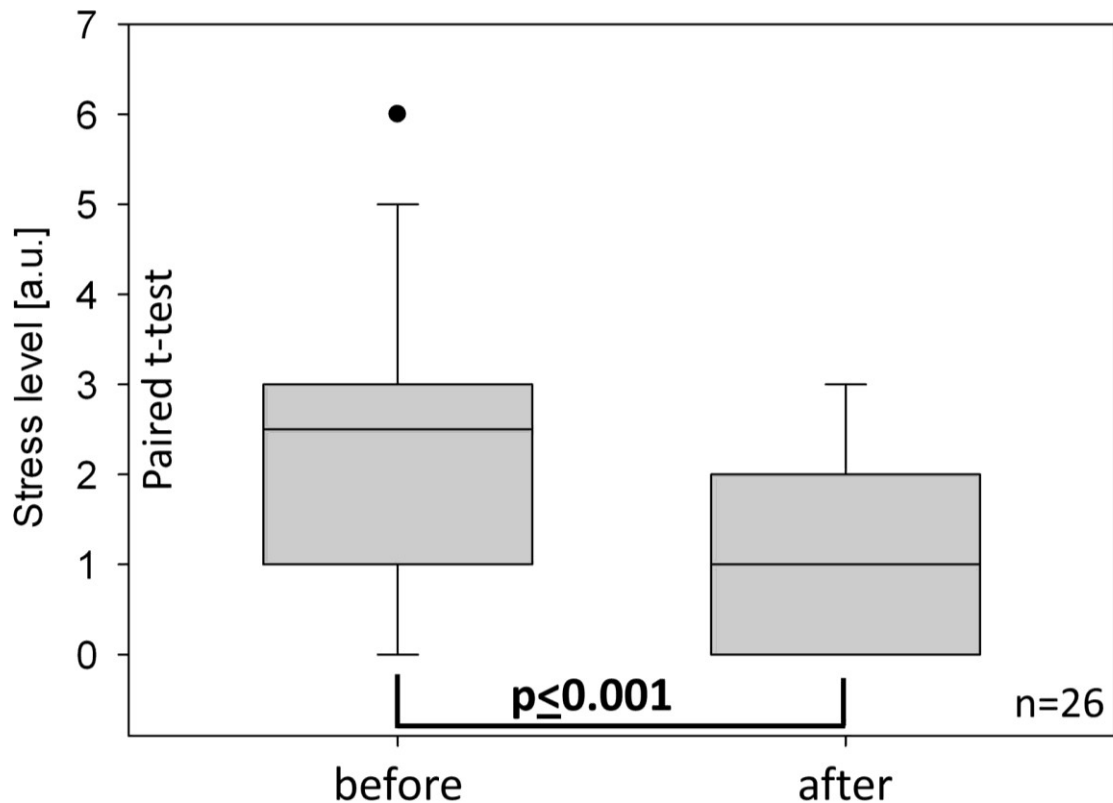


Fig. 1.12: Significant results of the stresslevel questionnaire before and after yellow laser acupuncture. For further explanations, see Fig. 1.5.

1.4 Discussion

Lifestyle-related diseases are a big problem in our society. For example stroke is one of the leading causes of death worldwide. But not only the elderly population is affected by this kind of diseases, also more and more young people are among the patients. A previous study had proven that the yellow laser could penetrate the skull, and therefore might be a promising new approach for the noninvasive stroke therapy [9]. Also dementia, another so called lifestyle-related disease, is a big problem for our population. Mainly because of the movement in the age pyramid, experts assume that the incidence of dementia will show an enormous increase in the coming decades [10].

Another big problem, which is related to many of those lifestyle-related diseases, is hypertonic blood pressure. In our study, which is the first trial worldwide using yellow laser acupuncture, we wanted to investigate the effects of yellow laser on BP in volunteers. A significant decrease of the systolic BP was found. This might be an effect of the combination of all acupoints we used. Therefore one can

assume that stimulation with yellow laser on the acupoints Baihui, Neiguan and Taichong could be useful in the treatment of hypertonic patients. We also compared the difference between female and male volunteers. In women the systolic BP decreased significantly, but not in men. This could appear because women might be more sensitive to the effects of yellow laser. Diastolic BP also decreased, but not significantly. Specific effects of laser stimulation on high BP have been reported in several studies [11].

Hong et al., for example, reported in 2014 about 126 cases of grade 1 essential hypertension [11]. They investigated different treatment methods. Beside a musical treatment group, laser acupuncture was also used once a day with positive effects. Similar to our study, the main acupuncture point was Taichong [11].

In another study, Zhang et al. [12] investigated the influence of laser acupuncture on high blood pressure. The authors from the USA and Europe concluded that low-level infrared laser acupuncture treatment of acupoints Hegu and Quchi resulted in lower blood pressure. Although the acupoints used in this study are different from those in our present investigation, a similar treatment duration (4 min) was used for each point (in our study: 5 min). In addition, Zhang et al. [12] did not observe a significant difference in body weight or HRV after laser acupuncture treatment. They applied laser treatment for 90 days (at least 12 treatments per subject). In our study, only acute (short term) effects were measured. However, we also found significant decreases in BP after only one treatment and no significant changes in total HRV, similar to the results of Zhang et al. [12] In another study, however, our group could provide evidence that there is a beneficial effect on HRV in patients with hypertension, and that there are some effects of laterality of the acupoint Quchi [13].

The demonstration of these short term effects can also have clinical significance; researchers from Russia report about the efficacy of laser acupuncture in controlling hypertensive crises in patients suffering from hypertensive diseases [14]. They also stated that laser therapy was more effective in patients with moderate and significant hypertrophy of the left ventricle compared to patients showing marked hypertrophy of the left ventricle [14].

HRV is used worldwide as a reliable indicator of the state of health. Special lifestyle related processes like stress or burnout could be counteracted by using different preventive methods like acupuncture. This could be demonstrated in previous investigations [15].

The results of our present investigation did not show significant differences regarding the total HRV in general. Nevertheless an overall increase of HRV was notable. This could be explained by the decreasing effect of the yellow laser on BP. Also the combination of the three acupoints should be useful. If we compare all single 5 min fragments, it can be seen that the highest variability is achieved at the end of the measurement and at the acupoints Neiguan and Taichong. For the treatment of hypertensive patients, a combination of the three acupoints and a simultaneous and continuous stimulation over a longer treatment period is therefore recommended.

We also investigated the LF/HF value. This parameter increased significantly. Taking into account the data of the thermographic measurements, there could be some possible explanations related to this. There was a significant temperature decrease during the stimulation and a significant increase after the stimulation. A higher LF/HF value indicates an increased function of the sympatheticus. The decreased surface temperature could be an indicator for a vasoconstriction in the vessels, and because of this there might be an increased function of the sympathetic system [8].

Within the LF band, the so-called “10-second-rhythm” (approx. 0.05 – 0.15 Hz) is manifested. This is related to the natural rhythm of cardiovascular active neurons in the lower brainstem. Analogous blood pressure waves (blood pressure waves of third order) prove the connection [7,8,16].

“HRV is associated with blood pressure levels, however, very few studies have correlated HRV to lifestyle in general population [17].” This statement was made by authors from Japan in 2014 [17]. They investigated 2040 women and 1418 men, aged 40 – 74 years, and measured HRV in the time and frequency domain, also using a 5-min RR interval recording as in our study. HRV indices in this study were not associated with systolic blood pressure levels in men, whereas in women, different HRV parameters were associated with a decrease in systolic blood pressure. The data of this study suggest that HRV reflects diastolic BP

better than systolic BP levels for both sexes, and that alcohol intake strongly affects systolic BP levels in men [17].

Our results of the temperature measurements in this study showed that there is a significant decrease of temperature during the stimulation with the yellow laser. Afterwards the temperature increases again. This effect has not been investigated in detail in scientific literature up to now. However, we found similar results in an animal experimental study. For this experiment in rats, red laser light (wavelength 658 nm, output power 40 mW, diameter 500 μm , duration 10 min) at Baihui and Zusanli also induced significant decreases in temperature values [18]. As stated in that paper, the mechanisms underlying these results are 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 [18]. Maybe the mechanisms are similar in the animal and the human experimental study. Quirico et al. [19], for example, also found that e.g. Taichong induced a rapid and marked vasoconstriction.

In the study we also analyzed the results of the stresslevel questionnaire. One can assume that yellow laser stimulation has a positive effect concerning the stresslevel. The volunteers felt significantly more comfortable after the measurement procedure. This positive effect of the yellow laser in combination with the three acupoints needs further investigation. This procedure can be performed in a double blind way. The volunteers or patients do not feel the stimulation and also the investigators need not be informed about active or deactivated yellow laser stimulation.

1.5 Conclusion

For the first time, yellow laser acupuncture was used in a scientific study. The stimulation of yellow laser on the combination of the acupoints Baihui, Neiguan and Taichong has a significant positive effect on BP and the well-being of the volunteers. In addition, HRV - especially the LF/HF ratio – was also influenced significantly. The first results are very promising, and therefore further studies on

the topic of yellow laser acupuncture will follow soon. The results can be very important especially for the treatment of lifestyle related diseases.

Acknowledgements

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2 Laser Therapy and Stroke: Quantification of Methodological Requirements in Consideration of Yellow Laser

[Ref. 2; D. Litscher et al.]

2.1 Introduction

There are various treatment methods, which should lead to the improvement of clinical outcomes after a stroke. In addition to conventional methods like drug therapy, there are currently some new methods like laser therapy in the testing phases. Hennerici et al. published an overview review article including these methods in the renowned scientific journal Lancet [1].

At the moment there is no certainty that the corresponding laser beam even penetrates the bone, so that the target (brain) can be reached. Currently no one knows laser parameters (wavelength, intensity, power, etc.) in detail, which should be selected on the intact skull, so that the laser light can show its potential efficacy inside the human skull.

The only study that could be found in the scientific literature for penetration of laser beam on the human skull dates from the year 1981 [2]. These authors investigated the so-called transmission of the laser radiation in the wavelength range from 400 nm to 856 nm, with some devices developed by them. No one has reviewed or quantified the penetration depth of the laser devices that are currently used for the planned large-scale stroke studies so far.

The goal of our present study was to calculate the transmission factors of different kinds of lasers through the human skull. For the first time the transmission of a yellow laser (589 nm, 50 mW) was tested.

2.2 Materials and Methods

For the application of laser light to human skulls (provided by the Department of Anatomy, Medical University of Graz) red, infrared, violet, green, and for the first time yellow lasers were used (Figure 2.1). The proof that these types of laser light penetrate the human skull was achieved with commercially available methods (BL-10 L lux meter, volt Craft, Hirschau, Germany). The measurements at the highest point of the skull (electroencephalogram (EEG) position C_z) were reproducible and in total nine measurements were performed per laser modality. The yellow laser is currently only available from weber medical (Lauenförde, Germany) and for research purposes at the Medical University of Graz (Figure 2.2).

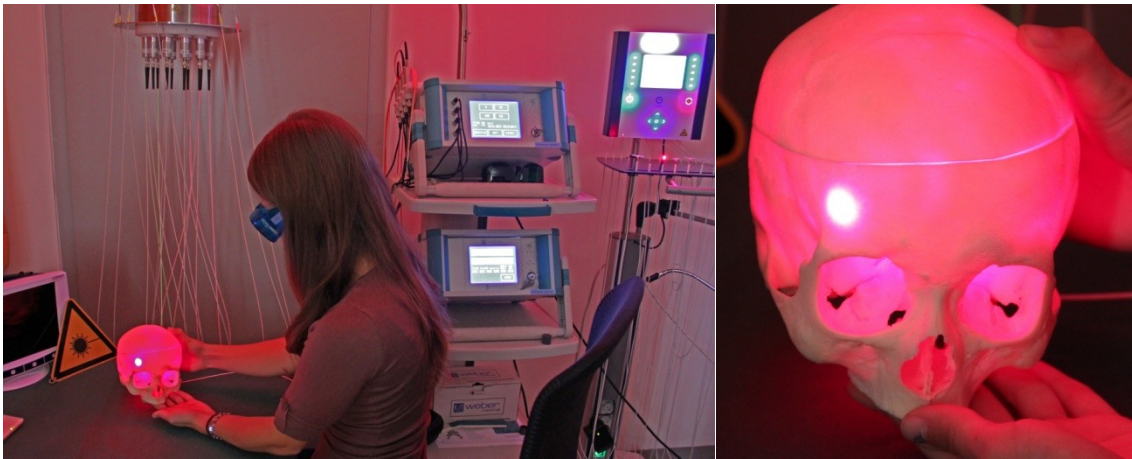


Fig. 2.1: Different kinds of laser equipment for transcranial laser stimulation.



Fig. 2.2: First yellow laser (589 nm, 50 mW) for future medical applications at the Medical University of Graz.

2.2.1 Statistical analysis

Data were analyzed using SigmaPlot 12.0 software (Systat Software Inc., Chicago, USA). The data are graphically presented as box plots.

2.3 Results

The results of this study are shown as box plot graphs and compared with the results of the only previous study on the subject [2]. Transmission is shown as a function of wavelength. In this way the percentage of the laser light which penetrates the skull bone can be measured. It can be seen that the values for the wavelengths 810 nm and 658 nm correlate well with the previous experimental findings (Figure 2.3).

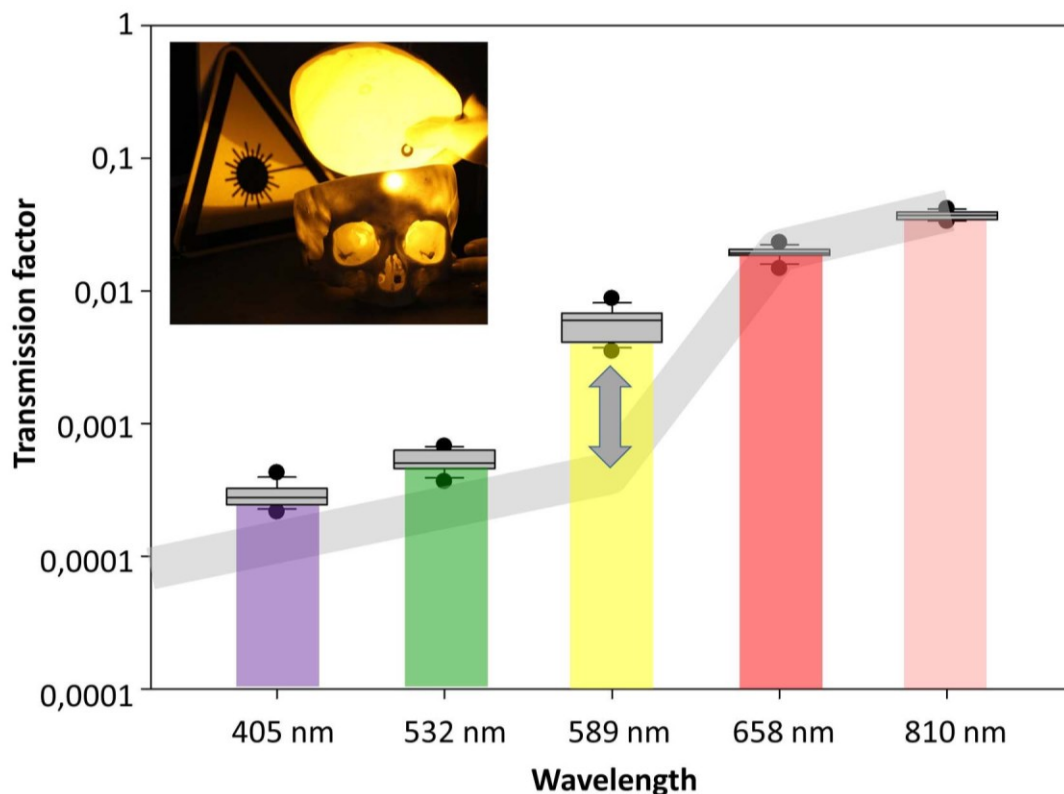


Fig. 2.3: Box plot diagram showing measurement values (box plot) and the schematic results of the previous study (light grey line) concerning the violet (405 nm), green (532 nm), yellow (589 nm), red (658 nm), and infrared (810 nm) laser. The lines in the boxes represent the median, the ends of the boxes define the 25th and 75th percentile, and the error bars define the 10th and 90th percentile. The arrow indicates the variation between our measured values and the estimated values of the previous study [2].

The information on the yellow laser was estimated incorrectly by the authors of the only existing paper mentioned before [2]. The values vary by a factor of ten.

2.4 Discussion

Stroke is one of the leading causes of death worldwide. According to very recent data, which were presented at the 21st World Congress of Neurology at the end of September 2013 in Vienna, every year more than 600,000 Europeans suffer a stroke. There are more and more young people among the patients. Even if someone survives a stroke, he or she often remains permanently disabled and so almost every third person concerned becomes a severe nursing case. Mainly because of the movement in the age pyramid, one can assume that the incidence of stroke will increase in the coming decades.

In most cases, a so-called ischemic stroke, caused by an acute circulatory disorder in the brain vessels, occurs. The acute care, for example, in Austria, takes place in the so-called stroke units, which should be normally reachable after 45 to 60 minutes. After intensive investigation, the treatment can be started 2 hours after the stroke.

Among other things, scientists are working with highly invasive methods, such as craniotomies (for pressure relief) or partial occlusion of the aorta in the abdomen (with the aim of making a larger volume of blood available in the area above the occlusion). Another method is the so-called sphenopalatine ganglion stimulation. Therefore an electrode will be implanted in the patients jaw, which stimulates the pterygopalatine ganglion electrically, and because of this, the cerebral blood flow should be improved. However, a significant disadvantage of all these methods is the high degree of invasiveness [1].

Furthermore, there are some additional nonpharmacological methods, like the therapeutic hypothermia and the transcranial laser irradiation [1]. In connection with the transcranial laser irradiation, invasive and noninvasive tests are currently

taking place. In the invasive variant, a piece of the skull is removed and replaced by transparent plastic, through which laser irradiation takes place afterwards [3].

The noninvasive laser procedures stimulate through the cranial bone. Currently, patients are treated with different lasers. Scientists try to demonstrate a possible therapeutic success with the collection of complex clinical scores and by using highly complicated measuring methods (e.g., near-infrared spectroscopy) [4, 5]. The studies with laser and partly “light-emitting diodes” (LED) [6–21] are currently being conducted worldwide with different success.

New LED-based systems use special physical effects, the so-called QIT-effect (quantum optical induced transparency), or optomechanically induced transparency [22–24]. Quantum interference effects in the amplitudes of optical transitions in atomic medium can lead to strong modifications of its optical properties. This effect well-known as electromagnetically induced transparency (EIT; [22]) has become an important tool to control the optical properties of dense media and has the potential to enhance the transparency contrast by a factor of five [24]. This means that bones like the skull, the spine, or joints can be penetrated even if the applied light is of relatively moderate intensity. Due to the QIT effect, the radiation should be able to reach deep tissue layers in muscles, connective tissue, and even bone, thus enabling noninvasive transcranial treatments, for example, for neurodegenerative diseases or stroke. However, to the best of our knowledge, no publications are available concerning light stimulation systems using this effect on human skulls.

This study is the first to prove that yellow laser light can penetrate the human skull. As photodynamic therapy (PDT) is one of the most promising treatment options for many kinds of tumors, the principle of PDT might also be used for treating brain tumors in future. It is well known that drugs like hypericin can stop or slow down the growth of brain tumors, but with yellow laser it can be used also for necrosis and apoptosis of such tumors, and this could open up a new strategy against brain tumors [25]. However, there are still many open questions, for example, the kind of photosensitizer [26] and especially the laser parameters like wavelength, intensity, dosage, and so forth.

2.5 Conclusion

Laser therapy is a promising new approach in poststroke treatment; however, there are still some methodological problems. We found that our own data concerning the human skull transmission factor correspond quite well to those of the researchers from 1981, apart from the data for the yellow laser, so this is certainly a question that deserves close attention. Further basic research on the entire topic is very important so that we can be sure to apply the correct kind or color of laser with the correct parameters. This can open up new dimensions in transcranial laser therapy.

Conflict of Interests

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

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3 Laser Therapy and Dementia: A Database Analysis and Future Aspects on LED-Based Systems

[Ref. 3; D. Litscher et al.]

3.1 Introduction

Dementia is a widespread disease. According to data from 2013 there are 44.4 million people suffering from dementia worldwide. Mainly because of the movement in the age pyramid, one can assume that the incidence of dementia will increase in the coming decades. Experts mentioned that in 2030 an estimated 75.6 million and, in 2050, 135.5 million people will suffer from dementia. The biggest problem is the increase in the developing countries. There are already 62% of people with dementia living in developing countries. The fastest growth in the elderly population is taking place in China, India, and their south Asian and Western Pacific neighbors. Experts warn that the whole percentage of people with dementia will increase to 71% in 2050 in those countries [1].

In Europe, for example, the Austrian society for Alzheimer's disease states that this particular disease is responsible for 60–80% of dementia cases. In Austria, currently 1 billion Euros is necessary for the therapy of dementia patients every year [2]. In Switzerland dementia is also a huge financial problem. On the homepage of the Swiss Alzheimer Union it is mentioned that every year about 7 billion Swiss francs is needed for the therapy of dementia patients [3]. Statistics from Germany (German Federal Ministry of Family Affairs, Senior Citizen, Women and Youth) show that one in three persons aged over 90 suffers from dementia. Experts warn that by 2050 the number of people in Germany suffering from dementia will increase to 3 million [4].

In the United States of America Alzheimer's disease (type of dementia) is the 6th leading cause of death. Total payments for health care, long-term care, and hospice for people with Alzheimer's and other types of dementia are projected to increase from \$203 billion in 2013 to \$1.2 trillion in 2050 (in current dollars) [5].

The goal of the present study was to give a short scientific overview on the topic laser therapy and dementia and to take new LED- (light emitting diodes-) based systems which use the quantum optical induced transparency effect into account. Therefore a database analysis in scientific databases was performed and the transmission factor (TF) of a new LED-based system through the human skull was calculated.

3.2 Current Scientific Research

A search of two scientific databases PubMed and Cochrane Library, on February 19, 2014, yielded more than 600 publications related to the topic laser and dementia. The topic laser and Alzheimer listed more than 450 scientific publications. The other search terms were “Alzheimer (or dementia) and laser; laser therapy; laser therapy transcranial; laser acupuncture; red laser; yellow laser” (see Figures 3.1 and 3.2).

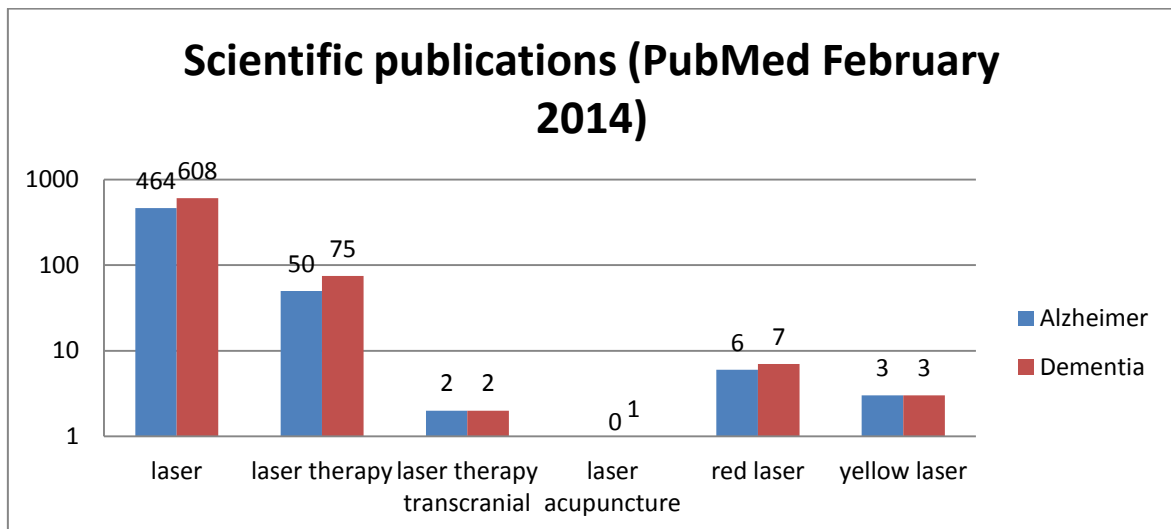


Fig. 3.1: Scientific publications related to the topic in the scientific database PubMed.

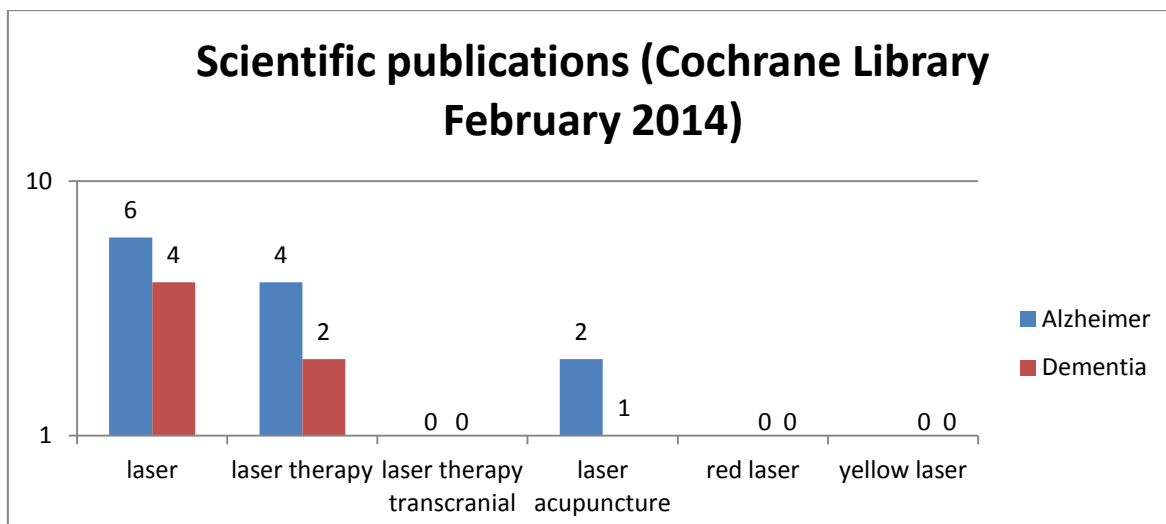


Fig. 3.2: Scientific publications related to the topic in the scientific database Cochrane Library.

Ten referenced publications (PubMed) with the terms “red laser” or “yellow laser” and “dementia” [6–15] are described in detail and cited in the list of references. Compared to laser or laser therapy in general this number of publications is very small. In the following, the 10 publications from the two databases will be discussed. At first 7 publications found with the search terms “dementia” and “red laser” are described.

Most of these studies deal with different laser methods for diagnostic purposes but are not directly related to laser therapy.

In the first paper, the authors state that occlusive brain ischemia and microstrokes are a major cause of dementia in older patients. In this in vivo animal experimental study two-photon laser-scanning microscopy and transgenic mouse models expressing cell type specific reporters were used to examine ischemia-related insults [6]. In the study by Sommer et al. [7] the authors wanted to show a potential relevance of their in vitro experiments in the treatment of Alzheimer’s disease (AD). Amyloid-beta ($A\beta$) was internalized into human neuroblastoma (SH-EP) cells which were then irradiated with moderately intense 670 nm laser light (1000 W/m^2) and/or treated with epigallocatechin gallate (EGCG). In the irradiated cells, the authors found that $A\beta$ (42) aggregate amounts were significantly lower than in nonirradiated cells. They concluded that irradiation with moderate levels of 670 nm

light and EGCG supplementation complementarily reduces A β aggregates in SH-EP cells.

An important study was published in the renowned scientific journal *Lancet* in the year 2003 [8]. The authors of the paper entitled “Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer’s disease” obtained postmortem specimens of eyes and brain from nine individuals with AD and eight controls without the disorder and samples of primary aqueous humour from three people without the disorder who were undergoing cataract surgery. Among others they used enhanced laser desorption ionisation. A β 1–40 and A β 1–42 were found in the lenses of all subjects, at concentrations comparable to those in the brain, and A β 1–40 was found in primary aqueous humour at concentrations comparable to that in cerebrospinal fluid. The authors concluded that since A β was found in the cytosol of lens fibre cells of people with Alzheimer’s disease, lens A β might promote regionally specific lens protein aggregation, extracerebral amyloid formation, and supranuclear cataracts [8].

A paper by Miyakawa et al. from the year 2000 also deals with the role of blood vessels and the deposition of A β protein in producing pathological changes in the brain with AD [9]. Laser scanning microscopy revealed a close relationship between A β 40 and the vascular network. The amount of mature plaques was reportedly correlated with the severity of dementia in Alzheimer’s patients. Therefore, the authors concluded that accumulation of A β 40 associated with blood vessels may play a critical role in the development of AD. Another paper entitled “Confocal observation of senile plaques in Alzheimer’s disease: senile plaque morphology and relationship between senile plaques and astrocytes” was published by Kato et al. in 1998 [10]. Senile plaques in the brains with AD were examined by confocal laser scanning microscopy [10]. Chiu and Liu [11] measured the erythrocytes deformability using a laser visco diffractometer (Vidometer). They found that the deformability of glucose-6-phosphate dehydrogenase- (G6PD-) deficient erythrocytes was drastically reduced by hydroxyl radicals and hypothesized that as a consequence of enhanced susceptibility to oxidative stress, G6PD-deficient individuals have lower antioxidant levels, particularly vitamin C, than normal individuals. An older publication from 1995 deals with the topic

“Mitochondrial constituents of corpora amylacea and autofluorescent astrocytic inclusions in senescent human brain” [12]. Immunofluorescence labeling and laser scanning confocal microscopy demonstrated consistent colocalization of the mitochondrial proteins, sulfite oxidase, and heat shock protein 60, to both corpora amylacea (CA) and the autofluorescent astroglial inclusions. These observations suggest that Gomori-positive granules may be structural precursors of CA in the senescent human brain, and degenerated mitochondria within periventricular astrocytes give rise to autofluorescent cytoplasmic granules and CA [12].

In the following 3 papers found with the search terms “dementia” and “yellow laser” are mentioned.

The two-photon laser scanning microscopy is often used for chronic in vivo studies. In this study Scheibe et al. used an upright two-photon microscope complemented with a software-controlled stage-rotation instead of a conventional stage for chronic in vivo imaging in the brain of transgenic mouse models of AD [13]. This experimental setup allowed the authors to eliminate deviations in sample orientations between consecutive imaging sessions, thus facilitating comparisons between three-dimensional image stacks taken at different time points [13]. One article by Li et al. deals with the “Construction of recombinant plasmid harboring APP717 mutation and preliminary study of APP proteolysis” [14]. The authors investigated the pathogenesis of AD and studied the enzymatic progress of amyloid precursor protein (APP) using laser confocal microscopy. In summary, the recombinant plasmid could be a useful tool to further study the cleavage mechanism of APP and to explore the pathogenesis of AD. The last publication which is listed in the literature deals with the “Use of YFP to study amyloid-beta associated neurite alterations in live brain slices” [15]. Again, laser scanning confocal microscopy was used by the authors of this study. Their acute slice preparation model should prove to be a useful tool to explore the pathophysiology of A β -related axonal, dendritic, and synaptic dysfunction.

A very important, maybe the most important paper related to the topic laser and dementia was released in 2013. “Low-level laser therapy rescues dendrite atrophy via upregulating BDNF expression: implications for Alzheimer’s disease” was

published in the Journal of Neuroscience [16]. The authors stated that the so-called low-level laser therapy (LLLT) was demonstrated to regulate neuronal function both in vitro and in vivo. They found that LLLT rescued neurons loss and dendritic atrophy via upregulation of brain-derived neurotrophic factor (BDNF) in both A β -treated hippocampal neurons and cultured APP/PS1 mouse hippocampal neurons. They also found that photoactivation of transcription factor CRE-binding protein (CREB) increased both BDNF mRNA and protein expression, since knockdown CREB blocked the effects of LLLT. Among other things these studies suggest that upregulation of BDNF with LLLT can ameliorate A β -induced neurons loss and dendritic atrophy, thus identifying a novel pathway by which LLLT protects against A β -induced neurotoxicity [16].

In addition to the ten papers mentioned before, there is another very interesting article listed in the scientific database PubMed [17]. In the progression of AD, oxidative stress and inflammation are important processes. The authors mentioned that recent studies have implicated the role of A β in mediating these processes. The objective of their study was to examine whether laser light with a wavelength of 632.8 nm was able to abrogate the oxidative and inflammatory responses induced by A β . Primary rat astrocytes were exposed to helium-neon laser, followed by the treatment with oligomeric A β . The author's data showed that, amongst others, laser light suppressed A β -induced superoxide production.

Another paper from the year 2012 deals with low-power laser irradiation (LPLI). Apoptosis induced by A β is thought to associate with the pathogenesis of AD. The mechanisms of LPLI to reduce A β -induced apoptosis are still unclear. The authors reported a novel molecular mechanism by which LPLI attenuates A β (25–35)-induced apoptosis. The data demonstrate that LPLI has a prosurvival effect on A β -induced apoptosis and may be an effective therapeutic strategy in treating AD [18].

3.3 Future Aspects—LED-Based Systems

At the moment there is only one paper concerning light emitting diodes and dementia (Alzheimer) in the scientific databases PubMed and Cochrane Library [19]. However, this paper uses LED not for therapeutic reasons but for stimulation

purposes. The authors investigated 12 patients with AD and their age and sex-matched controls. The goal of the study was to analyze whether Alzheimer's patients experienced a problem spontaneously planning an upcoming movement. The authors of the study state that the patients with AD revealed slower and less efficient movements than controls. To the best of our knowledge, up to now a LED-based system has never been used in extensive investigations concerning dementia, and therefore no other parameters are available.

Within this study, a new LED-based system was investigated with regard to its transmission factor on the human skull for the first time. Similar to a previous study [20], the transmission through the skull was measured with commercially available methods (BL-10L luxmeter, Volt Craft, Hirschau, Germany). The registrations on the highest area of the skull (1.6 x 6.5 cm around electroencephalogram position C₂) were reproducible, and in total nine measurements were performed using the new LED-based system medloux (laneg GmbH, Schönwalde, Germany) (see Figure 3.3). This system emits noncoherent, infrared LED radiation with a wavelength of 830 nm. The output power of the LED-applicator is 760 mW, and the LED class of the system is 1 M according to IEC 60825-1:A2. The absolute value of the measurement inaccuracy concerning the LED output power is 20%.



Fig. 3.3: The medloux system including the LED applicator.

The measurements were performed at a human skull provided by the Department of Anatomy, Medical University of Graz.

Data were analyzed using SigmaPlot 12.0 software (Systat Software, Chicago, USA) and are presented as box plots. One way repeated measures ANOVA was used for statistical analysis, and Tukey's test was used for post hoc analysis. A p-value of <0.05 was considered significant.

The results of the measurements can be seen in Figures 3.4 and 3.5.

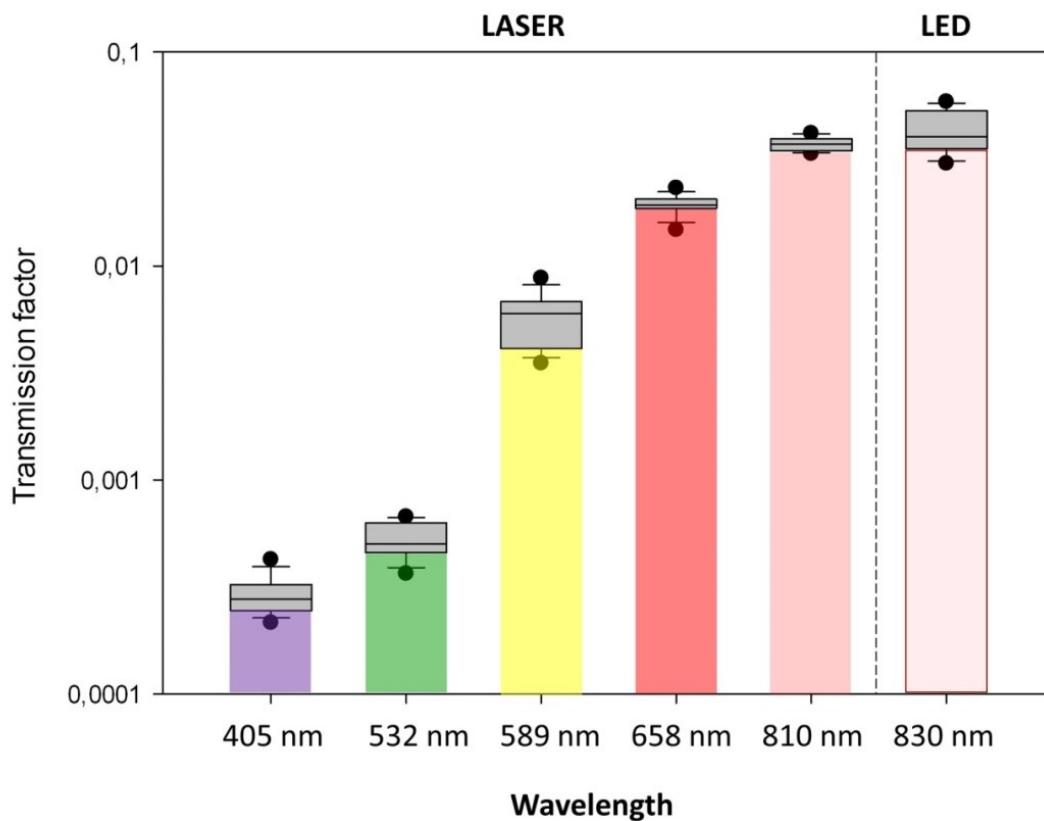


Fig. 3.4: Transmission factor of different types of laser (modified from [20]) and of the new medloux system (LED). The results from violet (405 nm), green (532 nm), yellow (589 nm), red (658 nm), and infrared (810 nm) lasers are based on a previous study [20]. The system based on LED showed the highest mean value of the transmission factor (830 nm infrared light). The lines in the boxes represent the median, the ends of the boxes define the 25th and 75th percentile, and the error bars define the 10th and 90th percentile.

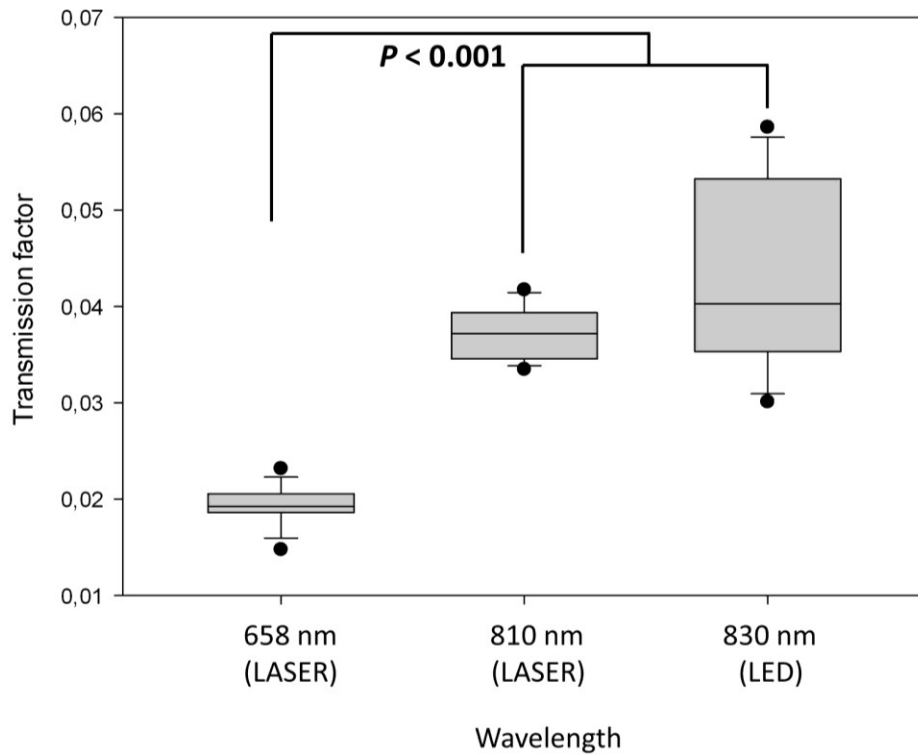


Fig. 3.5: Transmission factor of red (658 nm) and 810 nm infrared laser light and of 830 nm infrared LED radiation. For further explanations, see Figure 3,4.

It is interesting that the LED based system which uses a special physical effect, the so-called QIT- (quantum optical induced transparency-) effect [21–23], has the highest TF (0.0434 ± 0.0104 (SD)). In the literature, it is described that this electromagnetic effect has become an important tool to control the optical properties of dense media and that it has the potential to enhance the transparency contrast by a factor of five [23]. Compared to our laser measurements, the new LED system has indeed a high TF. There is a significant difference between red laser light (658 nm; $TF = 0.0194 \pm 0.0023$) and 830 nm infrared LED radiation (compare Figure 3.5). However, the TF of infrared laser light (810 nm; 0.0373 ± 0.00299) and infrared LED radiation does not differ significantly.

3.4 Conclusion

At first it should be mentioned that there are many scientific publications on the topic dementia (resp., Alzheimer) and laser diagnostics and therapy. However, there is one very important study about low-level laser therapy and Alzheimer's

disease in general [16]. The other described papers do not deal with laser irradiation as a treatment method but with the research on dementia with diagnostic laser methods [6–15]. We believe that the prerequisites (TF) for yellow, red, and infrared irradiation can be considered as given so that dementia research can be performed. In this context, also the new LED based system medloux fulfills the requirement “transmission factor.” Further research on this topic is absolutely necessary.

Conflict of Interests

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

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

[Ref. 4; D. Litscher et al.]

4.1 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.

4.2 Materials and Methods

4.2.1 Animals

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

4.2.2 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 4.1).

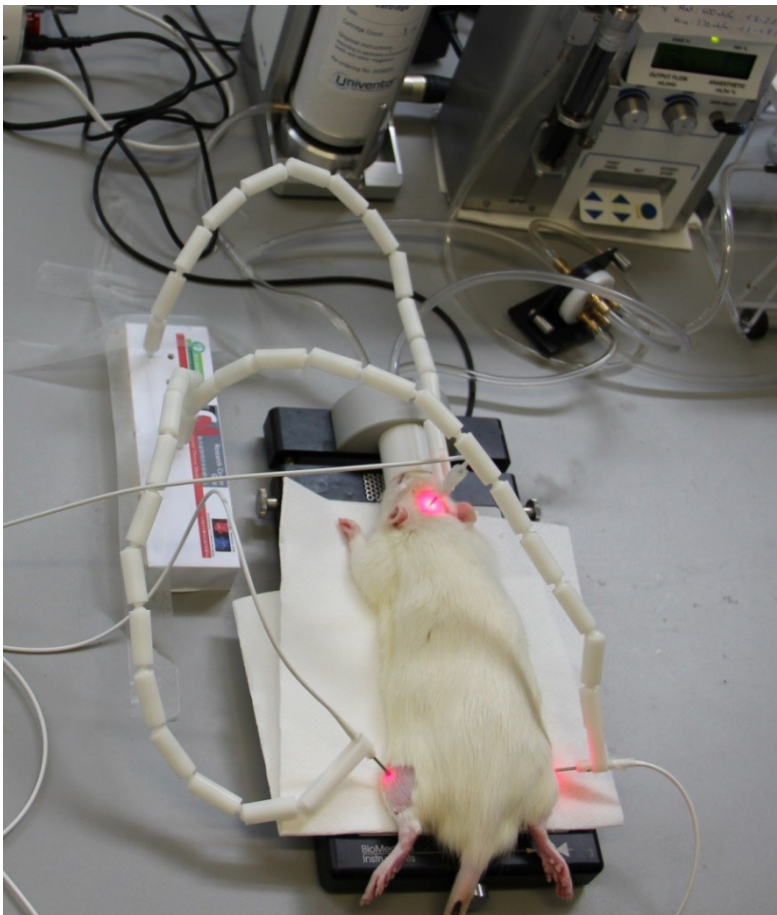


Fig. 4.1: Anesthetic procedure and laser stimulation.

4.2.3 Laser Acupuncture and Acupoints

Red laser stimulation was performed in 6 rats at the acupuncture points Baihui (GV20) and Zusanli (ST36, bilateral) (see Figure 4.2). 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 wave mode was used, and the duration of the stimulation was 10 min. The method is described in detail in previous publications [1].

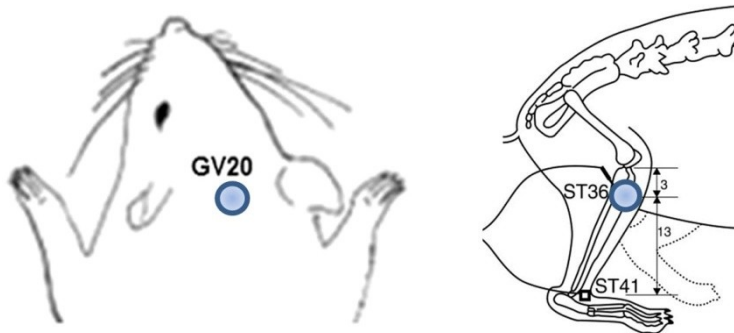


Fig. 4.2: Schematic diagram of the stimulated acupoints.

4.2.4 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 $+250^{\circ}\text{C}$. Its accuracy lies

at $\pm 2\%$ of the reading. Sensitivity is $<0.1^{\circ}\text{C}$ at 30°C , and the infrared resolution is 140×140 pixels. The system is ready for use in 15–20 seconds (see Figure 4.3).



Fig. 4.3: Temperature measurements using a Flir i7 infrared thermocamera.

4.2.5 Procedure

All experiments took place during daytime (between 09:00 and 14:00; room temperature: $\sim 21^{\circ}\text{C}$). Every rat completed the investigation. The rats were anesthetized throughout the laser acupuncture session with isoflurane (see section 4.2.2). At the end of a five-minute resting phase which served as baseline condition, the first thermal image (a) was taken (see Figure 4.4). 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.

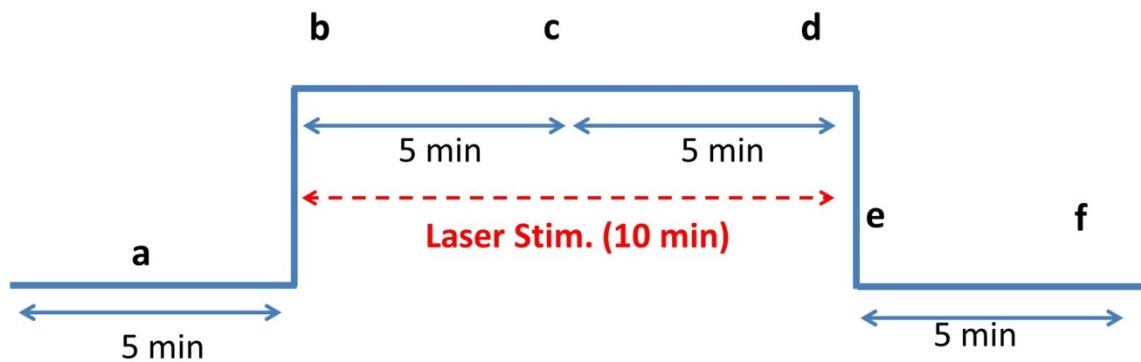


Fig. 4.4: Experimental protocol.

4.2.6 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 \pm SE (standard error). The criterion for significance was $p < 0.05$.

4.3 Results

Typical examples of the results of thermal imaging are shown in Figures 4.5, 4.6, and 4.7. The examples demonstrate anesthetized rats. The room temperature was $\sim 21^{\circ}\text{C}$. After red light stimulation the temperature at the Baihui acupoint (marker in Figure 4.5) decreased from 36.2°C to 34.5°C during the experimental procedure (compare Figure 4.4). 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 .

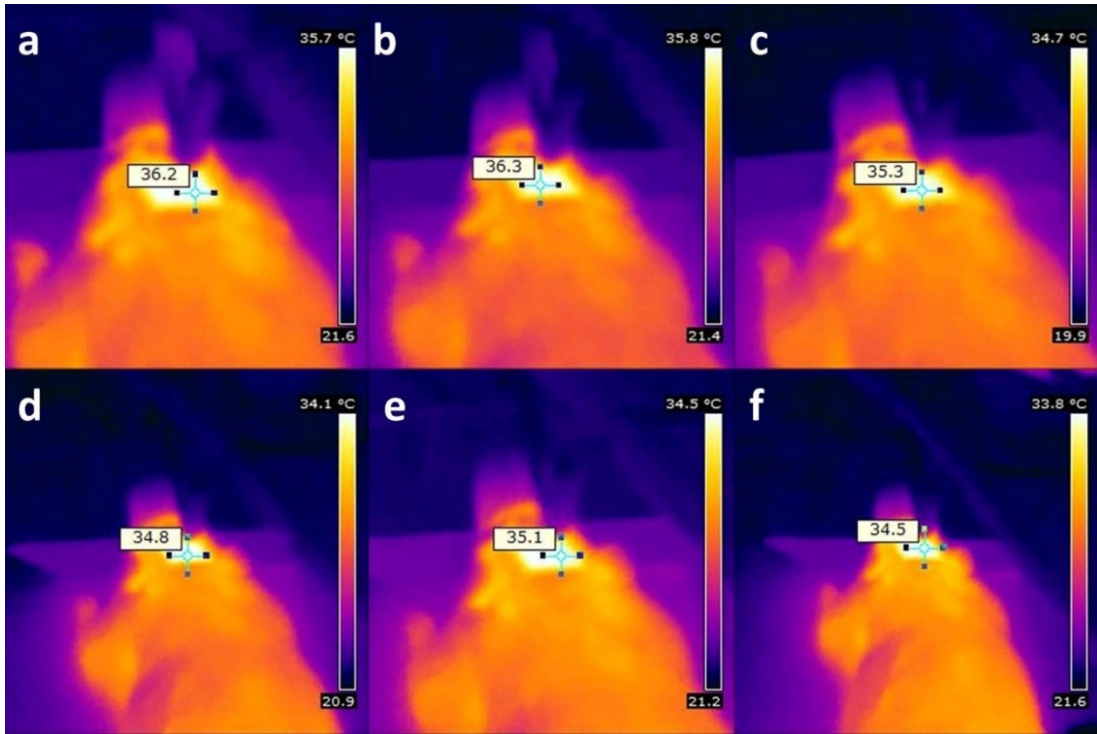


Fig. 4.5: Six thermal images from an anesthetized rat before (a), during ((b), (c), and (d)), and after ((e) and (f)) red light stimulation at the Baihui acupoint. Note the decrease of the temperature during and after red light irradiation.

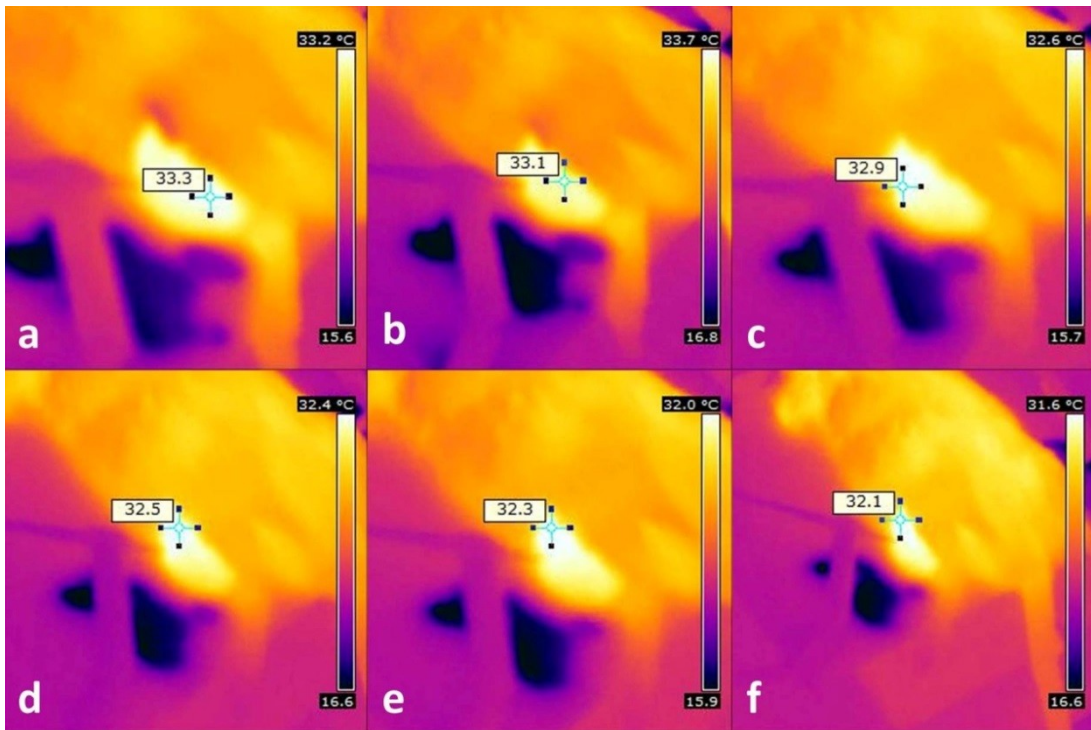


Fig. 4.6: Six thermal images from an anesthetized rat before (a), during ((b)–(d)), and after ((e) and (f)) red light stimulation at the left Zusanli acupoint. Note the decrease of the temperature during and after red light irradiation.

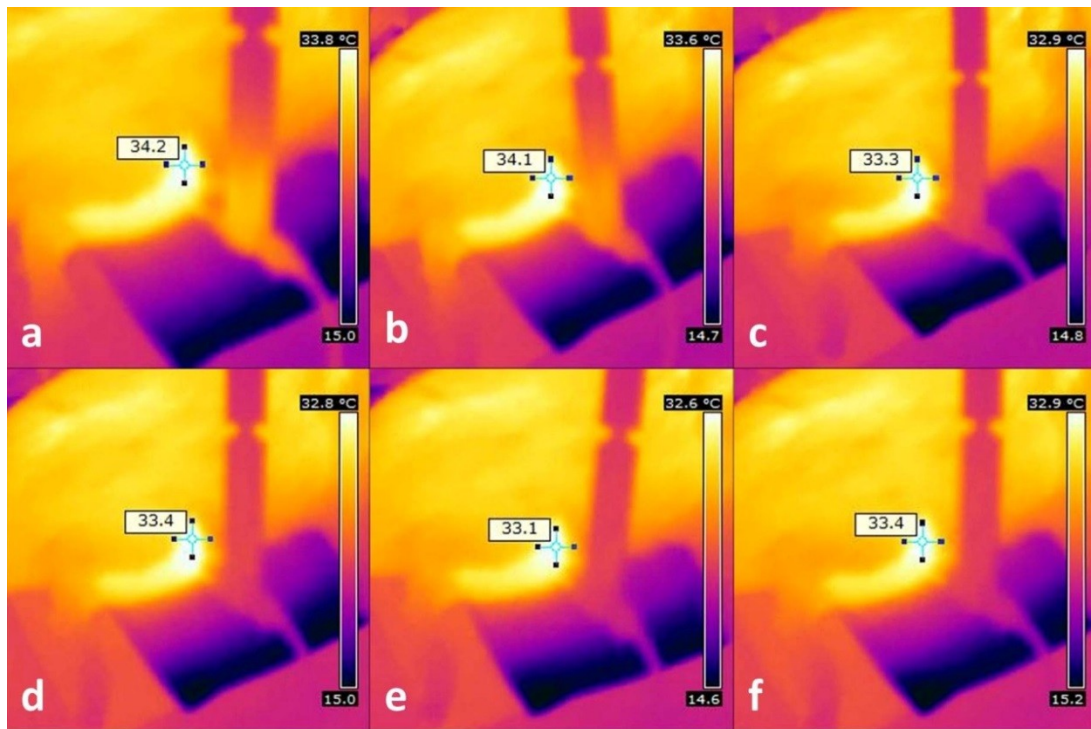


Fig. 4.7: Six thermal images from an anesthetized rat before (a), during ((b)–(d)), and after ((e) and (f)) red light stimulation at the right Zusanli acupoint. Note the decrease of the temperature during and after red light irradiation.

Figure 4.8 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.

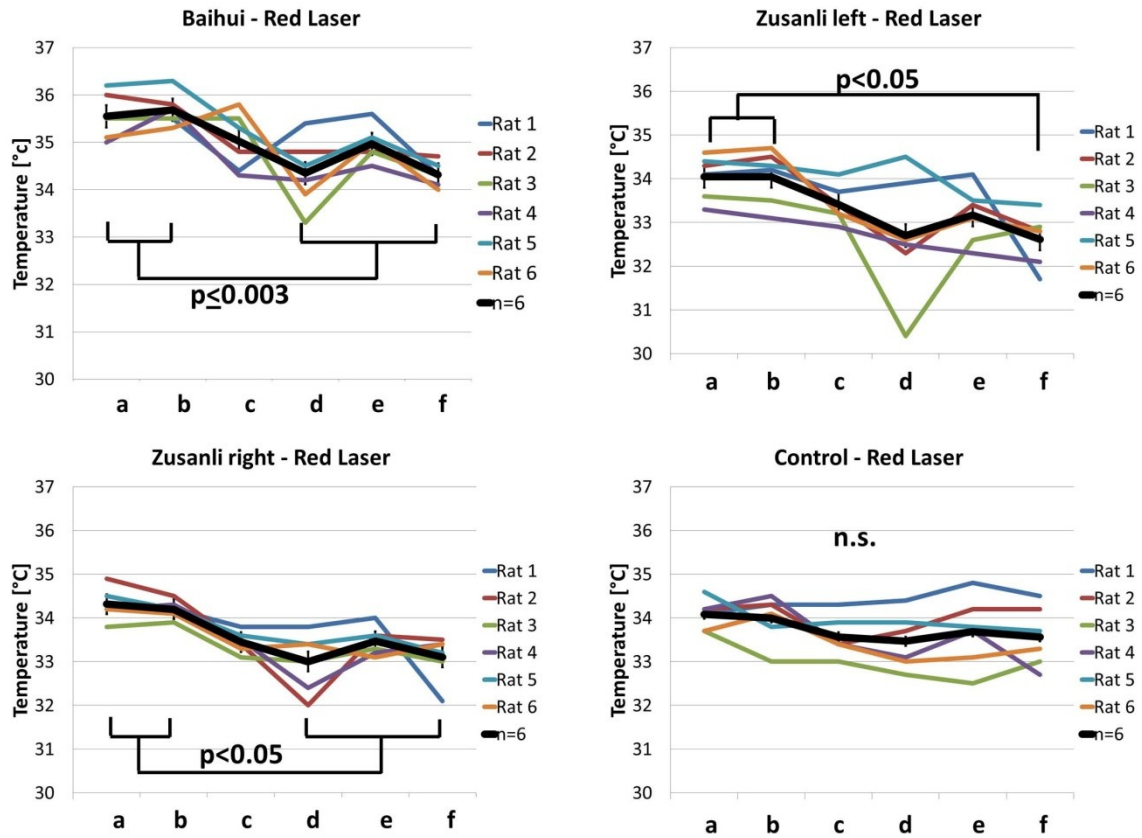


Fig. 4.8: Temperature values of all rats during the different phases (a–f, see Figure 4.4) of red laser stimulation. Significant changes were found for all acupoints, but not for the control point.

Figure 4.9 shows data extracted from the thermal images without laser stimulation (placebo control). No significant changes were found at the control point.

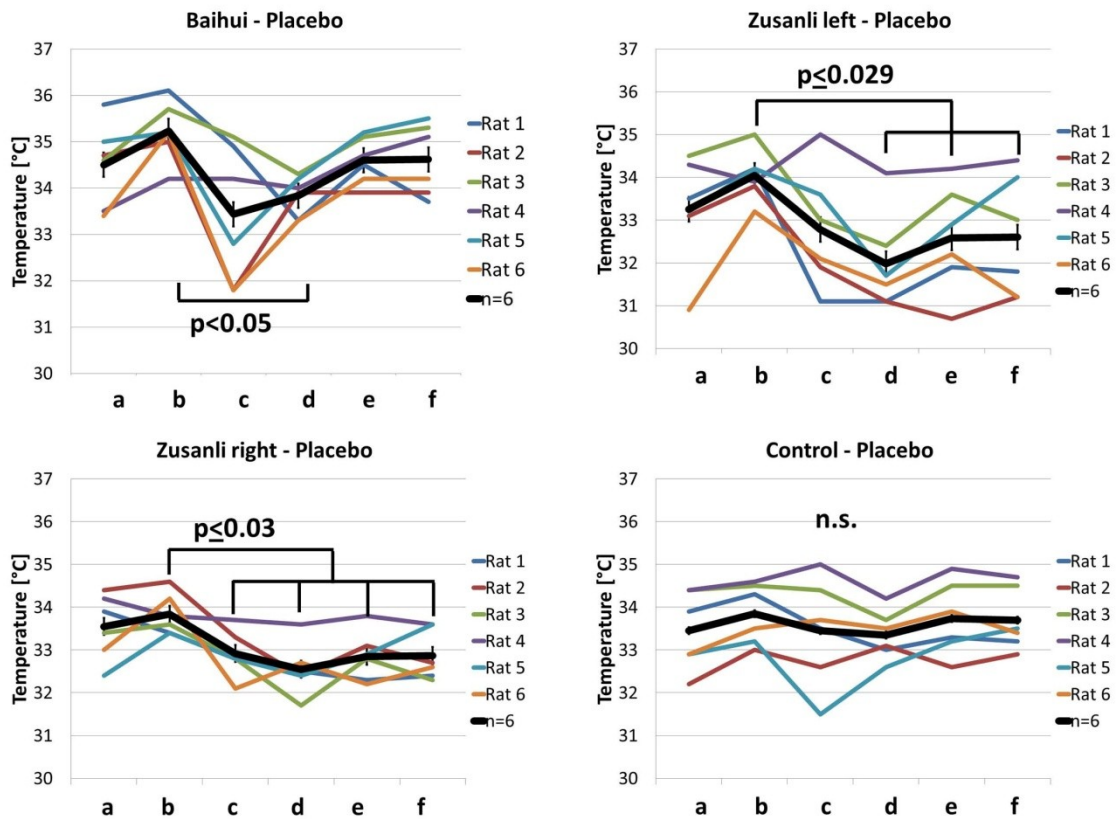


Fig. 4.9: Temperature values of all rats during the different phases (a–f, see Figure 4.4) of placebo stimulation.

4.4 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 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 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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5 The Influence of New Colored Light Stimulation Methods on Heart Rate Variability, Temperature, and Well-Being: Results of a Pilot Study in Humans

[Ref. 5; D. Litscher et al.]

5.1 Introduction

Human beings are very sensitive to light exposure, and changes of light intensity can shift many physiological parameters like melatonin, alertness, body temperature, heart rate (HR), and heart rate variability (HRV) [1]. In this context the effects of colored light have been investigated in few scientific studies [2–4], in addition to the alterations based on changes of white and bright light [5–7].

In previous investigations it has been found that colored light can influence the HRV within minutes and that the effects of individual colors can be differentiated by HRV [3]. It has also been reported that the HRV ratio of low frequency to high frequency (LF/HF) was decreased after illumination with so-called “cold colors” [2].

The goal of the present pilot study was to investigate acute HR, HRV, temperature, and state of mind reactivities after illumination with differently colored light (red and blue) during daytime in healthy volunteers with closed eyes.

5.2 Materials and Methods

5.2.1 Subjects

Seven healthy volunteers (5 females, 2 males; mean age \pm SD 34.1 \pm 11.9 years; range 23–55 years) were investigated at the Medical University of Graz. None of the subjects was under the influence of centrally active medication, and none had a history of heart or cerebrovascular disease, respiratory or neurological problems, or hypertension. All volunteers gave oral informed consent, and the study was carried out in compliance with the Declaration of Helsinki.

5.2.2 New Colored Light Stimulation Methods

Two color light panels (collaxx, mse elektronik, Frankenburg, Austria) were used in this study (see Figures 5.1(a) and 5.1(b)).

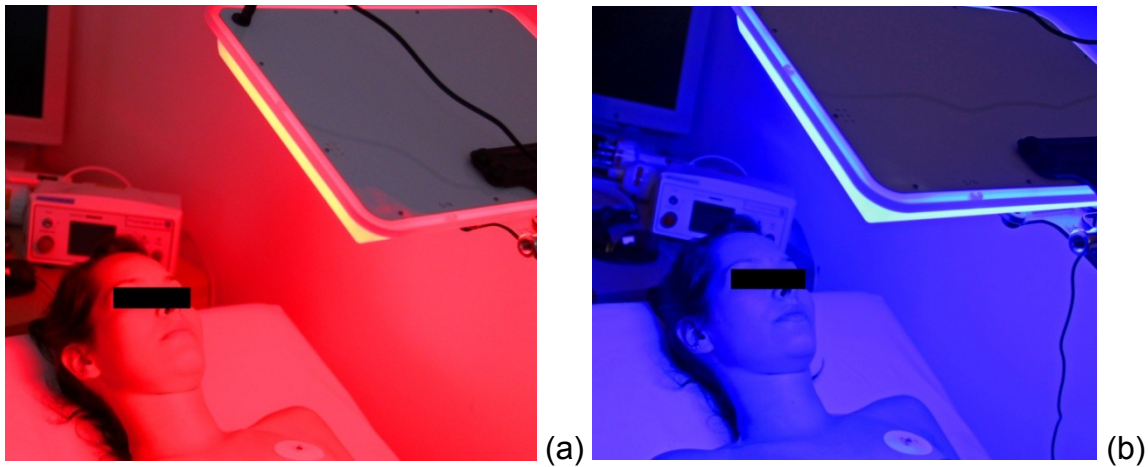
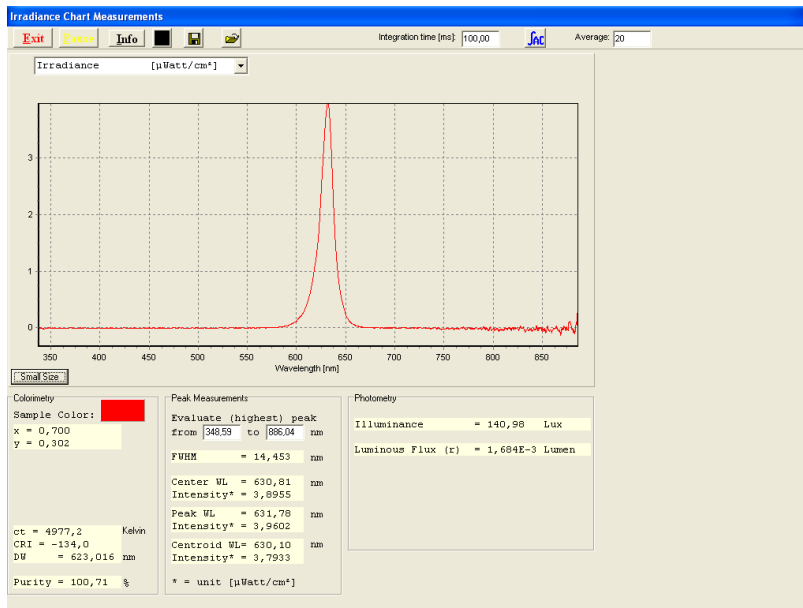
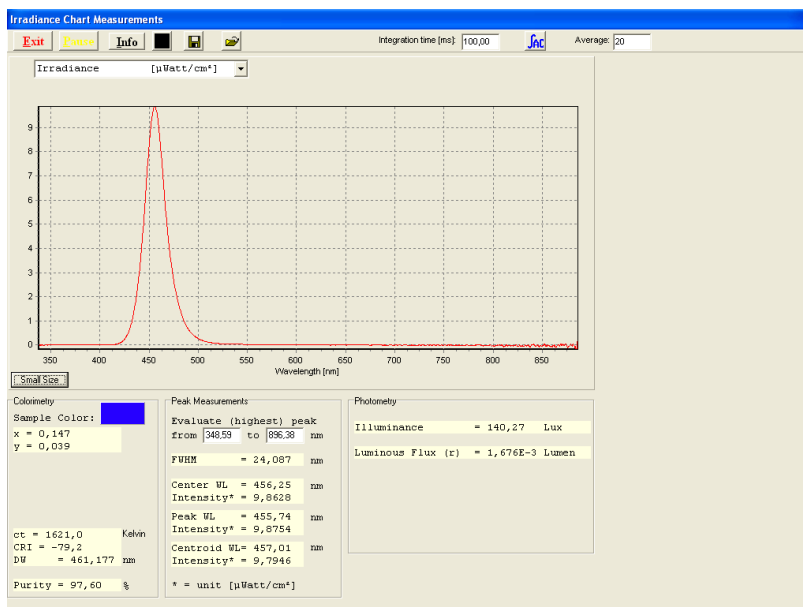


Fig. 5.1: Stimulation with red (approx. 631 nm; (a)) and blue (approx. 456 nm; (b)) color panels (approx. 140 lux).

Both colors had almost the same illuminance (red: 140.98 lux, and blue: 140.27 lux, measured at a distance of 40 cm). Figure 5.2 shows the spectra of the two colors. In addition, the dominant wavelengths ($DW_{red} = 623.0$ nm and $DW_{blue} = 461.2$ nm) are indicated. DW is the wavelength which is important for the sensitivity of the human eye. The peak wavelength differs from the DW because both colors, red and blue, are located at the (opposite) margins of the visible light spectrum, and the human eye's sensitivity for brightness is drastically reduced in these regions.



(a)



(b)

Fig. 5.2: Colorimetry and photometry of the two light panels used in the study. (a) 631 nm, red; (b) 456 nm, blue.

5.2.3 Temperature Measurements

The temperature measurements were performed using a Flir i7 (Flir Systems, Wilsonville, USA) infrared camera which operates at a wavelength range from 7.5–13 μm. The focal distance of the infrared lens is $f=6.8$ mm. The temperature measurement range is between -20°C and $+250^{\circ}\text{C}$. Its accuracy lies at $\pm 2\%$ of the reading. Sensitivity is $<0.1^{\circ}\text{C}$ at 30°C , and the infrared resolution is 140 x 140 pixels. The system is ready for use in 15–20 seconds. We chose the forehead and the tip of the nose as locations for the thermographic measurements. Both areas

were measured during illumination and also during the control phases before and after illumination.

5.2.4 Electrocardiographic Measurements

Electrocardiogram (ECG) is registered using three adhesive electrodes (Skintact Premier F-55; Leonhard Lang GmbH, Innsbruck, Austria) which are applied to the chest. The duration of RR-intervals is measured during time periods of 5 min, and on spectral analysis basis HRV is determined.

A medilog AR12 HRV (Huntleigh Healthcare, Cardiff, United Kingdom) system is used. The system has a sampling rate of 4096 Hz [8], and the raw data are stored on a memory card. Mean HR, total HRV, and the LF/HF ratio of HRV were chosen as preliminary electrocardiographic evaluation parameters, as such being recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [9].

5.2.5 Procedure

The experiment used a repeated-measures design with two different light conditions (see Figure 5.3) and took place during daytime (between 09:00 and 11:00) in July 2013 (room temperature: 28–30°C). Every volunteer completed the investigation. The persons were lying on a bed with closed eyes in a special lab of the Medical University of Graz, and the measurements started after a resting period of 5 minutes. The volunteers were exposed to the two differently colored stimulations for 10 minutes in randomized order. Between the two different stimulation modalities there was a resting period of 10 min. This duration was chosen for practical reasons (e.g., HR should return to baseline values in this time). HRV was measured during stimulation ((b) and (c) in Figure 5.3), and also for 5 min before (a) and after (d) illumination with red or blue light. After exposure to the red and blue light, respectively, the volunteers reported their state of mind for each modality. These reports were categorized on a scale from 0 to 10 (0: positive, comfortable, and relaxed; 10: negative, uncomfortable, and anxious).

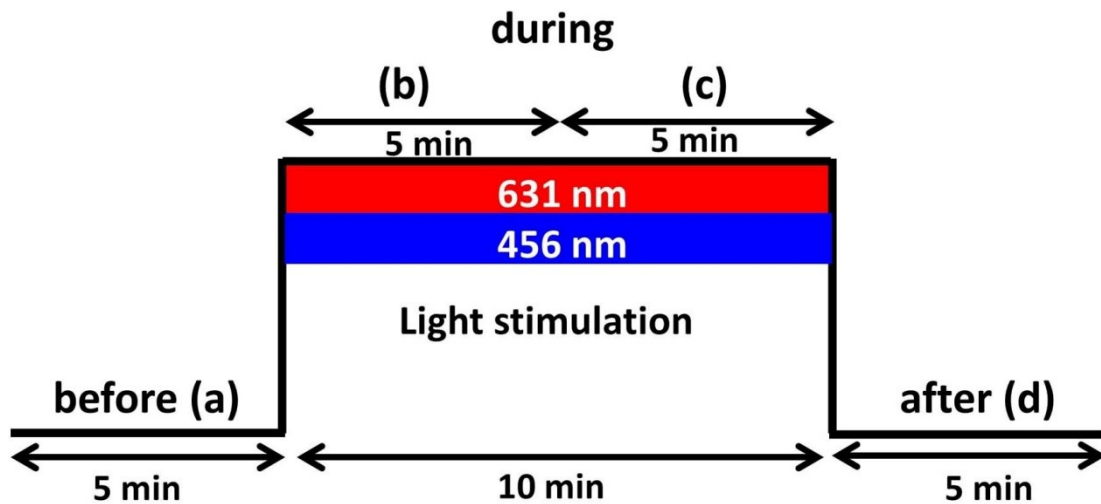


Fig. 5.3: Experimental protocol.

5.2.6 Statistical Analysis

Data were analyzed using SigmaPlot 12.0 software (Systat Software Inc., Chicago, USA). Testing was performed with one way repeated measures ANOVA and Holm-Sidak test. In addition, paired t-test was used. The data are graphically presented as mean \pm SE (standard error). The criterion for significance was $p < 0.05$.

5.3 Results

A typical example of the results of thermal imaging is shown in Figure 5.4. The example demonstrates the face of a 23-year-old female. The room temperature was 30°C. It is interesting that after red light stimulation the temperature of the nose (marker in Figure 5.4) increases from 34.4°C to 36.5°C. In contrast, after blue light stimulation the temperature of the nose of the same person decreases from 36.4°C to 34.6°C.

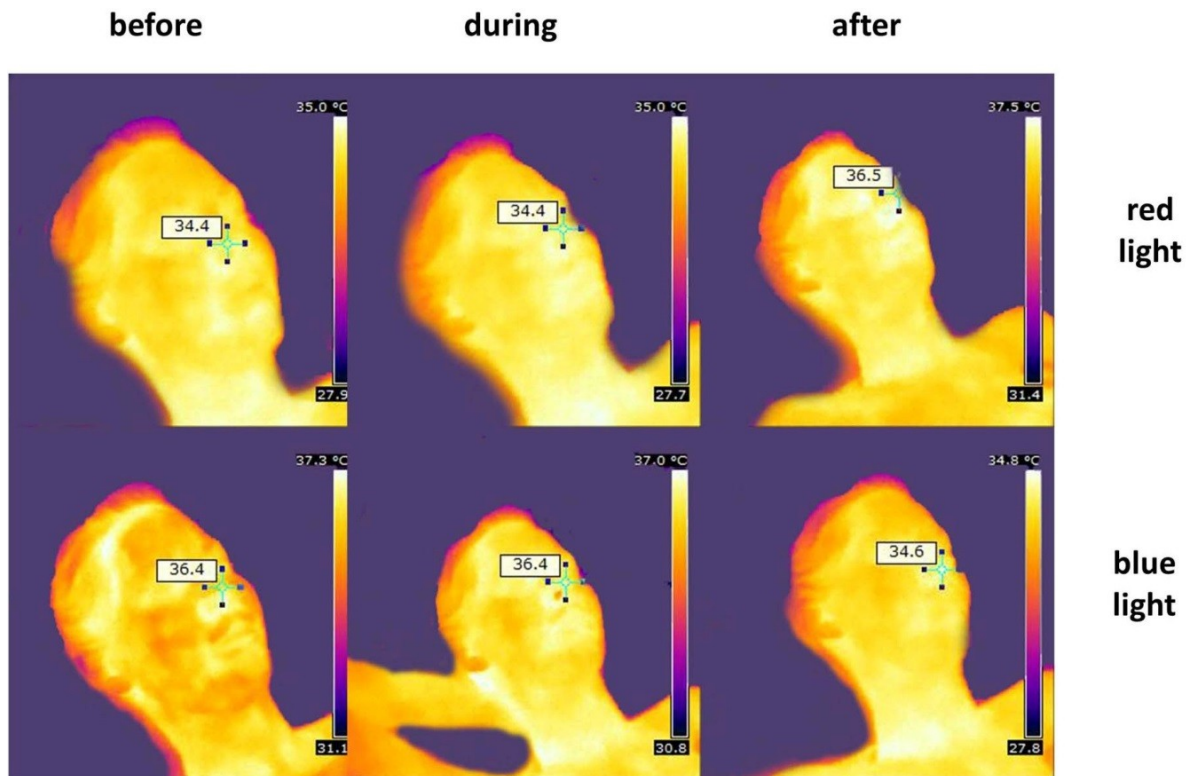


Fig. 5.4: Six thermal images from a 23-year-old healthy female volunteer before, during and after red (a) and blue (b) light stimulation with closed eyes. Note the increase of the temperature of the nose after red and also the decrease after blue light irradiation.

Figure 5.5 summarizes data extracted from the thermal images. Blue light decreases temperature in most of the volunteers (measured at the nose and forehead), whereas red light leads to a slight increase.

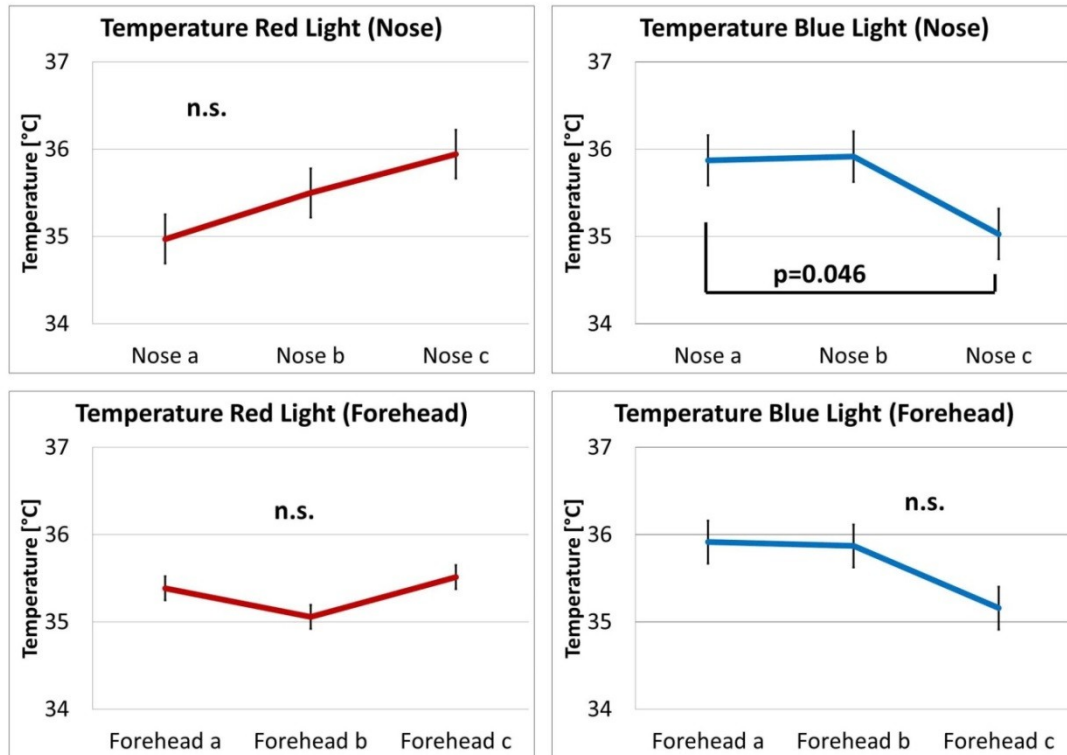


Fig. 5.5: Temperature values at the nose and forehead before (a), during (b), and after (c) irradiation with red and blue light. Note the significant decrease of the temperature at the nose after blue light stimulation.

Figures 5.6 and 5.7 show the mean HR and total HRV from the ECG recordings of altogether seven healthy volunteers during the four measurement phases (a–d). There was a slight but not significant decrease in HR after stimulation onset with red light. However, in the blue light session HR decreased significantly ($p<0.05$) during the second half of the stimulation phase and also in the 5 min period afterwards (Figure 5.6).

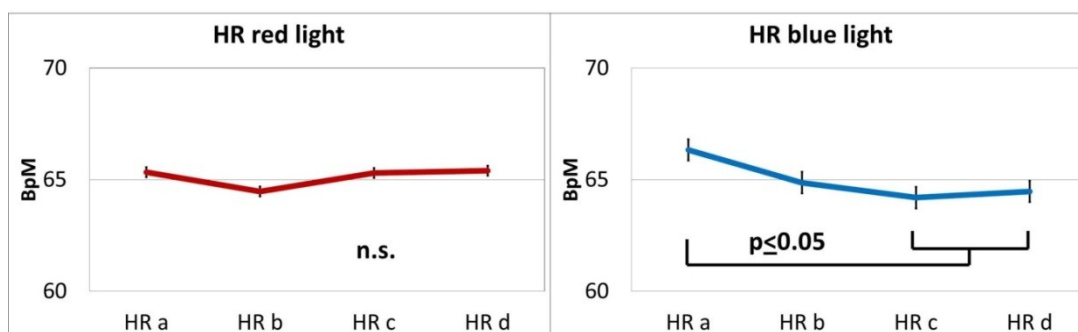


Fig. 5.6: Graphics displaying the changes in mean heart rate (HR) of the 7 subjects receiving red light stimulation (a) and the same persons receiving blue light stimulation (b). HR decreased significantly only following stimulation with a wavelength of 456 nm.

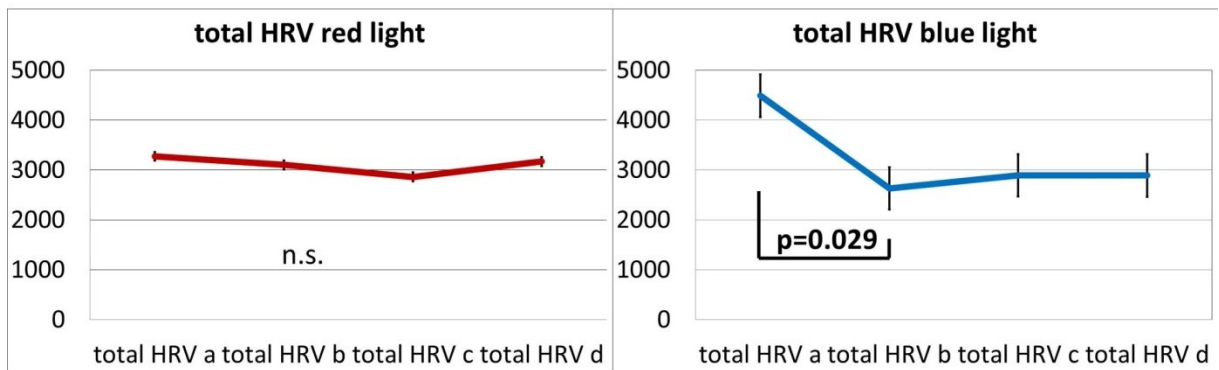


Fig. 5.7: Changes in total heart rate variability (HRV). Blue light (right) stimulation induced significant stimulation-related changes in total HRV in the seven subjects investigated in this study. No significant changes were found for red light stimulation.

In addition to HR, HRV also showed significant ($p=0.029$) alterations in the blue light session.

Continuous HRV monitoring also showed significant alterations in the LF/HF ratio during red light stimulation (see Figure 5.8).

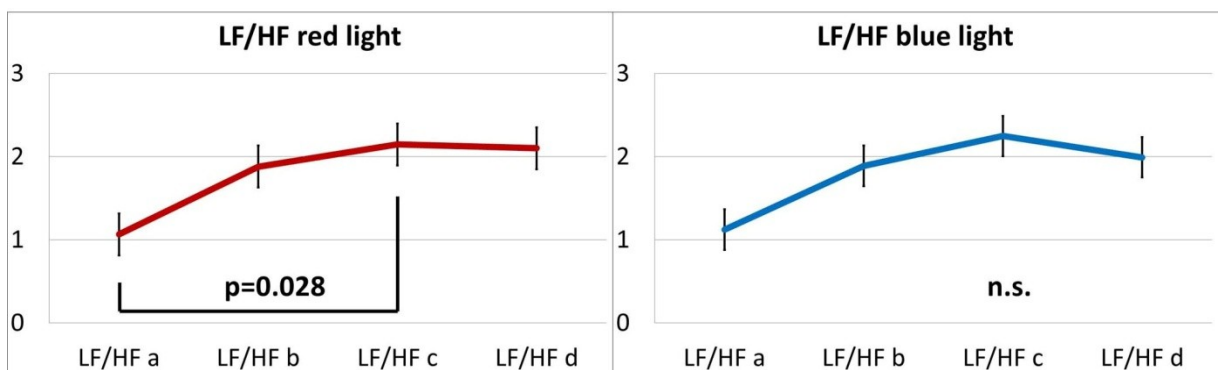


Fig. 5.8: The low frequency (LF)/high frequency (HF) ratio changed significantly during red light stimulation ($p=0.028$).

The results of the state of mind questionnaires are summarized in Figure 5.9. A significant ($p=0.006$) decrease in the sense of a positive effect of well-being was found after blue light stimulation.

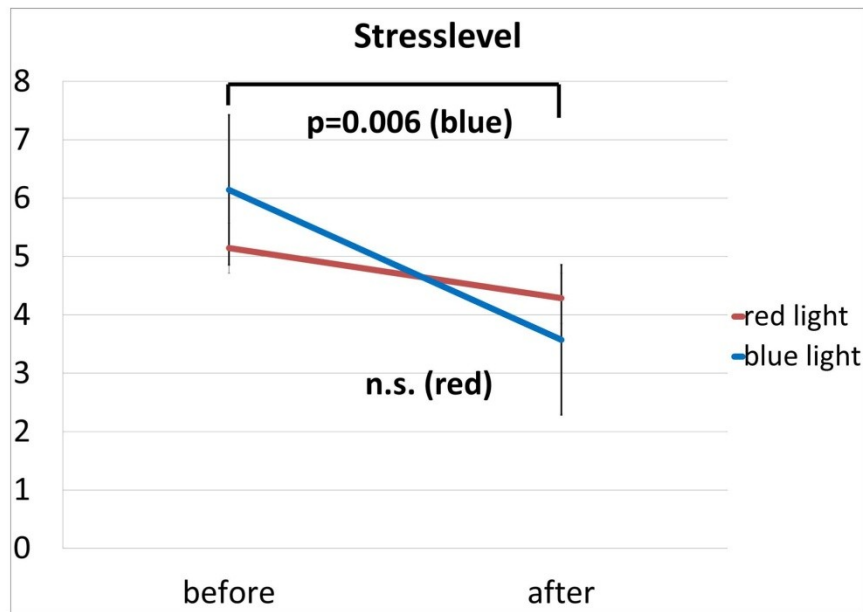


Fig. 5.9: Stress level score evaluated in the 7 subjects.

5.4 Discussion

Light plays a central role in life. Without sunlight there is no life on earth. Effects of light stimulation and light therapy on autonomic functions (e.g., body temperature, HR, or HRV) were already investigated in several human studies [5, 10, 11]. To the best of our knowledge, simultaneous recording of the three parameters has never been performed extensively during red and blue light stimulation. However, the results of this preliminary study should be regarded as those of a pilot study and thus require cautious interpretation.

The temperature results of the present study demonstrate that illumination with blue light for 10 minutes evoked significant changes in regional temperature at the nose. In addition, significant HR and total HRV reactivities were associated with alterations of the emotional state of the participants (stress level score). However, the red light stimulation did not induce significant changes in temperature, HR, and total HRV in the same persons. Furthermore the stress level score did not show significant alterations after red light stimulation with the same illuminance and distance to the eye.

Specific effects of different light colors have been reported in several studies. Exposure to low intensity blue light can have an acute alerting effect without melatonin suppression [12]. Other authors reported that red light activates avoidance, whereas blue light enhances approach [13], but it is also stated that the associated psychological processes have not been fully explored [2]. Our study is one of the first to demonstrate interactions between different parameters (temperature, HR, HRV, and a score) during and after exposure to two different light colors in the same persons during a relatively short time and during nearly identical steady-state laboratory conditions. This study design therefore minimizes intra- and interindividual subject variability.

There are reports about changes of nose temperature in evidence-based complementary studies, for example, after acupuncture stimulation. Zhang et al. [14] already showed in 1991 that nose temperature can be lowered immediately after acupuncture. This is also described in theories of ancient Chinese books [14].

Indicators of the functional state of the autonomic nervous system like temperature, HR, or HRV were also investigated by Suter and Kistler in the last century [15]. The authors pointed out the importance of studying basic regulatory mechanisms which are fundamental for most treatments in complementary medicine.

The sensitivity of HRV as an index of effective emotion regulation was demonstrated furthermore by Elliot et al. [16]. Participants who were exposed to red light (versus a control color) exhibited a decrease in HF-HRV, and this result was associated with worse cognitive performance [16].

In addition to HR and total HRV we also calculated the LF/HF ratio. Another study reported that this ratio was decreased after illumination with “cold” colors [17]. We could not confirm these results; in contrast, there was a slight but insignificant increase of the LF/HF ratio also under blue light stimulation. However, previous reports also mentioned that it is important to take the individual emotional state into account for such investigations [2].

Our results showed that the blue light altered total HRV, whereas the red light altered the LF/HF ratio. Maybe different pathways and activations in the brain are responsible for these results, however, our study design does not allow conclusions concerning the underlying mechanisms.

In addition it is also interesting that the changes in HRV during blue light stimulation appeared before HR changed significantly. This demonstrates that the HR changes per se were not the main factor influencing HRV.

The influence of sound and light on HRV was also demonstrated in several previous studies [18]. Authors from Japan found that the cardiac parasympathetic nervous activity during auditory excitation increases with elimination of visual stimuli and tends to be more pronounced in females than in males. In our study no comparison between females and males was performed due to the small sample size.

At this point it has to be mentioned that there are some limitations of this study. Firstly, as already stated, the number of persons included in the study is very small. Nevertheless there were significant changes in different parameters which made a common interpretation meaningful. Secondly, a possible response bias is always inherent in self-report information like the stress level score in our study, and thirdly, due to the small sample size, the baselines values of the temperature differed at the beginning of the blue and red light stimulation, respectively.

In conclusion of our study, blue light stimulation induced more significant effects in quantitative measurement parameters of the autonomic nervous system in comparison to red light stimulation with nearly the same illuminance and distance from the eye. The results also show that the objective, measurable effects were associated with subjective impressions of the test persons. However, it should also be stated very clearly that the different effects of colored light as environmental stimulation of human health are not clarified in detail at the moment and thus this topic deserves further studies.

Conflict of Interests

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

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6 Spectroscopic measurements in patients with completely ruptured anterior cruciate ligament before and after RegentK and physiotherapy

[Ref. 6; D. Litscher et al.]

6.1 Introduction

Every year more than 10,000 Austrians suffer an injury of the cruciate ligament, most of them during sports, especially skiing or playing football. According to Statistics Austria 2012, more than 8,600 of these ligaments were reconstructed surgically. Recent studies, however, show that a ruptured cruciate ligament can regrow even without surgical intervention. Normally this would take many months, but sometimes the healing process can be accelerated. In a small village, Hallein (close to Salzburg) in Austria, Mohamed Khalifa has been treating knee ligament injuries for more than 30 years with a non-invasive pressure therapy, the so-called regeneration therapy after Khalifa (RegentK). His patients include top athletes, but also surgeons [1-3]. However, at the moment Mohamed Khalifa has no students and therefore the therapy cannot be performed by other therapists. Our research tries to investigate the underlying mechanisms in order to create a new integrative treatment approach.

There are many different measurement methods in physical medicine and rehabilitation, and instrumental assessment is becoming increasingly important in clinical practice for quantification of the effects of different rehabilitation methods. Near infrared spectroscopy (NIRS) is one of these methods. It was described for the first time by Jöbsis in Science in 1977 [4]. It is a non-invasive technology and the multichannel monitoring equipment led to numerous clinical applications. In rehabilitation medicine, NIRS is also used for investigating changes in oxygen saturation of the lower extremities, however, there are only few reliable basic and clinical data available [2,5].

The goal of this randomized controlled study was to investigate acute effects of RegentK compared to standard physiotherapy on regional oxygen saturation of

knee tissues in patients with completely ruptured anterior cruciate ligament (ACL) using new modern technical equipment.

6.2 Materials and Methods

6.2.1 RegentK

Khalifa therapy has been reported in detail in a previous paper [2]: “Khalifa therapy is described as functional-pathological [6]. In this approach, function is the primary concern, not anatomy. The most important thing is not the ruptured ligament itself, but its function/dysfunction. Khalifa therapy restores the function of the knee in a natural way. During the 60 – 90 min of his manual therapy, he applies pressure to the injured knee in order to activate the self-healing processes of the human body, using his hands as an instrument for both measurement and therapy. Over periods of varying length, he applies increasing pressure on a spot before moving on to the next spot. The frequency of pressure application depends on the patient’s physiological reaction. The force of the pressure is not comparable to that normally used in acupuncture in Traditional Chinese Medicine [5]; it is much higher. Mohamed Khalifa’s method is based on manual pressure of varying frequency and does not damage the body, but supports it in its own natural healing activities. If one cuts through an elastic band and sews it together again, one cannot expect it to be as elastic at the stitching point as it was before. It is the same with human ligaments, and if the elasticity is disrupted anywhere in the human body, the whole system is affected [6,7].”

6.2.2 Physiotherapy

Physiotherapeutic intervention in order to mobilize/activate the knee joint included basic techniques of different myofascial treatment methods. Focus was put on the myofascial structures of tissues surrounding the knee joint; the upper part of the body and the upper extremities were not included in the treatment. Two basic techniques were used during the control intervention: (1) a static manual treatment, i.e. pressing trigger points usually to be found in the muscle belly (typically e.g. in the m. quadriceps, the m. biceps femoris, m. popliteus, m. gastrocnemius), and (2) static-dynamic stretching intermuscular techniques in the

areas of the tractus iliotibialis, the medial femoral muscles directed toward the knee or between (and in) the muscle bellies of the m. gastrocnemius.

Both techniques were performed at the patient's individual pain threshold, and static trigger points were pressed 3 – 5 times (for a maximum of 30 seconds each) until the tension and pain subsided. The myofascial longitudinal and crosswise stretchings were also repeated 3 – 5 times, slowly and dynamically.

Before and during the treatment (duration about 60 min), patients were informed about potential physiologic reactions of the interventions [8-12].

6.2.3 Patients

Twenty patients were randomly assigned to the two intervention groups (group A: RegentK; group B: physiotherapy). The demographical data of the two groups can be seen in Table 6.1.

Table 6.1: Demographical data of both patient groups. Data are given as means \pm SD.

	Group A (RegentK)	Group B (physiotherapy)
age [years]	31.3 \pm 8.5	34.8 \pm 10.2
range [years]	20-43	19-47
height [cm]	170.5 \pm 10.4	174.8 \pm 9.7
weight [kg]	67.7 \pm 12.2	66.1 \pm 12.0
sex	8 f, 2m	6 f, 4 m
cause of injury (no. of patients)	skiing (8), household (1), volleyball (1)	skiing (9), volleyball (1)
injured side (no. of patients)	left (5), right (5)	left (4), right (6)

Inclusion criteria:

- unilateral complete rupture of the ACL (less than 14 days ago), verified by magnetic resonance imaging
- age: 18 - 49 years
- normal body weight: BMI (body mass index) 18-25
- regular exercise level
- knee instability: experienced at least one giving-way
- dysfunction: knee range-of-motion: reduced or inhibited

Exclusion criteria:

- preceding surgical intervention (including arthroscopy) on the injured knee
- metabolic disorders like diabetes mellitus
- autoimmune diseases

The patients were informed about the nature of the investigation as far as the study design allowed and were not paid for their participation. The study was approved by the ethics committee of the University of Salzburg, Austria (21-232 11-12 sbg + amendment), and registered at clinicaltrials.gov under the ID-no. NCT01762371. All participants provided written informed consent.

6.2.4 Evaluation parameters

The NIRS measurements were performed using a four-channel INVOS 5100C Oximeter (Somanetics, Troy, USA). The principle of this system is based on NIRS technology, a non-invasive method for measuring regional oxygenation through the intact skin [13]. Near-infrared light (730 and 805 nm) is emitted through the skin, and after passing different kinds of tissue (muscle and bone), the returned light is detected at two distances from the light source (3 and 4 cm). Based upon this principle, the spectral absorption of blood in deeper structures (2 – 4 cm) can be determined and defined as regional oxygen saturation (rSO₂) [5,13]. The different tissues of the human body have different absorption characteristics, and therefore it is not easy to define in which kind of tissue the rSO₂ value is measured [2].

6.2.5 Procedure

The NIRS measurement procedure was the same for both patient groups. Approximately 3 hrs before starting the measurement, both legs were shaved. The skin was then cleaned with the enclosed skin-prep pad and dried with a gauze pad. After removing the protective backing label from the adhesive side of the sensor, four sensors were applied below the right and left lateral side of the patella of both legs. Calibration is performed automatically. To minimize external light influence, the knees were covered with a dark surgical cloth during the recording procedure. The rSO₂ data were recorded before the start and after the end of the

respective manual therapy (three readings each within approximately 2 min; for graphical presentation, only the mean values “before” and “after” were chosen).

All patients were investigated in a supine position under similar conditions. The parameter rSO_2 was simultaneously measured at four sites: laterally and medially at both knees, the injured one and the healthy one (control; this knee did not receive any therapy or manual manipulation, only the measurement sensors were applied). To avoid a potential technical recording bias, the four channels of the equipment were used in a randomized order, as in a previous study [2].

6.2.6 Statistical analysis

The rSO_2 values of both legs were tested with one way repeated measures ANOVA (SigmaPlot 12.0, Systat Software Inc., Chicago, USA). The Tukey test was used for post-hoc analysis. The level of significance was defined as $p < 0.05$.

6.3 Results

In all patients who fulfilled the inclusion criteria, neither the initial conditions (knee instability and dysfunction) nor the demographic data showed significant differences between group A and group B. All 20 patients completed the study procedure, and the technological NIRS measurements could be performed without problems.

Figure 6.1 shows the highly significant ($p < 0.001$) increase of rSO_2 values after RegentK on the injured knee. It has to be mentioned that this increase was a little bit more pronounced on the anteromedial side of the injured knee (Fig. 6.1, right).

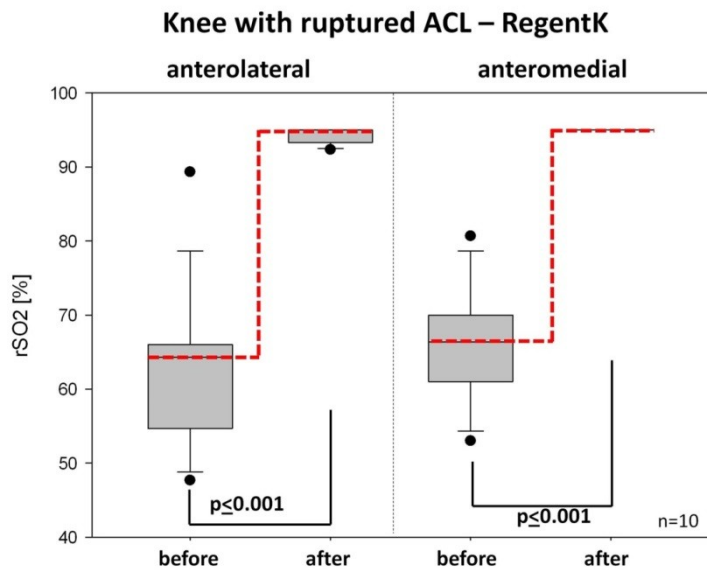


Fig. 6.1: Box plot presentation of changes in regional oxygen saturation (rSO₂) on the injured knee after RegentK (ends of boxes: 25th and 75th percentile; line at the median; error bars: 10th and 90th percentile).

The rSO₂ values on the injured knee before and after physiotherapy are shown in Figure 6.2. The baseline values before treatment were within the same range as those in group A. However, the increase after physiotherapy was far less than after RegentK. The level of statistical significance was reached on the anterolateral side (p=0.015), but not on the anteromedial side of the injured knee.

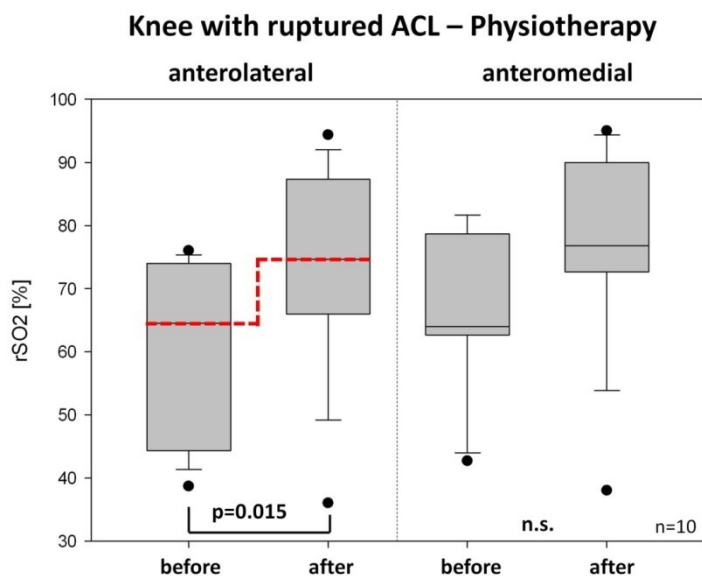


Fig. 6.2: Changes in regional oxygen saturation (rSO₂) on the injured knee before and after physiotherapy. Further explanations see Fig. 6.1.

The next figures (Figs. 6.3 and 6.4) show the values of the NIRS oximeter on the control (healthy) knee. It is interesting that the baseline values before therapy were lower than those of the injured knee. In addition, there was also a highly significant increase on both measurement sites (anterolateral and anteromedial) of the healthy knee after RegentK.

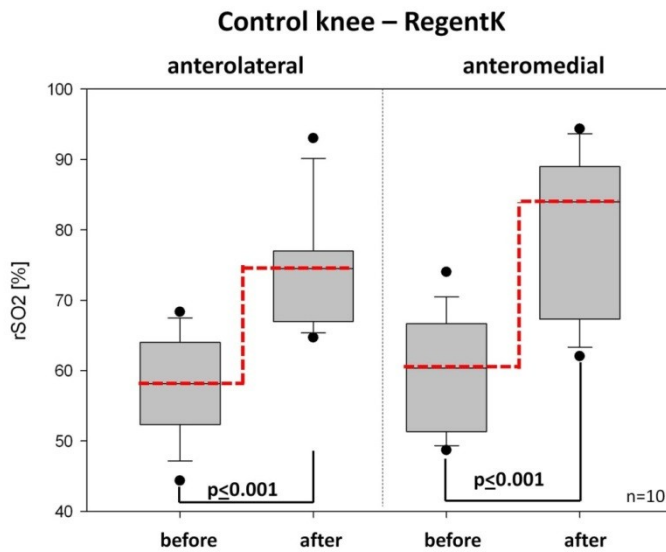


Fig. 6.3: Changes in regional oxygen saturation on the healthy control knee before and after RegentK. For further explanations see Fig. 6.1.

Figure 6.4 summarizes the rSO₂ results of the control knee before and after physiotherapy. No significant changes were found anterolaterally and anteromedially.

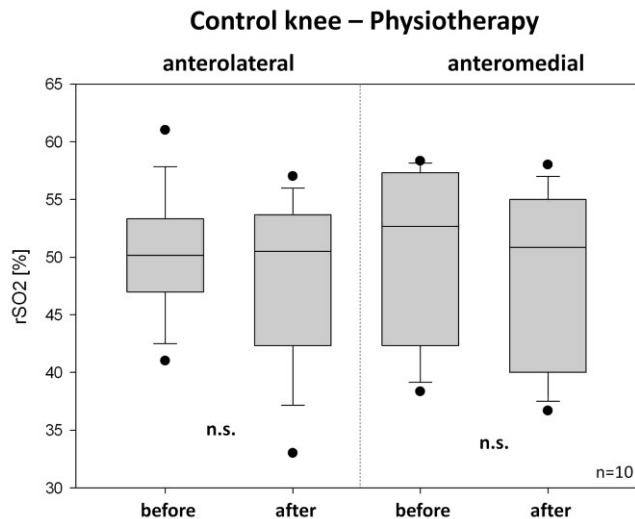


Fig. 6.4: Changes in regional oxygen saturation on the healthy control knee before and after physiotherapy. For further explanations, see Fig. 6.1.

Within this randomized controlled trial, the function of all ten knees with a ruptured ACL was restored after 60-90 minutes of RegentK, but not after about 60 minutes of physiotherapy. All patients in group A were able to bend their knees immediately after the end of RegentK. The biomechanical data will be described in future publications by another research team. In group B, some success could also be seen, but not to the same extent as in group A.

6.4 Discussion

The first study to report an immediate close-to-normal functional restoration after one non-surgical intervention was published recently [1]. Three months after the intervention, 47 % of the patients with an initially MRI-verified complete ACL rupture showed an end-to-end continuous ACL, again MRI-verified [1].

An increase in tissue blood flow is one of the most acknowledged potential effects of massage [14]. Up to now, research studies investigating this phenomenon are rare, inconsistent and inconclusive [15]. Many studies in the field of manual therapy and regeneration employed laser Doppler techniques [16,17]. The problem is that laser Doppler can only estimate blood flow at the skin level. On the other hand there is also the possibility of measuring with Doppler ultrasound, but this method is not sensitive to blood flow in the smaller vasculature [14,18].

However, there are also two preliminary studies from our research team at the Medical University of Graz investigating these effects using non-invasive methods. One of these two studies deals with NIRS measurements before and after acupuncture at an acupoint on the knee [5], the other one is a preliminary study from the first phase of the current research project on RegentK [2].

In our opinion – and also that of others – NIRS may be a more accessible measurement option for manual therapy and researchers focusing on regeneration. The method provides dynamic information about oxy- and deoxyhemoglobin concentrations and blood oxygen saturation in deeper tissues [19].

The instrument used in our study is a commercial one, but many prototypes with different functions are also available. One system will measure massage and manual therapy effects on skeletal muscle hemodynamics using a novel hybrid near-infrared optical instrument that combines a commercial NIRS system with a custom-made one for simultaneous measurement of muscle blood flow and oxygenation [14]. The principle of this hybrid instrument is described elsewhere [20].

The Swiss Medical Board recommends that ACL ruptures should preferably be treated with physiotherapy because not significant differences in outcome were found between the surgical and the conservative treatment group [21]. However, the difference in costs is huge. It is estimated that more than 35 million Swiss francs could be saved per year by applying conservative treatment for ruptured ACLs [21]. Using conventional physiotherapy requires usually several treatment sessions. RegentK, on the other hand, consists of only one treatment session.

In conclusion, spontaneous healing of ACL ruptures is possible within three months of RegentK therapy [1], and basic research on its mechanisms is now proceeding at full speed. Based on the results of the current study in patients after RegentK or physiotherapy, we conclude that manual therapy influences the hemodynamics of muscles and deeper structures. These results are in accordance with the only previous measurements on this topic [2,5]. They will also serve as a

tool to quantify the effects of non-pharmacological and non-surgical interventions (e.g. RegentK) on microvascular circulation in deep tissue after a complete rupture of the ACL.

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Conflict of Interest

The authors declare that there is no conflict of interest concerning the publication of this article. The authors assume responsibility for the overall content and integrity of this paper.

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