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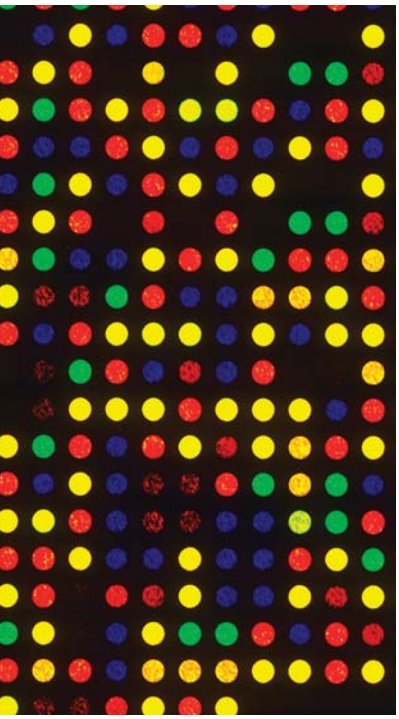
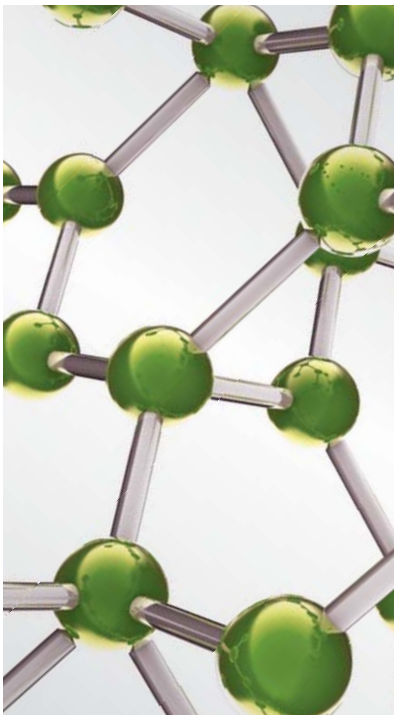
High-Tech Acupuncture and Integrative Laser Medicine

Guest Editors: Gerhard Litscher, Xin-Yan Gao, Lu Wang, and Bing Zhu



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Editorial Board

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Editorial

High-Tech Acupuncture and Integrative Laser Medicine

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Basic research on high-tech acupuncture and integrative laser medicine has been successfully performed all over the world during the last decades, using a broad spectrum of innovative biomedical engineering methods. One of the main goals is to combine basic research on high-tech acupuncture with necessary further experimental and clinical pilot studies for the first time. Acupuncture has been used for medical treatment for thousands of years. Using electroacupuncture, needle or laser needle stimulation, and modern biomedical techniques, it was possible for the first time to quantify changes in biological activities caused by acupuncture.

The patient is in China—the analysis for the efficacy of acupuncture is performed by experts in Europe. This "*transcontinental teleacupuncture*" is a way which was realized by our research team within joint Sino-Austrian and Sino-European projects. The investigations are carried out over thousands of kilometers; for example, 24-hour electrocardiographic (ECG) recordings from patients are registered in China, and the data are transferred directly after the acupuncture treatment to an analysis computer at the Medical University of Graz. The acupuncturists in China are informed about the results immediately based on the analysis protocol.

These Sino-European studies included this special issue that contains 26 interesting publications, of which 12 are related to needle acupuncture, 5 to laser acupuncture, and 9 to electroacupuncture. Apart from body acupuncture, special emphasis is also given to auricular acupuncture. The investigations cover animal experimental studies and studies in healthy volunteers and patients, as well as basic and clinical research on evidence-based high-tech acupuncture, integrative laser, and translational medicine based on acupuncture and moxibustion. A first introduction of new developments of acupuncture stimulation equipment is also featured among the papers.

It has to be mentioned that this special issue has a total impact factor of 124.124 and contains, among others, the following topics: (i) modernization of acupuncture (evidence-based medicine, integrative laser medicine), (ii) high-tech acupuncture, (iii) development of innovative acupuncture stimulation methods (needle, laser, and electroacupuncture), (iv) methods for the quantification of peripheral and central effects of acupuncture, (v) scientific evaluation of complementary medical methods (acupuncture, acupressure, moxibustion, and laser therapy), (vi) computer-controlled acupuncture, (vii) teleacupuncture, (viii) laser needle acupuncture, (ix) red- and infrared laser stimulation, (x) violet laser acupuncture, (xi) biomedical assessment of acupuncture.

Modernization of acupuncture is a contemporary issue. The bridging between Eastern and Western medicine was successful using modern biomedical engineering technology, as described in this special issue. The next task is to make the arising possibilities and results useable for all involved persons.

Acknowledgments

The lead guest editor thanks the other three guest editors, Professor B. Zhu, M.D. Ph.D. (Director of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing, China), Associate Professor Xin-Yan Gao, M.D. Ph.D. (Head of the Department of Physiology, Institute of Acupuncture and Moxibustion, China Academy

of Chinese Medical Sciences, Beijing, China), and Professor L. Wang, M.D. L.Ac. (Stronach Research Unit for Complementary and Integrative Laser Medicine and TCM Research Center Graz, Medical University of Graz, Graz, Austria), for the excellent cooperation. In this context, he would also like to thank all authors for their excellent contributions and patience during the review process. Of course, the work of all reviewers on the papers within this special issue is highly appreciated. We want to thank Ms. Ingrid Gaischek, M.S. (Stronach Research Unit for Complementary and Integrative Laser Medicine, Research Unit of Biomedical Engineering in Anesthesia and Intensive Care Medicine, and TCM Research Center Graz, Medical University of Graz, Graz, Austria) for her valuable support in every respect. All guest editors would like to thank Hindawi Publishing Corporation, and especially Eman Bastawy, for the excellent cooperation.

Gerhard Litscher
Xin-Yan Gao
Lu Wang
Bing Zhu

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Research Article

Longitudinal Anti-Müllerian Hormone in Women with Polycystic Ovary Syndrome: An Acupuncture Randomized Clinical Trial

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Others have studied acupuncture treatment for polycystic ovary syndrome (PCOS). Anti-müllerian hormone (AMH) is positively correlated with the ovarian follicle pool, thus making it a useful ovarian reserve measure. AMH is elevated in women with PCOS and has been suggested as a diagnostic tool. This study examined the impact of electroacupuncture on AMH concentration in women with PCOS. Seventy-one women with PCOS participated in a randomized, double-blind, sham-controlled clinical trial of acupuncture. Three longitudinal AMH samples over the 5-month protocol were compared with objective ovulation parameters primarily using nonparametric statistics. Results indicated that AMH levels in PCOS were higher than published norms in women without PCOS. There was no difference between the true and sham acupuncture arms in the change in AMH longitudinally. Baseline AMH, but not the change in AMH over time, was inversely correlated with ovulation and menstrual cycle frequencies in both arms combined ($P < 0.001$). In conclusion, AMH correlated with an increased likelihood of monthly ovulation, as expected from the literature on women without PCOS. The lack of difference by intervention in AMH was consistent with the underlying clinical trial. AMH may be clinically useful to predict which PCOS women are more likely to respond to an intervention.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age with incidence ranging from 8.7% to 17.8% depending upon which criteria are used [1]. Diagnosis of PCOS is made when androgen excess, ovulatory dysfunction, and/or polycystic ovaries are identified after the exclusion of other disorders that can cause these signs and symptoms [2]. Although not a part of the formal diagnosis, a number of endocrine abnormalities are often seen, including an increased ratio of luteinizing hormone (LH) to follicle stimulating hormone (FSH) [3] and insulin resistance [4]. There is emerging evidence that serum anti-müllerian hormone (AMH) may be useful in the diagnosis of PCOS [5].

AMH is expressed by the ovarian preantral and early antral follicles and reflects the size of the follicular pool. AMH levels gradually decline as the follicle pool declines, and it is thus useful as a marker of ovarian reserve, although there is no consensus on a threshold value for diagnosis of diminished ovarian reserve [6–8].

Emerging evidence has linked elevated AMH levels with women who have PCOS. AMH concentration correlates well with clinical, endocrine, and ultrasound markers associated with PCOS and may be a useful marker for the extent of disease [5, 9]. Although growing data support AMH as a possible clinical diagnostic and/or prognostic marker, there is little known about changes in AMH level in response to an intervention.

Acupuncture has been shown to be efficacious for a number of medical conditions. Of women seen in a reproductive endocrinology and infertility clinic, 22% had tried acupuncture therapy within 18 months of their initial clinic visit in the USA [10] and 12.5% within 6 months in Australia [11]. Researchers found an 8% use of acupuncture among infertility patients in the UK [12]. Interestingly, we

found no difference in ovulation rates or ovarian hormones levels between the active acupuncture and sham acupuncture arms of a randomized clinical trial, although there was a suggestion that both arms benefited from improved frequency of ovulation during the study [13].

Given these results, we set out through a secondary analysis of a randomized clinical trial to determine if AMH levels can predict response to acupuncture and/or can predict ovulation among oligoovulatory and anovulatory untreated, adult female patients with PCOS. Additionally, we aimed to characterize the levels of AMH of this subset of women relative to women without PCOS by age, as reported by others.

2. Materials and Methods

2.1. Population. This is a secondary analysis of AMH results from a randomized, double-blind, sham-controlled clinical trial of acupuncture in women diagnosed with PCOS. The original trial is described in detail elsewhere [13]. In summary, the 5-month protocol involved baseline questionnaires and biological sampling, 2 intervention months, post-intervention repeat questionnaires and biological sampling, 3 months of follow-up without intervention, and post-follow-up questionnaires and biological sampling. Women provided urine or blood samples weekly throughout the entire 5 months for objective assessment of ovulation. Menses were self-reported. This trial was approved by the University of Virginia's Institutional Review Board (no. 12045).

Inclusion criteria were (a) a diagnosis of PCOS, as confirmed by the presence of both oligomenorrhea and hyperandrogenism per the US National Institutes of Health criteria [14], (b) aged 18 to 43 years, (c) at least one menses in the past six months but no more than eight periods in the most recent 12 months without hormonal intervention, and (e) agreement to not take hormonal contraceptives, metformin, or fertility medication for the 5 months of study participation. Exclusion criteria were (a) diagnosed with Cushing's Syndrome, uncontrolled thyroid disease, hyperprolactinemia, congenital adrenal hyperplasia, and diabetes mellitus, (b) use of metformin or hormonal contraceptives in the 60 days prior to enrollment, (c) use of any other hormonal drug in the 30 days prior to entry into study, including fertility medications, and over-the-counter hormonal supplements or herbs (i.e., black cohosh, clover, soy, dong quai/Chinese angelica root, fructus rubi, and white peony root), (d) currently pregnant or breastfeeding during the prior 30 days, (e) any acupuncture treatment for ovulatory disorders in the prior 30 days, (f) weight > 113.4 kg (250 pounds), (g) currently taking anticoagulation medication other than low-dose (≤81 mg) aspirin, (h) immune deficient, and (i) history of any bleeding disorder.

2.2. Interventions and Ovulation Assessment. Subjects were randomized to 12 acupuncture or sham sessions: twice each week for the first four weeks followed by once per week for an additional four weeks. For the true acupuncture treatment, the following bilateral points were stimulated with electroacupuncture (EA): Bladder 23, Bladder 28, Spleen 6, and Spleen 9. The following points were manually stimulated: Pericardium 6, Triple Energizer 5, and Governor Vessel 20. The sham acupuncture was performed with the validated Park Sham Device [15, 16]. The sham device was placed on the skin at standardized points on all four extremities (Achilles tendon and lateral head of the triceps) chosen in order to avoid standard acupuncture meridians and acupuncture points [17]. For further details, the reader is referred to a prior publication [13].

The participants provided weekly blood samples for serum progesterone measurement or collected first-void urine samples at home (stored in their home freezer) for pregnanediol glucuronide (PDG) measurement, for the entire 5-month protocol. Ovulation was defined as progesterone ≥3 ng/mL or a ratio of the peak urinary PDG to the basal PDG level in the follicular phase ≥4.0.

2.3. AMH Assays. Serum AMH was measured longitudinally on all study participants using samples collected at their three study center visits (preintervention, postintervention, and after 3 months of follow-up). The assays were conducted by the Clinical Laboratory Research Core at Massachusetts General Hospital (Boston, MA, USA) using an AMH Gen II ELISA kit from Beckman Coulter according to the manufacturer's protocol. The sensitivity of the assay was 0.05 ng/mL.

2.4. Statistical Analyses. Medians and interquartile ranges were calculated by intervention arm, rather than means and standard deviations, due to the modest sample size. Graphs were created to investigate the distributions. Potential differences between the intervention arms were assessed with Kruskal Wallis, Wilcoxon Rank Sum, or Sign tests (continuous variables) and Spearman chi-square tests (categorical variables). After zero-skewness log transformation of AMH, linear regression was used to develop lines-of-best fit. *t*-tests were used to compare this cohort to the literature. Power calculations were not run *a priori* due to the fact that this was a secondary analysis. Statistical significance was judged by a two-sided alpha ≤ 0.05, unless otherwise specified. All statistical analyses were conducted with STATA/IC 12 software (STATA Corp, TX, USA).

3. Results

Ninety-six women were eligible, consented, and were randomized for the underlying acupuncture clinical trial [13], and 72 had more than one AMH sample as required for this longitudinal analysis. Of those 72 women, one was subsequently dropped from the dataset due to perimenopausal AMH levels (<0.05 mg/mL) despite a normal FSH level at baseline (2.7 mIU/mL) (Figure 1). Of the final cohort of 71 women diagnosed with PCOS, 32 received active acupuncture and 39 received sham acupuncture. These women were randomized between February 2006 and August 2009.

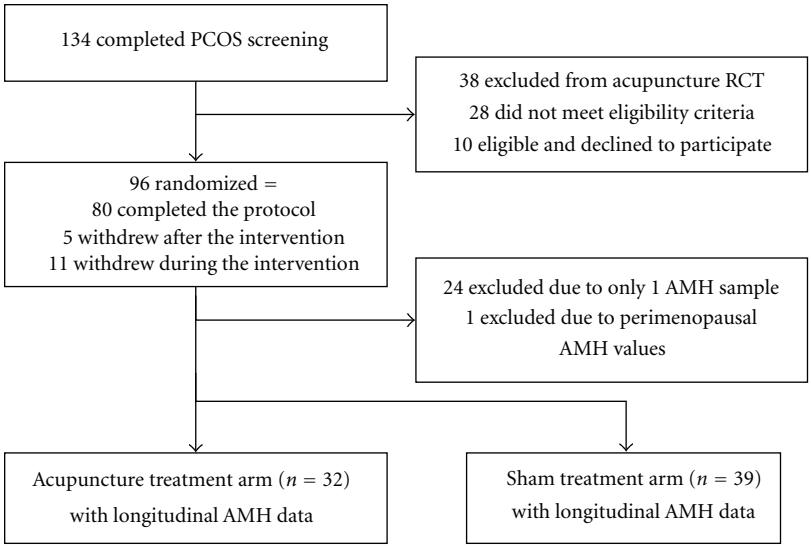

Figure 1: Flowchart of study participation.

Most of the women had some college education and were Caucasian (Table 1). The median body mass index was 29-30 kg/m^2. There were no differences by intervention in age, education, BMI, race, or Hispanic ethnicity ($P > 0.10$). Selected eligibility data and endocrine results are also displayed in Table 1. On average, the participants had 5-6 menses in the most recent 12 months without hormonal intervention before enrollment.

The preintervention AMH concentration did not vary between the intervention arms ($P = 0.79$, Table 2), nor were there differences in AMH by intervention at the other two time points ($P > 0.80$). The change in the AMH concentration was not clinically relevant by intervention arm. AMH increased by 0.02 and decreased by 0.005 ng/mL in the true and sham arms, respectively, after the intervention ($P > 0.30$). AMH decreased by 0.05 and increased by 0.01 ng/mL in the true and sham arms, respectively, after the entire 5-month protocol (data not displayed, $P > 0.40$).

No differences were detected between the true and sham acupuncture interventions in terms of the relationship between AMH concentration and both ovulation and menses. There was no correlation between the pre- and postintervention change in AMH level and the ovulatory frequency or menstrual cycle frequency in either the acupuncture arm ($P > 0.30$) or the sham arm ($P > 0.50$). Similarly, there were no corresponding correlations when the timeframe was expanded to include the entire 5 months of the protocol ($P > 0.30$). The preintervention AMH concentration did not predict who would become a "responder" to the true acupuncture, defined as at least a 60% monthly ovulation rate over the entire study timeframe ($P = 0.29$). The change in the AMH concentration over the 5-month protocol was not associated with being a "responder" in the true acupuncture arm ($P = 0.66$).

Combining the intervention arms in Table 3, the preintervention AMH concentration was significantly inversely related to both the ovulatory frequency during the trial (Spearman's $r = -0.54$, $P < 0.001$) and the frequency of menses during the trial (Spearman's $r = -0.50$, $P < 0.001$). Combining the intervention arms, there was no correlation between the change in AMH level either during the intervention only or the entire 5-month trial and the ovulatory frequency or menstrual cycle frequency ($P > 0.55$).

Age approached statistical significance in terms of being associated with the preintervention AMH levels in this PCOS cohort ($P = 0.053$). Figure 2 displays the log-transformed AMH concentration by age with a line-of-best-fit, which corresponds to the equation

$$\text{Ln}\ (\text{AMH}_0) = 2.6 - 0.02\ (\text{Age}). \quad (1)$$

The AMH concentration by age was significantly higher in the PCOS cohort ($P < 0.0001$) in comparison to a cohort of 17,120 women from infertility clinics across the USA [6]. The comparison cohort is graphed as a heavy red line in Figure 3. For comparison purposes, the equation underlying the line-of-best-fit for this cohort from Seifer et al's is

$$\text{AMH}_{\text{Seifer}} = 6.21 - 0.15\ \left(\text{Age}_{\text{Seifer}}\right). \quad (2)$$

AMH was not related to BMI (Spearman's $P = 0.45$). The median AMH by BMI tier is as follows: 6.4 for 20.0–24.9 BMI, 8.4 for 25.0–29.9 BMI, 7.6 for 30.0–34.5 BMI, 7.5 for 35.0–39.9 BMI, and 4.3 for 40.0–44.9 BMI.

4. Discussion

4.1. Summary. Our investigation revealed that this acupuncture protocol and this sham protocol individually did not significantly alter the serum AMH concentrations in this cohort of women with PCOS. Combining the two protocols, the preintervention AMH concentration was positively correlated with both the ovulation frequency and the menstrual cycle frequency during the 5-month clinical trial protocol ($P < 0.0001$). The AMH concentration was significantly higher

Table 1: Participant demographics and biochemical data by intervention arm.

Factor	True acupuncture (n = 32)	Sham acupuncture (n = 39)	P value
Age: median (IQR)	27.5 (22–33)	25 (23–29)	0.14
Education: n (%)			
HS or less	2 (6%)	3 (8%)	0.89
Some college	14 (44%)	15 (38%)	
College degree	7 (22%)	13 (33%)	
More than college	9 (28%)	8 (21%)	
Body mass index: Median (IQR)	29.3 (23.5–36.3)	29.9 (24.4–34.9)	0.99
Race: n (%)			
Caucasian	25 (78%)	32 (82%)	0.67
African-American	3 (9%)	4 (10%)	
Other	4 (13%)	3 (8%)	
Hispanic: n (%)	0 (0%)	3 (8%)	0.11
Menses in the 12 months prior to enrollment without hormonal medications	6 (3.5–7)	5 (3–7)	0.31
Fasting plasma glucose (mg/dL): median (IQR)	93 (88–96)	94 (89–98)	0.62
Fasting serum insulin (mIU/mL): median (IQR)	7.8 (3.5–13.3)	6.9 (2.7–10.9)	0.46
TSH (uIU/mL): median (IQR)	1.36 (0.84–1.91)	1.51 (1.07–2.02)	0.50
17 OHP (ng/dL): median (IQR)	121 (81–148)	124 (76–150)	0.62
HbA1C: median (IQR)	5.3 (5.1–5.5)	5.3 (5.1–5.6)	0.34
DHEAS (μg/dL): median (IQR)	129 (101–231)	174 (126–214)	0.75
Free testosterone (pg/mL): median (IQR)	11.3 (7.6–14.6)	11.1 (7.6–18.7)	0.67
SHBG (nmol/L): median (IQR)	33.1 (21.7–58.0)	33.5 (23.0–53.4)	0.90

Table 2: Longitudinal AMH concentrations (ng/mL) in women with PCOS by intervention: median (interquartile range).

Intervention arm	Preintervention baseline	Postintervention	P value[a]	Three-month follow-up	P value[a]
True acupuncture (n = 32)	6.5 (4.4–9.9)	6.4 (4.5–10.9)	0.36	6.2 (5.0–9.2)	0.57
Sham acupuncture (n = 39)	7.4 (4.1–9.6)	6.4 (4.5–9.0)	0.63	5.8 (4.2–10.4)	0.43
P value[b]	0.79	0.90		0.84	

[a]One-sided test for a decline in AMH since the preintervention value.
[b]Two-sided test comparing true versus sham acupuncture.

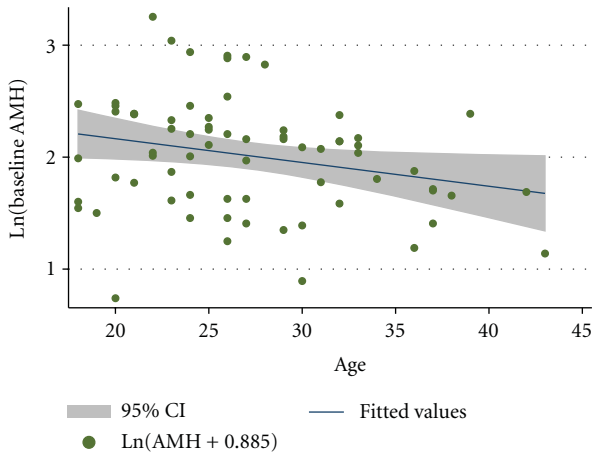

Figure 2: Preintervention log-transformed AMH concentration by age in PCOS women.

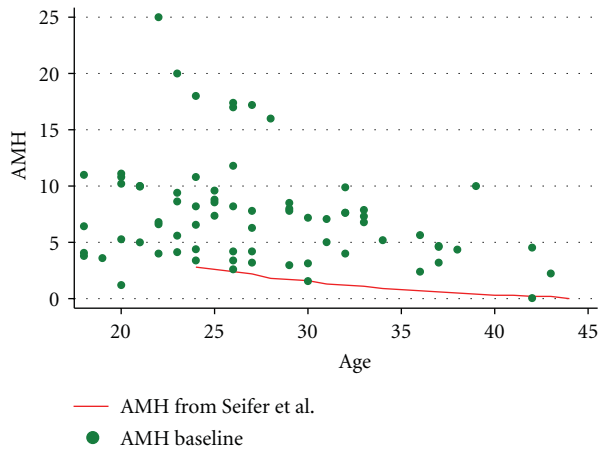

Figure 3: Preintervention AMH concentration (ng/mL) by age in PCOS women and compared to Seifer et al.'s [6] population of 17,120 women.

Table 3: Correlation between AMH concentrations and both ovulation and cycle frequency for the entire PCOS cohort: Spearman's rho (P value).

AMH variable	Ovulatory frequency	Menstrual cycle frequency
AMH preintervention	−0.54* (<0.0001)	−0.50* (<0.0001)
Change in AMH post- versus preintervention	−0.02 (0.90)	−0.07 (0.58)
Change in AMH 3 month follow-up versus preintervention	0.08 (0.53)	−0.01 (0.92)

* $P \leq 0.05$.

across all ages in the PCOS cohort in comparison to a very large cohort of fertility clinic patients ($P < 0.001$).

4.2. Comparison to Relevant Literature. Low serum AMH levels have been shown to be predictive of infertility treatment in women without PCOS. Lower serum AMH concentrations during assisted reproduction treatment are strongly associated with a reduced oocyte yield and low oocyte quality [18, 19]. For women with PCOS who had AMH levels that were 2 to 3 times the level produced by normal ovaries [20] the opposite appeared to be true in that women with high serum AMH predicted poor response to treatment [21, 22]. It was not surprising that the AMH concentrations were not associated with the real or sham acupuncture protocols, as the underlying randomized clinical trial found that there was no difference in the ovulation rate by intervention arm [13]. We predicted a decline in the AMH concentrations toward non-PCOS levels in women who responded to the intervention; however, any decline was not statistically significant. In one study with 65 PCOS cases, serum AMH concentrations declined an average of 7% after six months of metformin treatment ($P < 0.01$) [23]. A 7% decline in our measurements would have corresponded to a half point.

Preintervention serum AMH concentrations predicted success in terms of regular ovulation and menses, as has also been reported in women without PCOS [18, 19]. This finding suggests that AMH levels could serve as a marker of the likelihood of PCOS resolution without treatment or may support the underlying clinical trial's original report suggesting that both interventions provided benefit [13]. One prior report did find that baseline AMH levels in women with PCOS predicted the response to treatment; in their case the treatment arms were laparoscopic ovarian drilling and clomiphene citrate ($P < 0.01$, [24]).

As expected, there was an inverse association between age and AMH concentration, as reported by others [5, 23, 25, 26]. If we were to impose a linear structure on our AMH values in order to compare to the literature, then the slope of the AMH/age association in our PCOS cohort was −0.19. This slope was somewhat steeper than a prior report (−0.11 [25]) and was surprisingly similar to the slope of the AMH decline among women without PCOS who were seen in a fertility clinic (−0.15) [6]. This implies that the rate of decline in AMH as a woman ages is similar in both PCOS and non-PCOS populations. One area of future research, unrelated to the acupuncture intervention, is a description of the AMH concentrations in women with PCOS as they progress through the menopausal transition to their final menstrual period.

Most of the prior research did not find an association between AMH and BMI in women with PCOS [9, 23, 25], although one publication did report an inverse correlation [26]. The varying results in the literature on this item may be due to different presentations of PCOS as reported by Lin et al. in 2011 [26] or may be due to serum versus plasma AMH measurements.

4.3. Strengths and Limitations. The findings are limited by the fact that the acupuncture protocol did not allow for individualization, thus the findings are not reflective of "real world" acupuncture practice. Therefore, the interpretation of the results is limited to this particular acupuncture (and sham) protocol. There was no consideration of phenotypes within the PCOS diagnostic framework, as was investigated by others [26]. The sham procedure might not have been inert, as the underlying clinical trial results implied a benefit to both the true and sham acupuncture groups. Thus there may be a true impact of acupuncture on AMH, which might have been observed if the comparison had been an observation-only cohort. Our sample was also limited to women with longitudinal AMH samples, thus women who became pregnant or dropped out of the study during the 2 months of the intervention ($n = 13$) could not be included, and this may have influenced our findings if those women had lower AMH levels than the median of the population analyzed.

A strength of this study is the RCT design, with its prospective data collection and blinding of the intervention. This is the only clinical trial of acupuncture to measure AMH in women and one of only a few reports [23, 24] with longitudinal assessments of AMH in a cohort of women with PCOS. The diagnosis of PCOS was conducted objectively through this study on all women, thus avoiding bias at the time of the eligibility assessment. Ovulation was measured objectively through serum progesterone and urinary pregnanediol glucuronide, as reported previously [13]. This study included PCOS women from the general community (approximately 40% had not been diagnosed with PCOS prior to study enrollment) as opposed to a clinic population with potentially more severe disease. The cohort represented a wide range of ages and BMI, which improves the generalizability of our findings.

4.4. External Validity. These findings are applicable to women with PCOS as diagnosed with NICHD criteria. As stated above, the interpretation of the results is limited to this particular acupuncture (and sham) protocol.

4.5. Clinical Significance. In women without PCOS, AMH has been increasingly used as a marker of ovarian reserve [27] and has been shown to be a potential predictor of

success in assisted reproduction [18, 19]. AMH has been shown to be elevated in women with PCOS [20] and may become a useful marker of PCOS. Our study showed elevated levels of AMH, consistent with other reports [5, 18, 20, 21], and it appears that this elevation may be an arithmetic deviation (as opposed to a multiplicative relationship) that is fairly consistent over a wide age range. Importantly, preintervention AMH levels were associated with ovulatory rates during the course of the trial, lending further evidence that AMH could become a potential marker for severity of disease in women with PCOS.

AMH concentrations were not associated with the real or sham acupuncture protocols consistent with the findings from the underlying randomized clinical trial which found no difference in the ovulation rate by intervention arm [13]. Thus, we were unable to define clinical or serum markers associated with successful treatment with this acupuncture protocol in women with PCOS. More research is indicated in this particular area of fertility treatment of women with PCOS.

Conflict of Interests

No conflicts of interest are declared by the authors.

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Review Article

How to Design the Control Group in Randomized Controlled Trials of Acupuncture?

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In evidence-based medicine, randomized controlled trials (RCTs) are the preferred method for evaluating the efficacy of interventions. In regard to acupuncture RCTs, the most difficult issues are the design of the control group and implementation of the principle of "double-blinding." We compared the advantages and limitations associated with different control group designs in acupuncture RCTs, to assist researchers in this field.

1. Introduction

1.1. The WHO Promotes the Use and Research of Acupuncture. Acupuncture is a convenient, effective, and simple form of traditional Chinese medicine that has few side effects [1]. Thus, it is often the first therapy that patients consider for a wide range of complaints where medicine is lacking and is the most widely used complementary and alternative medicine worldwide. In 1979, the World Health Organization (WHO) issued its first report on traditional medicine and described 43 categories of symptoms treatable by acupuncture; in 1996, the list was expanded to 64 [2]. This fact alone shows the value of acupuncture worldwide. In 1995, the WHO "Guidelines for Clinical Research on Acupuncture" [3] stipulated three criteria for good acupuncture studies: validity, reliability, and statistical significance (P value). Thus, at every stage of research, from basic studies to clinical trials, these criteria are paramount in any attempt to explain the function and effect of acupuncture.

1.2. Acupuncture RCTs Are Needed. Evidence-based medicine (EBM) [4] has become the gold standard among researchers, ever since the term was coined by Guyatt et al. in his paper published in *JAMA* in 1992 [5]. Randomized clinical trials (RCTs) belong to the Ib [6] level of EBM and therefore provide reliable criteria for study validity. Factors influencing the validity of RCTs include [3] patient selection, study size, site(s) of investigation, blinding techniques, randomization, design of the control group, crossover studies (usually inappropriate for acupuncture), and the strategic approach. Well-designed, properly conducted RCTs also include statistical analysis by a biostatistician to ensure that the results are statistically valid.

1.3. The Importance of a Control Group in Acupuncture RCTs. The ongoing debate about the therapeutic effects of acupuncture for some diseases [7–12] means that RCTs are needed to evaluate the efficacy of acupuncture. Such trials must include an appropriate control group [13]. A well-designed control group not only increases reliability of the study but also improves its blinding, which further affects the study results [14].

One researcher [15] has questioned the consistently positive clinical trials of acupuncture from countries in East Asia. His systematic review of controlled trials noted that

six studies had insufficient blinding. Inadequate concealment from the control group and lack of randomization yielded a higher ratio of positive results in these studies. Furthermore, any mistakes in selection or having a poorly designed control group may lead to misinterpretation of results or misleading statements [16].

The purpose of this study was to systematically review the construction of control groups in RCT acupuncture studies published over the last few decades.

2. Methods

A literature search was performed using the PubMed English language database, using the search keywords "acupuncture" and "placebo needle." We included studies that met the following criteria: randomized control trials (RCTs) that adopted a double-blind, single-blind, or nonblind design. We chose studies that we considered to be representative for each category of control group design. We focus our analysis on the design of control groups in acupuncture studies, instead of evaluating the efficacy of acupuncture in each study.

3. Results

3.1. The Types, Advantages, and Limitations of Control Groups in Acupuncture RCTs. A survey of the literature reveals that 11 different designs exist for control groups in acupuncture RCTs, as shown in Table 1. Among these 11 designs, those classified under 1–5 have no needle intervention, those under 6–8 vary from the treatment group according to the location and depth of needle insertion, those under 9-10 use assistant tools on the skin surface, while the last design comprises multiple methods using paired subjects/controls. Advantages and limitations associated with each type are listed in Table 1.

4. Discussion

4.1. The Ideal Control Group. The purpose of the control group is to determine the effect of the intervention by properly eliminating any placebo effect produced by the test group. Therefore, RCT study designs must include at least 1 control group. Importantly, the control group must experience the same placebo effect as the test group, and ideally participants and researchers are blinded as to whether participants receive treatment or placebo.

An ideal acupuncture control group must include three conditions. First, the design should be accepted by the study subjects, who must be blinded as to their assigned treatment group. Second, the curative effects should be minor and not give rise to special therapeutic effects that influence the results. Third, all other conditions for controls, excluding the intervention, should be identical to those used for the test group.

4.2. Design Strategy. Four different strategies for designing control groups have been used alone or in combination in recent acupuncture RCTs: (1) absence of acupuncture needle insertion, (2) different location of inserted acupuncture needles, (3) different depth of insertion, and (4) the use of assistant tools. In regard to the absence of acupuncture needle insertion, the control group employed an imitative puncture action and no acupuncture, performed at specific acupoints, nonspecific acupoints, or sham acupoints. In regard to different locations for inserted acupuncture needles, specific acupoints consisted of the acupoints applied in the test group according to the meridian method of acupuncture, while sham acupoints comprised the points drifting off the specific acupoints or meridian above 0.5 B-cun and not on the meridian or known acupoints. However, the optimal distance for drifting off the specific acupoints or meridian remains controversial. Nonspecific acupoints are acupoints with nontherapeutic effects or minor effects that have been observed in previous research. In regard to different depth of insertion, needle insertion depths are categorized as superficial, minimal, or deep acupuncture. Superficial acupuncture does not pierce the epidermis. In minimal acupuncture, the needle penetrates to a depth of less than 4 mm and does not cause "de-chi." Deep acupuncture involves needle passage through the hypodermis to a depth of more than 5 mm, usually 10–20 mm, and often causes "de-chi." In reference to the use of assistant tools, placebo needles [17–20] such as the Streitberger's needle, Park Sham Device, or Takakura's needle use a blunt tip and are tapped onto the skin. It is important to note that, although these four strategies were adopted, the control group in acupuncture RCTs is less of an ideal comparator than when it is used in drug RCTs.

4.3. Laser Acupuncture and Transcutaneous Electrical Nerve Stimulation (TENS). Laser acupuncture and TENS usually serve as nonacupuncture contrasts, meaning that they are not involved in needle insertion. Laser acupuncture has been clinically applied since the 1970s [21]. Laser acupuncture is defined as the simulation of traditional acupuncture points with low-intensity, nonthermal laser irradiation. The therapeutic effects are related to not only wavelength, irradiance, and beam profile, but also skin properties such as thickness, age, and pigmentation [21]. Laser acupuncture may be beneficial for conditions ranging from pain relief, hiccups to enuresis, and postoperative nausea and vomiting [22, 23]. These possible therapeutic effects may mean that using laser acupuncture as an intervention in the control group may cause underestimation of acupuncture effects.

TENS is a noninvasive analgesic technique that is usually used to relieve nociceptive, neuropathic, and musculoskeletal pain [24]. Evidence from animal studies has shown that TENS reduces ongoing nociceptive cell activity and sensitization in the central nervous system when applied to somatic receptive fields. It is hypothesized that low-intensity TENS relieves pain by a segmental mechanism, while higher intensity TENS activates extrasegmental descending pain inhibitory pathways [25].

Because of these possible therapeutic effects, using TENS as the control group intervention may cause underestimation of acupuncture effects. Moreover, the procedure of TENS differs from acupuncture. Therefore, blinding is impossible. It is noted that TENS should not be applied on the

Table 1: Acupuncture study control group designs.

Number	Categories	Method	Purpose	Advantage	Limitation
(1)	Nontreatment contrast	CG: not receiving any treatment; TG: acupuncture [40]	To assess the effects of acupuncture	Observe the progression of the condition and patient recovery	No blinding; placebo effects of acupuncture are not eliminated. This treatment would be contrary to medical ethics when treatment involves acute or severe conditions
(2)	Complementary contrast	CG: standard western medicine (with [41] or without [42] placebo acupuncture); TG: standard western medicine and acupuncture	To compare the effects of western medicine and western medicine plus acupuncture	Clarify the costs and side effects of acupuncture as the complementary therapy	Blinding is inadequate unless placebo acupuncture is used. Comparisons with standard treatments cannot be made, and the β error may not be correct
(3)	Alternative contrast	CG: standard western medicine; TG: acupuncture [43]	To compare the effects of acupuncture and western medicine	Demonstrate the effectiveness of acupuncture as the alternative therapy and assess the costs and side effects	No blinding; placebo effects of acupuncture are not eliminated, resulting in the β error
(4)	Nonacupuncture contrast	CG: nonpenetrating intervention, for example, TENS or laser acupuncture; TG: acupuncture [44, 45]	To assess the different effects of acupuncture, TENS, and laser acupuncture	Similar amounts of time and attention are spent on each group, thereby helping to eliminate some of the placebo effect	It is impossible to ensure blinding because of the substantial differences between the CG intervention and acupuncture. The CG intervention may have therapeutic effects. The effects of acupuncture are underestimated
(5)	No effects of nonacupuncture contrast	CG: mock nonpenetrating intervention, for example mock-TENS or mock laser acupuncture; TG: acupuncture [46]	To observe whether the therapeutic effects of acupuncture are greater than those of a nonpenetrating placebo intervention	If the TG receives TENS or laser acupuncture, elimination of the placebo effect and blinding can be assured. The CG intervention has no therapeutic effects and allows the observation of the progression of the condition and patients' recovery	It is impossible to ensure blinding because of the substantial differences between the CG intervention and acupuncture
(6)	Sham acupuncture contrast	CG: insertion points are not acupoints or meridians; TG: acupuncture at acupoints [47, 48]	To compare the effects of acupoints with sham points	The needling methods are the same in the CG and TG, resulting in an optimal elimination of placebo effect, and blinding can be performed	The effects of acupuncture on pain are underestimated. Having no uniform protocol for the sham points precludes accurate comparison of the results and conditions. Participants with previous acupuncture experience may be conscious of the difference in acupoint site(s), which influences the blinding
(7)	Nonspecific sites contrast	CG: acupuncture at acupoints which are considered to produce no or only minor effects; TG: acupuncture at specific acupoints related to the objective illness [49, 50]	To compare the specific effects of acupuncture points	Needling methods are the same in the CG and TG, resulting in an optimal elimination of placebo effect, and blinding can be performed	The effects of acupuncture on pain are underestimated. Selecting an unsuitable acupoint in the CG would render the result invalid

Table 1: Continued.

Number	Categories	Method	Purpose	Advantage	Limitation
(8)	Minimal acupuncture contrast*	CG: insertion points are not acupoints, and needle penetration is to a depth of less than 4 mm; TG: standard acupuncture [48, 51]	To assess the effects of acupuncture at acupoints with manipulating stimulation	The elimination of placebo effect, blinding is ensured; the procedure is easily manipulated	Potential therapeutic effects in the control group. To increase the efficacy of blinding, acupuncturists decreased the manipulation in the TG which then reduced the effects and confounded the results, and analysis. "De-chi" was not attained
(9)	Superficial acupuncture contrast	CG: dull needles or other tools (e.g., needle tube, toothpicks) are slapped on acupoints and tapped on them; dull needles do not prick the skin; TG: standard acupuncture [52]	To examine whether acupuncture is more effective than placebo acupuncture	The physiological reaction in the control group is minimal; the procedure is easily manipulated and suitably applied without requiring a novel study design; the blinding is effective	The operating locations were restricted to areas patients could not see, such as the neck, upper back, and dorsal side of limbs. The sensation was minimal and "de-chi" was not attained, which influenced the blinding for patients with previous experience of acupuncture; thus, the protocol cannot be used in long-term research
(10)	Placebo needle contrast	CG: Streitberger's needle, Park Sham Device, or Takakura's needle with a blunt tip was tapped onto the skin; TG: Streitberger's needle, Park Sham Device, or Takakura's needle with a real needle [17–20]	To observe whether real acupuncture is more effective than placebo acupuncture	The placebo effect and blinding are regarded as optimal, and, thus, the protocol has been widely used with good confidence	The major limitation of placebo needles is the associated lack of the "de-chi" sensation. Certain body sites cannot undergo acupuncture with these devices, such as the fingers, toes, and scalp, as well as sites that require transverse insertion or oblique insertion. These devices do not overcome the problem of double-blinding [33]. The needling methods in the TG had to be limited, thereby reducing the effects; thus, this procedure cannot be used in long-term research
(11)	Combined multiple methods contrast				
	(a)	CG: medicine and acupuncture placebo; TG: acupuncture and placebo medicine [53]	To reduce the psychological influence	The blinding and elimination of placebo effects were enforced to contrast the specific therapeutic effects between acupuncture and medicine	The procedure is difficult to use in long-term research
	(b)	CG: placebo needles inserted into acupoints and real needles inserted into sham points; TG: real needles inserted into acupoints and placebo needles inserted into sham points [54]	To produce similar therapeutic experience in the 2 groups; promote blinding and eliminate placebo effects	Blinding was effective, and the nonspecific effect of placebo needles was reduced	Participants with previous acupuncture experience may be conscious of the difference in acupoint site(s), which affects the blinding

CG: Control group; TG: Test group; TENS: Transcutaneous electrical nerve stimulation; *Minimal acupuncture was termed superficial acupuncture in some studies [51].

anterior and posterior areas of the chest because TENS may compromise pulmonary ventilation due to the stimulation of the intercostal muscles [25]. TENS should not be placed over areas where there has been recent haemorrhage because the currents are able to cause further haemorrhage.

4.4. Needle Pricking May Induce Nonspecific Physiological Reactions. Needle pricking may induce nonspecific physiological reactions and diffuse noxious inhibitory controls (DNICs) [26]. When a nociceptive stimulus is applied to any part of the body that is distinct from the excitatory receptive fields, discharges of neurons in the dorsal horn of the spinal cord are strongly inhibited. Sham needles penetration into sites other than acupoints or nonspecific needles into non-therapeutic acupoints n may act as nociceptive stimuli. These stimuli then activate the A delta and/or peripheral C-fibers, triggering DNIC [27]. Hence, in pain research, acupuncture may decrease pain sensation among subjects in the control groups using sham and nonspecific contrast designs and thereby reduce any between-group differences. Therefore, we suggest that acupuncture analgesia RCTs adopt minimal acupuncture contrast, superficial acupuncture contrast, or a placebo needle contrast design for the control group.

4.5. Minimal Acupuncture and Superficial Acupuncture. Minimal acupuncture requires that insertion points are not acupoints and needle penetration is to a depth of less than 4 mm, while superficial acupuncture requires that dull needles or other tools (e.g., needle tube and toothpicks) are tapped onto acupoints; dull needles do not prick the skin. It was assumed that both interventions have no therapeutic effects. However, recent studies did not agree with this assumption. Lightly touching the skin stimulates mechanoreceptors coupled to slow conducting unmyelinated C afferents that causes activity in the insular region, but not in the somatosensory cortex [28, 29]. This afferent nerve activity may affect brain function resulting in a "limbic touch response," which causes emotional and hormonal reactions. It is likely that, in many studies, when minimal acupuncture and superficial acupuncture are used as control interventions, they are able to alleviate the pain condition. However, compared to nonspecific and sham acupuncture involving real needle insertion, minimal acupuncture and superficial acupuncture are considered to have less influence upon levels of pain.

4.6. Nonspecific and Sham Acupuncture. Nonspecific acupuncture involves acupuncture performed at acupoints that are considered to produce only minor or no effects. However, care must be taken as to choice of nonspecific acupoints: inappropriate selection will render the study invalid. Not only does it remain controversial as to the optimal distance for drifting off specific acupoints or meridian when using sham acupuncture, but also, if two meridians are sited closely together, the sham acupoints may be located on an unintended meridian. For long-term study, both nonspecific and sham acupuncture are considered to be more appropriate as control interventions because they involve real needle insertion.

4.7. Placebo Needling. Placebo needling is considered to be a credible technique for use in subjects with little or no experience of acupuncture [17–20]. However, studies have found that placebo needling had a greater placebo effect on subjects than did placebo pills [30, 31]. The more knowledge and experience among study participants have as to acupuncture the deeper the controversy. Moreover, the therapeutic procedure may produce a psychological placebo effect, instead of real physiological phenomena that are associated with true acupuncture. Selection of acupuncture points and the visual impact of needling also influence the applicability of placebo needling [32]. More importantly, those devices (the placebo needle) do not overcome the problem of double-blinding [33]. However, the blinding effect is increased when placebo needles are used together with needle tubes [34–39]. While Takakura's placebo needle blinds the acupuncturist to a greater degree compared with the Park Sham Device (PSD) and Streitberger's needles, the blinding is less effective for participants, because the needle sensation is less than that produced by the PSD and Streitberger's needles.

5. Conclusions

We list 11 different designs and four strategies associated with control group design in acupuncture RCTs. In clinical practice experience, efficacy is closely related to the manipulation of acupuncture performance, including the lifting and thrusting of needles or needle rotation. For example, in acupuncture analgesia, stronger stimulation (rotating needles with higher frequency and a thicker needle) will have greater efficacy than a weaker stimulation (rotating needles with lower frequency and with a thinner needle). Acupuncture manipulation is not easy to describe very clearly, so most acupuncture RCTs are performed with electroacupuncture. We suggest that acupuncture RCTs may be successfully conducted by hand manipulation if the method is properly described, including details such as the reinforcing and reducing method and acupuncture dosage. In this way, acupuncture research will be more closely related to clinical practice.

Hence, the choice of control in an RCT depends on the type of research and the therapeutic effects of acupuncture being examined. The research design should take into consideration the placebo effect, a blinded design, subject selection, measurements chosen, and any contrast between the treatment and placebo groups. Such a design will correctly reflect the result without underestimating the effect of acupuncture.

Authors' Contribution

J.-G. Lin and C.-H. Chen contributed equally to this work as cofirst authors.

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Research Article

Local and Systemic Cardiovascular Effects from Monochromatic Infrared Therapy in Patients with Knee Osteoarthritis: A Double-Blind, Randomized, Placebo-Controlled Study

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Infrared (IR) therapy is used for pain relief in patients with knee osteoarthritis (OA). However, IR's effects on the cardiovascular system remain uncertain. Therefore, we investigated the local and systemic cardiovascular effects of monochromatic IR therapy on patients with knee OA in a double-blind, randomized, placebo-controlled study. Seventy-one subjects with knee OA received one session of 40 min of active or placebo monochromatic IR treatment (with power output of 6.24 W, wavelength of 890 nm, power density of 34.7 mW/cm^2 for 40 min, total energy of 41.6 J/cm^2 per knee per session) over the knee joints. Heart rate, blood pressure, and knee arterial blood flow velocity were periodically assessed at the baseline, during, and after treatment. Data were analyzed by repeated-measure analysis of covariance. Compared to baseline, there were no statistically significant group x time interaction effects between the 2 groups for heart rate ($P = 0.160$), blood pressure (systolic blood pressure: $P = 0.861$; diastolic blood pressure: $P = 0.757$), or mean arterial blood flow velocity ($P = 0.769$) in follow-up assessments. The present study revealed that although there was no increase of knee arterial blood flow velocity, monochromatic IR therapy produced no detrimental systemic cardiovascular effects.

1. Introduction

Osteoarthritis (OA) generally involves articular cartilage, anabolic and catabolic mechanisms, and bony structures in the synovial joints [1]. Weaker quadriceps muscle strength, lower knee proprioception, and poor balance with increased postural swaying were noted in subjects with knee OA than in age- and gender-matched controls [2]. Pain and decreased postural stability may be accompanied by difficulties in performing basic and instrumental daily activities, increased fall risks among community-dwelling elderly [3, 4], and a decreased quality of life [5].

Physical modalities are commonly used to treat older patients with knee OA to ameliorate pain and improve functional performance in the rehabilitation medical field. Physical modalities, such as hot packs, pulse ultrasound, transcutaneous electrical nerve stimulation, and phototherapy, are commonly applied to patients with musculoskeletal pain to increase local circulation [6]. However, there are few high-quality clinical studies with randomized placebo-controlled designs on physical modalities' therapeutic effects in the rehabilitation medicine field [7].

Light encompasses a portion of the electromagnetic spectrum. Infrared (IR) radiation wavelengths range from 750 nm to 1 mm. In 2002, the US Food and Drug Administration approved IR therapy for pain relief associated with neck and head pain, arthritis, and carpal tunnel syndrome [8]. IR therapy is commonly used for patients with wounds,

lower-limb peripheral neuropathies, and musculoskeletal disorders such as knee OA [7, 9–19]. Photoenergy exerts bioenergetic, biostimulating, biochemical, and bioelectrical effects on cells [20, 21]. The biological effect of phototherapy is related to photochemical cellular reactions rather than thermal reactions [22]. Phototherapy has been found to improve microcirculation by increasing arterioles diameter and blood flow velocity [23–25]. Improving microcirculation at the local and systemic levels is one of the most important phototherapy effects [26, 27]. It is speculated that vessel dilatation, increase of blood flow rate, and improved blood rheologic properties are mediated by NO, prostacyclin, and endothelial-derived hyperpolarizing factors, all of which are produced by endothelial cells [26, 28]. NO causes rapid transduction and increases local blood flow followed by prostacyclin and endothelial-derived hyperpolarizing factors in changing microcirculation at the systemic level [26, 29]. In addition to mediation by enhancing NO synthesis and increasing microcirculation, phototherapy also relives pain by other pathways and mechanisms, such as by modulating inhibitory cyclooxygenase and prostaglandin E2, modulating nerve transmission, increasing endorphin and serotonin release, and stimulating metabolism [8, 21, 30].

A series of IR treatments had been confirmed to have significant efficacy in improving pain, function, and quality of life in patients with knee OA [17, 31, 32]. Possible mechanisms include peripheral nerve stimulation, microcirculation enhancement, analgesic effects, inflammation resolution, chondrocyte proliferation enhancement, and increased matrix synthesis [17, 33]. Due to significant efficacy of OA knee treatment with IR therapy as a series of sessions, it is necessary to provide evidence that IR therapy does not produce any detrimental systemic cardiovascular effect. However, to our knowledge, no comprehensive study has focused on IR therapy's cardiovascular effects in patients with knee OA [32, 34, 35].

If IR therapy in patients with knee OA can improve the knee arterial blood flow velocity without producing detrimental systemic cardiovascular effects, then the increased blood flow in and/or around the knee joint may infer benefits to the knee joint such as pain reduction in patients with knee OA by long term, repeated IR treatments. Therefore, we hypothesized that IR therapy presumably would influence knee joint tissue perfusion by increasing the local arterial blood velocity at the knee without producing detrimental systemic cardiovascular effects. In our research, we conducted a double-blind, randomized, and placebo-controlled study to examine local and systemic cardiovascular effects from monochromatic IR therapy in patients with knee OA.

2. Materials and Methods

This study was conducted at Shin Kong Wu Ho-Su Memorial Hospital, a teaching hospital with 921 beds located in northern Taiwan. In total, 73 subjects confirmed to have knee OA were identified and recruited from the clinic of the Department of Physical Medicine and Rehabilitation at the hospital. All patients fulfilled the combined knee OA clinical and radiographic criteria established by the American College of Rheumatology [36]. Anteroposterior radiographic views of both knees were taken while bearing weight. A qualified senior physiatrist was in charge of reading the X-rays to classify subjects' Kellgren-Lawrence scores. The hospital's Institutional Review Board for the Protection of Human Subjects approved this study. Written informed consent was obtained from each subject. Subjects with a history of stroke, peripheral vascular disease, peripheral neuropathy, a previous knee operation with an implant, a malignancy, or who were pregnant or planning to become pregnant were excluded.

General information, including age, gender, educational level, marital status, work status, smoking and drinking habits, and comorbidities, was recorded. The body mass index (BMI) was calculated. The self-reported OA knee-specific health status was assessed with the Chinese version of the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) [37]. Using a visual analog scale, total WOMAC scores of pain, stiffness, and physical function, respectively vary from 0 to 500, 0 to 200, and 0 to 1700. Higher scores represent worse symptoms with greater functional limitation. The reliability and validity of the three visual analog scale versions are excellent [37–40].

After completing basic data recording, patients were allocated to a treatment group (active treatment) or a placebo group (inactive treatment) following the block randomization principle (with a block size of four). The allocation was initially concealed. An envelope was opened for each consecutive subject to reveal his or her group assignment at the time when he or she was recruited to the study. All patients, regardless of group assignment, underwent 40 min of monochromatic IR therapy with either power on (treatment group) or power off (placebo group) (Figure 1).

Each subject laid down on a standard bed with socks, shoes, and pants removed and rested for 15 min before the intervention in a quiet room with air conditioning. An Anodyne Therapy Professional System (Anodyne Therapy Professional System 480) was used in this study. The device has a main power unit with 8 flexible therapy pads. Each pad contains 60 superluminous gallium-aluminum arsenide diodes that emit an 890 nm light energy wavelength. Eight therapeutic pads were used in this study for both knees, and subjects in the treatment group received a total energy of 41.6 J/cm^2 per knee per session (with a radiant power output of 6.24 W, at a wavelength of 890 nm, and a power density of 34.7 mW/cm^2 for 40 min).

Four therapy pads were placed over the following sites in each knee: the anterior knee joint, the posterior knee joint, and the medial and lateral knee joints (Figure 2). The pads were held in place with neoprene straps supplied by the manufacturer. All subjects were told that they may or may not feel anything from the treatment. Subjects received 1 session of monochromatic IR therapy for 40 min with either the power on or off. The manufacturer checked the monochromatic IR device before the intervention began on the first participant.

The heart rate and blood pressure were measured over the brachial artery with an automated sphygmomanometer (Tango$^+$ Stress BP, Sun Tech Medical Instruments) by

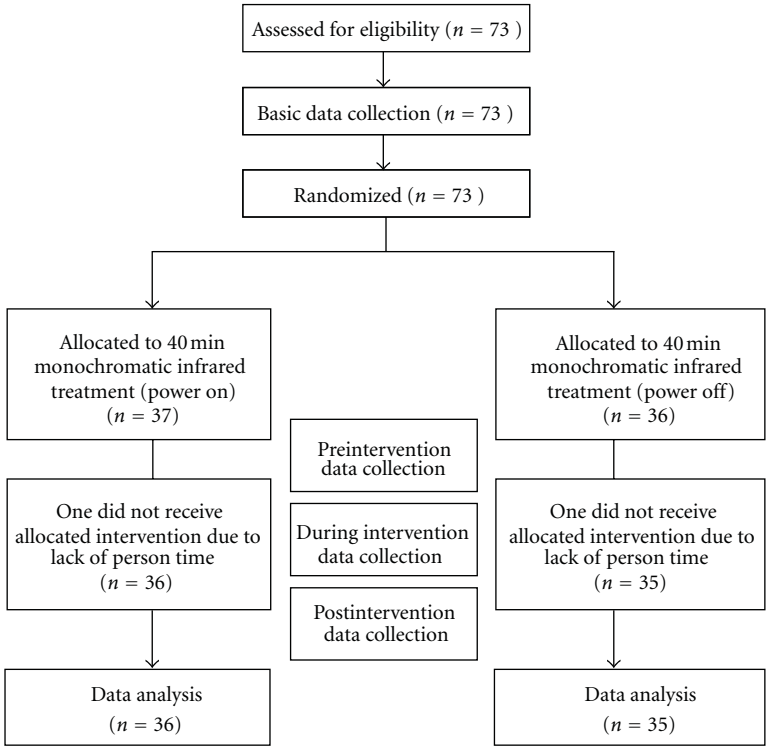

Figure 1: Consort flow diagram.

Figure 2: Monochromatic infrared therapy application.

a well-trained technician. The blood pressure measurement had good reliability and validity [41–43]. The instrument was calibrated, and the same cuff was used for all subjects. The heart rate was monitored before treatment, immediately after completing 40 min of treatment, and 5, 10, and 15 min after completing treatment. Systolic and diastolic blood pressures were automatically recorded before monochromatic IR treatment, every 10 min during the 40 min of treatment, and 5 and 15 min after completing treatment. No conversation was allowed between the participants and the technician during the whole course of heart rate and blood pressure measurements.

Color Doppler ultrasonography (LOGIQ P5, GE Ultrasound Korea, General Electric) was performed on patients in a prone position by a qualified senior physiatrist who was not informed of each patient's group allocation. The peak popliteal arterial systolic blood flow velocity (meters/second) was measured in each subject by high-resolution B-mode ultrasound images using standardized parameters with a 7.5 MHz linear array transducer. The peak popliteal arterial blood flow velocity was measured before IR radiation treatment; immediately after completion of 40 min of treatment; and 5, 10, and 15 minutes after treatment. It has high reliability [44, 45].

Except for the physical therapist performing the monochromatic IR treatments, neither the subjects receiving the treatment nor the investigators (including the technician who measured patients' heart rates and blood pressures, and the physiatrist who conducted the Doppler study) were aware of the monochromatic IR therapy's operating status during the study's treatment and data collection periods.

The results are expressed as the mean ± the standard deviation. A chi-squared test or t-test was used to analyze demographic data such as age, gender, educational level, marital status, occupation, comorbidities, smoking and drinking habits, BMI, Kellgren-Lawrence scores, and knee OA-specific measures of pain, stiffness, and physical function of the treatment and placebo groups. Repeated-measure analysis of covariance (ANCOVA) was used to assess the heart rate, systolic and diastolic blood pressures, and mean arterial knee joint blood flow in patients with knee OA between the follow-up assessments in each group, using the pretreatment baseline as the covariate. The group effect, time effect, and group x time interaction effect for the 2 groups at the follow-up assessments were analyzed. We report the

Table 1: Patient's basic demographics.

Variables	Groups		P value
	Treatment $n = 36$	Placebo $n = 35$	
Gender			
Female	33 (92%)	28 (80%)	0.189
Male	3 (8%)	7 (20%)	
Age (yr)	61.1 ± 9.3	61.3 ± 12.0	0.931
Body mass index (kg/m^2)	26.4 ± 5.0	26.0 ± 4.5	0.765
Married			
Yes	28 (78%)	26 (74%)	0.730
Educational level			
Below 9th grade	21 (58%)	19 (53%)	0.650
Above 9th grade	15 (42%)	16 (47%)	
Work status			
Yes	6 (17%)	4 (12%)	0.735
Comorbidities			
Yes	18 (50%)	21 (62%)	0.322
Smoking			
Yes	0 (0%)	0 (0%)	1.000
Alcohol consumption			
Yes	3 (8%)	3 (9%)	1.000
Kellgren-Lawrence scores	2.7 ± 0.7	2.7 ± 0.7	0.962
WOMAC*			
Pain	130.0 ± 87.9	116.9 ± 84.4	0.493
Stiffness	40.4 ± 47.2	40.6 ± 40.6	0.986
Physical function	413.3 ± 318.1	413.5 ± 326.8	0.999

Note: the scores are presented as the number of cases (percentage) or the mean (standard deviation) for each variable.
*WOMAC: Western Ontario and McMaster University Osteoarthritis Index.

results of the ANCOVA by providing the F statistic, degrees of freedom, and the P value for all 71 participants. The level of statistical significance was set at $P < 0.05$.

3. Results

Seventy-three subjects were enrolled in this study. Two subjects refused to participate after completing basic data collection due to personal time constraints. There was no statistically significant difference in the 2 groups in age, gender, educational level, marital status, occupation, comorbidities, smoking and drinking habits, BMI, or severity of knee OA according to the Kellgren-Lawrence scores and WOMAC assessments. Detailed demographic data for both groups are shown in Table 1.

Compared to pretreatment, there was no statistical significance demonstrated in the heart rate between the 2 groups (group effect: $P = 0.918$; time effect: $P = 0.340$; group x time interaction effect: $P = 0.160$) during the 4 follow-up assessments (after 40 min of treatment; and 5, 10, and 15 min after treatment) (Table 2, Figure 3).

As shown in Table 2 and Figure 4, there was no statistically significant difference in systolic blood pressure (group effect: $P = 0.281$; time effect: $P = 0.180$; group x time interaction effect: $P = 0.861$) or diastolic blood pressure (group effect: $P = 0.262$; time effect: $P = 0.663$; or group x time interaction effect: $P = 0.757$) between the 2 groups during the 6 follow-up assessments (after 10, 20, 30, and 40 min of the treatment; and 5 and 10 min after the treatment was completed).

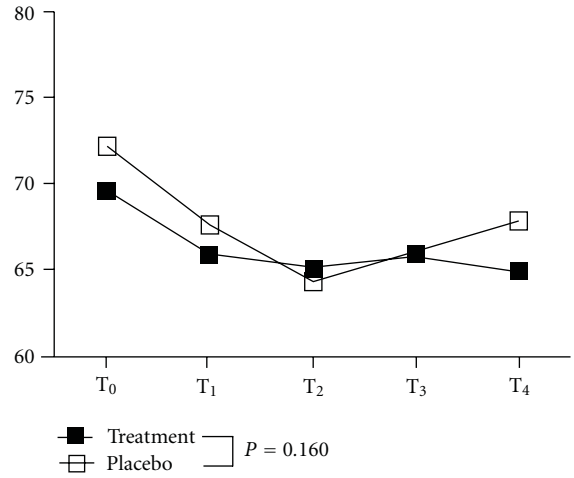

Figure 3: Changes in the heart rate with monochromatic infrared treatment. Solid square: treatment group; hollow square: placebo group; T_0: before treatment; T_1: after 40 min of treatment; T_2: 5 min after treatment; T_3: 10 min after treatment; T_4: 15 min after treatment. Between groups by repeated-measure ANCOVA: group effect: $P = 0.918$ ($F_{1,68} = 0.01$); time effect: $P = 0.340$ ($F_{3,204} = 1.12$); group x time interaction effect: $P = 0.160$ ($F_{3,204} = 1.74$).

As for the popliteal arterial blood flow velocity, compared to pretreatment, there was no statistically significant difference in the blood flow (group effect: $P = 0.666$, time effect: $P = 0.323$, group x time interaction effect: $P = 0.769$) at the 4 separate follow-up assessments between the 2 groups (after 40 min of treatment; and 5, 10, and 15 min after treatment) (Table 2, Figure 5).

No local or systemic side effects were reported during or after the intervention.

4. Discussion

A series of IR treatments are demonstrated to have significant efficacy in improving pain in patients with knee OA. However, the cardiovascular effects by these treatments remain uncertain. To our knowledge, this is the first study to demonstrate the local and systemic cardiovascular effects of monochromatic IR therapy in patients with knee OA. Our results revealed that although there was no knee arterial blood flow velocity increase, monochromatic IR therapy produced no detrimental systemic cardiovascular effects.

A significant microcirculation increase began after 20 min of IR therapy and reached a maximal level 15 min after treatment termination [46]. Therefore, we conducted 40 min of monochromatic IR therapy and followed up for 15 min after treatment termination to examine the local and systemic cardiovascular effects in patients with knee OA in the present study. There has been a tendency to shift from

Table 2: Changes in heart rate, blood pressure, and blood flow velocity.

Time point	Treatment group	Placebo group	Mean between group difference	Repeated-measure ANOVA P value (F value)		
	Mean (SD)	Mean (SD)	(95% confidence interval)	Group	Time	Time × group
Heart rate (beats/min)						
Initial score	69.6 (10.2)	72.2 (11.5)	−2.3 (−7.4, 2.8)	0.918	0.340	0.160
After 40 min of treatment	65.8 (8.0)	67.6 (11.2)	−1.8 (−6.4, 2.8)	($F_{1,68}$ = 0.01)	($F_{3,204}$ = 1.12)	($F_{3,204}$ = 1.74)
5 min after treatment	65.1 (8.5)	64.3 (10.4)	−0.2 (−4.8, 4.5)			
10 min after treatment	65.8 (9.2)	65.8 (10.7)	−0.9 (−5.9, 4.1)			
15 min after treatment	64.9 (10.1)	67.8 (11.7)	−3.6 (−8.7, 1.6)			
Systolic blood pressure (mmHg)						
Initial score	120.3 (16.5)	121.1 (17.6)	−0.8 (−8.9, 7.3)	0.281	0.180	0.861
After 10 min of treatment	111.8 (17.0)	114.0 (20.7)	−2.2 (−11.1, 6.8)	($F_{1,68}$ = 1.18)	($F_{5,335}$ = 1.53)	($F_{5,335}$ = 0.38)
After 20 min of treatment	109.3 (17.3)	114.6 (24.8)	−5.3 (−15.4, 4.8)			
After 30 min of treatment	111.6 (17.5)	117.3 (21.0)	−5.7 (−14.8, 3.5)			
After 40 min of treatment	112.8 (20.4)	116.4 (14.5)	−3.6 (−12.0, 4.8)			
5 min after treatment	116.1 (16.8)	117.4 (19.6)	−1.3 (−10.0, 7.3)			
15 min after treatment	114.1 (15.0)	119.9 (17.3)	−5.9 (−13.6, 1.8)			
Diastolic blood pressure (mmHg)						
Initial score	73.8 (12.0)	70.8 (11.3)	3.0 (−2.5, 8.5)	0.262	0.663	0.757
After 10 min of treatment	73.1 (12.0)	70.5 (12.0)	2.6 (−3.1, 8.3)	($F_{1,68}$ = 1.28)	($F_{5,335}$ = 0.65)	($F_{5,335}$ = 0.53)
After 20 min of treatment	69.7 (12.7)	70.1 (12.7)	−0.4 (−6.5, 5.6)			
After 30 min of treatment	70.6 (10.8)	72.9 (14.5)	−2.3 (−8.4, 3.7)			
After 40 min of treatment	72.3 (14.1)	72.8 (11.8)	−0.5 (−6.6, 5.7)			
5 min after treatment	73.7 (11.1)	74.6 (10.7)	−0.9 (−6.1, 4.3)			
15 min after treatment	73.8 (12.0)	74.9 (12.1)	−1.1 (−6.8, 4.7)			
Blood flow velocity (meters/sec)						
Initial score	36.3 (11.0)	40.5 (10.9)	−3.7 (−8.9, 1.5)	0.666	0.323	0.769
After 40 min of treatment	39.6 (10.8)	41.1 (11.3)	−1.4 (−6.6, 3.8)	($F_{1,68}$ = 0.19)	($F_{3,204}$ = 1.17)	($F_{3,204}$ = 0.38)
5 min after treatment	40.6 (11.6)	41.6 (11.5)	−1.0 (−6.4, 4.3)			
10 min after treatment	40.8 (11.6)	40.3 (10.5)	−0.2 (−5.5, 5.2)			
15 min after treatment	39.7 (10.6)	40.9 (10.5)	−1.2 (−6.2, 3.8)			

Note: scores are presented as the mean (standard deviation) for each variable.

treatment with laser-based devices to treatment by light-emitting diodes in recent years due to the lower cost, lack of coherence, and larger spot size in light-emitting diode devices [8, 47, 48]. Therefore, we used the light-emitting diodes in this study. Color Doppler sonography was used for local blood flow velocity evaluation, and it is widely used in clinical medicine because it is a rapid, simple, accurate, and noninvasive method of objectively monitoring blood flow [49, 50]. However, this study did not demonstrate an increase in the local arterial blood flow velocity after 40 min of monochromatic IR therapy over the knee joints in patients with knee OA.

Measuring blood pressure with a conventional manual sphygmomanometer used by a physician in routine clinical practice often reported inconsistent and imprecise blood pressure readings due to patient-physician interaction, failure to minimize patient anxiety, or poor measurement techniques [51]. The "white coat" bias has been demonstrated to be 15% to 20% in patients with hypertension [52]. Therefore, blood pressure measurement taken outside the clinic using ambulatory blood pressure monitoring is the gold standard measure [53]. However, due to cost and convenience considerations, automatic devices are commonly used in clinics for blood pressure measurements.

Measuring blood pressure using an automatic device in a clinic and leaving the patient alone in a quiet room for at least 14 minutes rest before measurement were found to minimize the white coat effect and yielded values that were comparable to the ambulatory blood pressure measurements [54–56]. The most innovative features to measure blood pressure by automatic devices were that the cuff must be wrapped snugly around the arm, and the patient must keep proper posture during measurement [57]. Therefore, in this study, to avoid the resting effect on heart rate and blood pressure (as the subjects were lying down for up to 60 min for treatment and followup) and the white coat effect, all

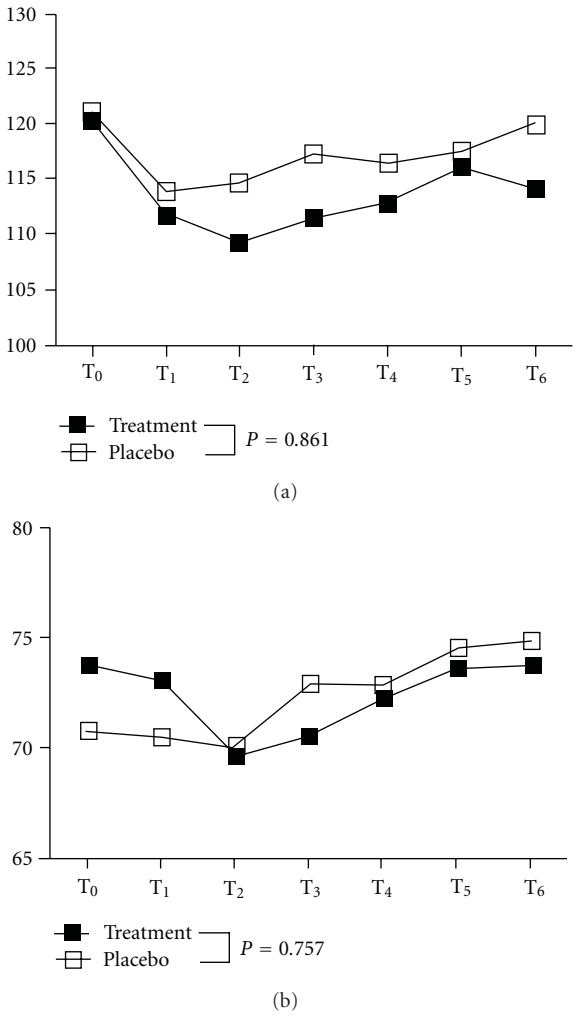

Figure 4: Changes in blood pressure with monochromatic infrared treatment. Solid square: treatment group; hollow square: placebo group; T_0: before treatment; T_1: after 10 min of treatment; T_2: after 20 min of treatment; T_3: after 30 min of treatment; T_4: after 40 min of treatment; T_5: 5 min after treatment; T_6: 15 min after treatment. (a) systolic blood pressure; (b) diastolic blood pressure. Between groups by repeated-measure ANCOVA: systolic blood pressure: group effect: $P = 0.281$ ($F_{1,68} = 1.18$); time effect: $P = 0.180$ ($F_{5,335} = 1.53$); group x time interaction effect: $P = 0.861$ ($F_{5,335} = 0.38$); diastolic blood pressure: group effect: $P = 0.262$ ($F_{1,68} = 0.19$); time effect: $P = 0.663$ ($F_{5,335} = 0.65$); group x time interaction effect: $P = 0.757$ ($F_{5,335} = 0.53$).

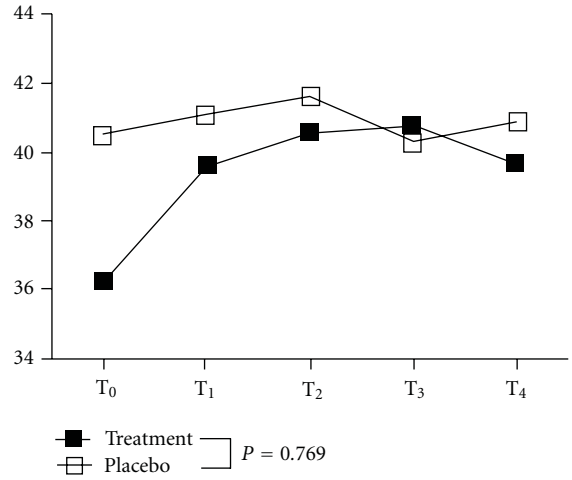

Figure 5: Changes in the blood flow velocity with monochromatic radiation treatment. Solid square: treatment group; hollow square: placebo group; T_0: before treatment; T_1: after 40 min of treatment; T_2: 5 min after treatment; T_3: 10 min after treatment; T_4: 15 min after treatment. Between groups by repeated-measure ANCOVA: group effect: $P = 0.666$ ($F_{1,68} = 0.19$); time effect: $P = 0.323$ ($F_{3,204} = 1.17$); group x time interaction effect: $P = 0.769$ ($F_{3,204} = 0.38$).

participants were asked to lie on the bed in a quiet room for 15 min before beginning the blood pressure measurement and intervention. The blood pressure measuring point was 8 cm above the right elbow joint [57], and all measurements were taken on the right arm. A technician rather than a physician completed the blood pressure measurement. In terms of heart rate and blood pressure, there was no significant difference between the treatment group and placebo group during the 40 min of treatment or 10 to 15 min after treatment termination.

Compared to a previously conducted, community-based cohort study in Taiwan [58], although the mean age of participants was relatively older, the systolic blood pressure and the diastolic blood pressure mean value ranges were relatively lower in the present study (the respective values for the previously-conducted study in contrast to the present study are as follows: mean age (years): 56.3 in contrast to 61.2; mean heart rate (beats/min): 64.8 in contrast to 67.1; mean systolic blood pressure (mmHg): 120.2 in contrast to 115.4; mean diastolic blood pressure (mmHg): 74.1 in contrast to 72.4). In contrast to participants' blood pressures being measured by a conventionl, manual sphygmomanometer in a clinic by a physician without mentioning full rest as in the previous study, the participants' blood pressure monitors were applied by a technician, blood pressure was measured automatically in a quiet room after resting for 15 minutes, and conversation between the technician and participants during heart rate and blood pressure measurements was prohibited in the current study. All of these procedures would effectively minimize the white coat effect and measure blood pressure more accurately. This could partially explain why the ranges of systolic and diastolic blood pressure are lower than the average ranges for 56 years old.

Although all participants were told that they may or may not feel anything from monochromatic IR therapy, active monochromatic IR therapy actually emits mild, tangible heat. Therefore, subjects would expect a difference in perception from monochromatic IR therapy between the 2 groups, which could have given subjects in the treatment group a greater perception that they were being treated in contrast to those in the control group. The percentage of patients who perceived heat in the control group in contrast to the experimental group was 33% versus 58% ($P = 0.101$).

Stratified analysis according to whether the participants perceived heat feeling or not was further performed to control for this factor. For the participants who perceived a heat feeling, we found statistically significant group x time interactions for heart rate, with higher trends in the control group ($P = 0.033$), and no statistically significant group effect ($P = 0.762$) or time effect ($P = 0.708$). There was no statistically significant group x time interaction effect for systolic blood pressure ($P = 0.543$), diastolic blood pressure ($P = 0.940$), or knee arterial blood flow velocity ($P = 0.323$). For participants who did not perceive a heat feeling, there was no significant group x time interaction for heart rate ($P = 0.523$), systolic blood pressure ($P = 0.779$), diastolic blood pressure ($P = 0.574$), or knee arterial blood flow velocity ($P = 0.444$). Although the perceived heat feeling by active monochromatic IR therapy could compromise the experiment's blindness on the patients' side, it does not affect the results after we had controlled for that factor.

Because the effects of monochromatic IR therapy are time-dependent [59], the level of photoenergy delivered would have affected the study's results. Compared to previous studies that applied total energies of 52.0–58.5 J/cm^2 [11, 18, 19], the present study used 34.7 mW/cm^2 for 40 min for a higher total energy of 83.2 J/cm^2. At this higher energy, monochromatic IR therapy still had no detrimental systemic cardiovascular effects on patients with knee OA as measured by the heart rate and blood pressure.

OA is often associated with obesity and several cardiovascular conditions, including coronary artery disease, hypertension, and diabetes mellitus [60]. Because obesity, hypertension, and cardiovascular diseases are present in metabolic syndrome, it is hypothesized that OA may represent another aspect of metabolic syndrome [61, 62]. Potential mechanisms for joint OA include the following: (1) reduced blood flow from small vessels and interstitial fluid flow in the subchondral bone and (2) subchondral ischemia with compromised gas and nutrient exchange in the articular cartilage [63]. A higher rate of blood flow is associated with an increased bone remodeling rate [63]. On the contrary, compromised blood flow in the subchondral bone could have deleterious effects on the bone and have implications for the cartilage's integrity [63]. There was a positive association between increased popliteal artery vessel wall thickness and generalized OA [62]. These evidences showed that vascular pathology plays a role in joint OA initiation and/or progression [63]. There may be common pathogenic mechanisms that affect the vascular system and joints [61]. Furthermore, most patients with knee OA who require medication for pain relief are likely to be older and at high risk for both adverse cardiovascular and gastrointestinal effects [60]. Therefore, from the point of no detrimental systemic cardiovascular effects, monochromatic IR therapy can be safely applied to elderly people with knee OA and cardiovascular diseases.

Although the present study did not support our previous hypothesis and found no evidence that monochromatic IR therapy increased knee arterial blood flow velocity in patients with knee OA, we acknowledge that there are many factors that could have affected the study results: the photosource, wavelength, power, energy density, duration of treatment, method of application (noncontact mode in contrast to contact mode), site of stimulation, size of the exposure area, and so forth. Therefore, these results cannot be considered conclusive. Our research presents a reasonable initial foray into the local and systemic cardiovascular effects of clinical monochromatic IR therapy application in patients with knee OA.

There are some limitations to the present study. First, no direct NO, prostacyclin or endothelial-derived hyperpolarizing factor productions were measured. Second, whether the increased popliteal blood flow velocity was related to the increased knee joint blood flow and/or arteriole dilatation remains uncertain.

5. Conclusions

In this study, we applied monochromatic IR therapy in a double-blind, randomized, and placebo-controlled trial to subjects with knee joint OA. Our results revealed that although there was no increase in knee arterial blood flow velocity, monochromatic IR therapy produced no detrimental systemic cardiovascular effects. Therefore, it can be applied to patients with knee OA and cardiovascular diseases safely. Further studies on the effects of monochromatic IR therapy are warranted in the future using different settings for the power, wavelength, energy density, stimulation duration, and stimulation location.

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Review Article

Evaluation of the Effects of Acupuncture on Blood Flow in Humans with Ultrasound Color Doppler Imaging

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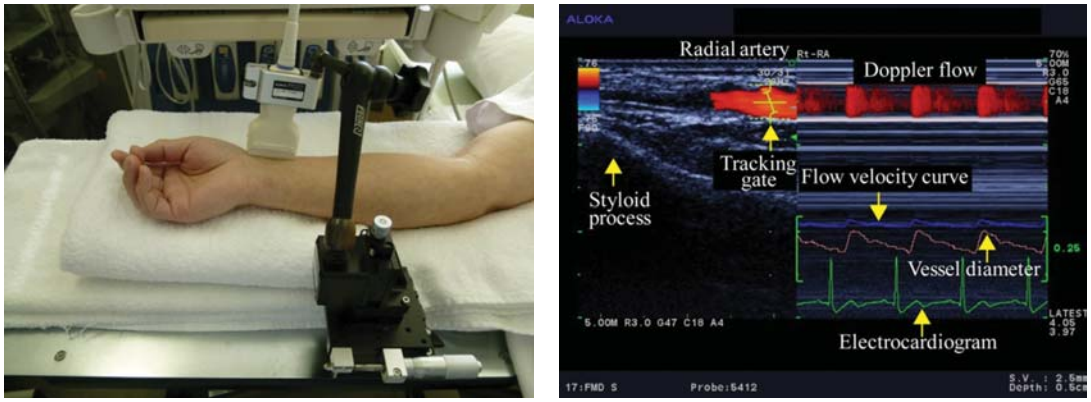
Color Doppler imaging (CDI) can be used to noninvasively create images of human blood vessels and quantitatively evaluate blood flow in real-time. The purpose of this study was to assess the effects of acupuncture on the blood flow of the peripheral, mesenteric, and retrobulbar arteries by CDI. Statistical significance was defined as P values less than 0.05. Blood flow in the radial and brachial arteries was significantly lower during needle stimulation on LR3 than before in healthy volunteers, but was significantly higher after needle stimulation than before. LR3 stimulation also resulted in a significant decrease in the vascular resistance of the short posterior ciliary artery and no significant change of blood flow through the superior mesenteric artery (SMA) during acupuncture. In contrast, ST36 stimulation resulted in a significant increase in blood flow through the SMA and no significant change in the vascular resistance of the retrobulbar arteries. Additionally, acupuncture at previously determined acupoints in patients with open-angle glaucoma led to a significant reduction in the vascular resistance of the central retinal artery and short posterior ciliary artery. Our results suggest that acupuncture can affect blood flow of the peripheral, mesenteric, and retrobulbar arteries, and CDI can be useful to evaluate hemodynamic changes by acupuncture.

1. Introduction

To date, no quantitative evaluation methods have been established for determining the physiological effectiveness of acupuncture. Therefore, researchers conduct experiments using a variety of approaches. In this study, we focused on the physiological reactions to acupuncture and investigated blood flow changes that result from acupuncture [1–5].

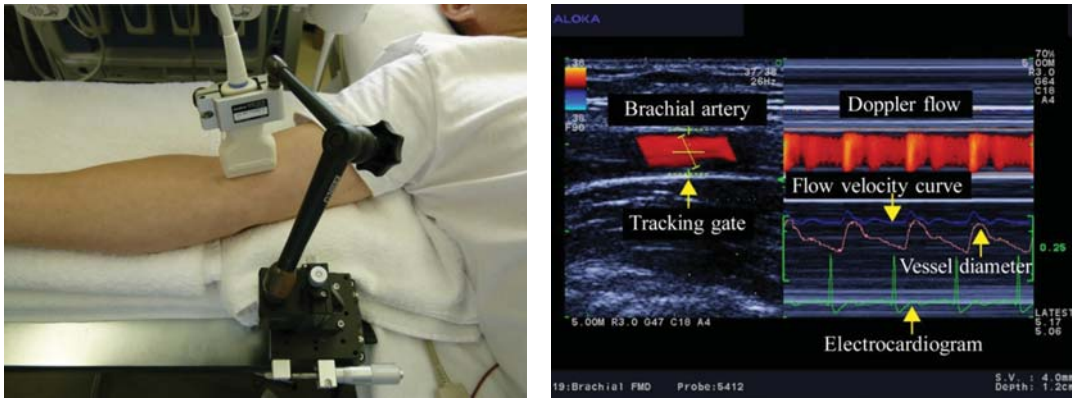
Many studies of acupuncture efficacy have been based on the results of animal experiments with anesthesia. These studies indicate that acupuncture works through physiological mechanisms that occur primarily in the autonomic nervous system [6–12]. When acupuncture is performed in human clinical practice, the conditions are very different from those in animal experiments. Additionally, because the invasive examination techniques that are often used to evaluate the results of acupuncture treatments affect the efficacy of those treatments, it is difficult to distinguish physiological reactions caused by acupuncture from those caused by the invasion necessary for examination. To determine the efficacy of acupuncture in humans, it is important that the examination method be noninvasive. We therefore used noninvasive color Doppler imaging (CDI) with ultrasound to evaluate blood flow. CDI is an examination technique that is widely used in the practice and research of Western medicine [13–21]. CDI can quantitatively measure intravascular blood flow in the extremities and in various organs in real-time. It is useful in the investigation of vessels, such as the peripheral, coronary, splenic, adrenal, and superior mesenteric arteries (SMA) [22]. In addition, the reproducibility of real-time and noninvasive hemodynamic measurement with CDI is reported elsewhere [23].

In traditional Chinese medicine, LR3 (Taichong, located on the foot, 1.5–2 units above the web between the



(a) (b)

Figure 1: (a) Ultrasound measurement of the radial artery. 13 MHz linear transducer is fixed along radial artery with a special probe holder (MP-PH0001, Aloka Co., Ltd., Tokyo, Japan). (b) Display of CDI. Left: the vessel image and the position of the artery tracking gate. Right: changes in vessel diameter, Doppler flow, and flow velocity as determined by an automated edge-detection device and computer analysis software (e-Tracking system; Aloka Co., Ltd., Tokyo, Japan).



(a) (b)

Figure 2: (a) Ultrasound measurement of the brachial artery. 13 MHz linear transducer is fixed along brachial artery with a special probe holder (MP-PH0001, Aloka Co., Ltd., Tokyo, Japan). (b) Display of CDI. Left: image of the vessel image and position of the artery tracking gate. Right: changes in vessel diameter, Doppler flow, and flow velocity, as determined by an automated edge detection device and computer analysis software (e-Tracking system; Aloka Co., Ltd., Tokyo, Japan).

first and second toes [24]) is an acupoint on the liver meridian, which has the functions of "soothing the liver," "regulating the blood," and "opening into the eyes" [24]. We therefore hypothesized that LR3 acupuncture would affect hemodynamics in the peripheral arteries and the retrobulbar arteries. ST36 (Zusanli, located on the lower leg, 3 units below the lateral "eye" of the knee, approximately 1 finger width lateral to the tibia [24]), in contrast, is an acupoint on the stomach meridian, and is associated with the functions of gastrointestinal organs [25]. We therefore hypothesized that ST36 acupuncture would affect hemodynamics in the SMA. Because glaucoma prognosis and retrobulbar circulation are related [26–29], we also investigated the effects of acupuncture on retrobulbar circulation in open-angle glaucoma (OAG) patients. In this study, we introduce the noninvasive CDI with ultrasound to evaluate blood flow changes by acupuncture.

2. Materials and Methods

2.1. Ultrasound Technique for Blood Flow Measurement. We measured circulation in the upper limb, SMA, and retrobulbar vessels using an ultrasound system (Prosound α10; Aloka Co., Ltd, Tokyo, Japan). The system had a 13 MHz linear transducer and a 5 MHz convex transducer. We used the linear transducer to examine peripheral arteries and the retrobulbar vessels. We used the convex transducer to measure SMA circulation.

The radial artery was examined just medial to the radial styloid process (Figure 1). The brachial artery was monitored immediately proximal to the elbow (Figure 2). The SMA supplies blood to the whole small intestine, except for the superior part of the duodenum. It also supplies blood to the cecum, the ascending colon, and most of the transverse colon. SMA measurements were acquired within 2-3 cm of

(a) (b)

Figure 3: (a) Ultrasound measurement of the SMA. 5 MHz convex transducer is positioned on the abdomen. (b) Display of CDI. Left: image of the vessel and the position of the artery tracking. Right: Doppler flow and flow velocity.

the artery origin (Figure 3) [30, 31]. Avoiding any pressure on the eye, CDI was performed for the retrobulbar vessels, including the ophthalmic artery (OA), central retinal artery (CRA), and nasal or temporal short posterior ciliary artery (Figures 4 and 5). The OA was examined approximately 20 mm behind the globe (Figure 5(b)), the CRA was examined within 5 mm of the retrolaminar portion of the optic nerve (Figure 5(c)), and the nasal or temporal SPCA that obtained clear image was examined approximately 5–10 mm behind the globe (Figure 5(d)). Blood flow was monitored continuously [32, 33] and we employed a Doppler angle of 60° or less for each measurement [34, 35]. Each Doppler waveform was automatically drawn and calculated using the software included with the ultrasound system. The following calculations were used to determine the hemodynamic parameters at each site [30, 31].

(i) Vessel diameter (VD).

(ii) Cross-sectional area (CSA) = $(\mathrm{VD}/2)^2 \times \pi$.

(iii) Peak systolic velocity (PSV).

(iv) End-diastolic velocity (EDV).

(v) Resistive index (RI) = (PSV − EDV)/PSV.

(vi) Mean flow velocity (MV).

(vii) Blood flow volume = CSA × MV.

2.2. Statistical Analysis. Statistical analysis was performed with SPSS software (version 16.0, SPSS Japan Inc., Tokyo, Japan). Repeated measure analysis of variance, followed by Dunnett's post hoc test, was used for statistical comparison between the measure points. Comparison between rest and after acupuncture was done by paired t-test. Results are presented as the mean ± SD and $P < 0.05$ was taken to indicate significance for all statistical analysis.

2.3. Experiment 1: Effects of LR3 Acupuncture on Upper Limb Circulation [1]. This study was employed to investigate the upper limb circulation after acupuncture at LR3 acupoints on foot. The participants were recruited by the poster recruitment in Tohoku University. Eighteen healthy volunteers (mean age: 32 ± 5 years; 14 males and 4 females) were enrolled in this study. A disposable fine stainless-steel needle (diameter: 0.16 mm; length: 40 mm; Seirin Co., Ltd., Shizuoka, Japan) was inserted on LR3 bilaterally and maintained at a depth of 10 mm during the test. After the needle was inserted, stimulation (rotating the needles manually within an angle of 90 degrees) was performed for 18 seconds. The needles were removed 200 seconds after acupuncture. Radial and brachial CDI were performed before acupuncture; during acupuncture treatment; 30, 60, and 180 seconds after acupuncture.

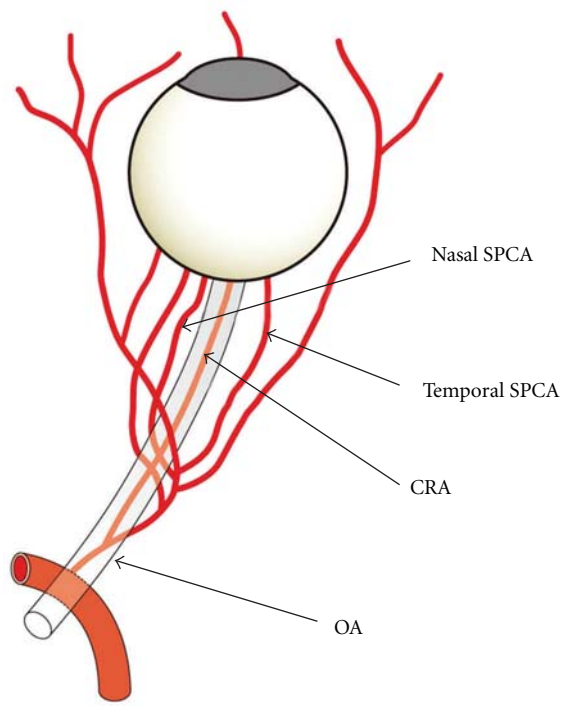


Figure 4: Schema of the retrobulbar arteries (OA: ophthalmic artery, CRA: central retinal artery, and SPCA: short posterior ciliary artery).

Figure 5: (a) Ultrasound measurement of retrobulbar arteries. 13 MHz linier transducer is attached on the eyelid. Horizontal scans by CDI through the ocular globe showing the (b) ophthalmic artery (OA), (c) central retinal artery (CRA), and (d) temporal short posterior ciliary artery (SPCA). Left: image of the vessel and the position of the artery tracking. Right: Doppler flow and flow velocity (b, c, and d).

2.4. Experiment 2: Effects of LR3 Acupuncture on Blood Circulation to the Eye and through the SMA. This study was employed to clarify the hemodynamic changes by acupuncture in two different organs (intestine and eye) with simultaneous evaluation by ultrasound. The participants were recruited by the poster recruitment in Tohoku University. Thirteen healthy volunteers (mean age: 36 ± 9 years; 10 males and 3 females) were enrolled in this study. Acupuncture was performed bilaterally on LR3 with manual needle rotation and the disposable stainless steel needles (0.16 mm × 40 mm; Seirin Co. Ltd., Shizuoka, Japan) were kept at the same site for 15 minutes. Retrobulbar vessels and SMA circulation were measured simultaneously at rest and 15 minutes after the start of acupuncture using ultrasound.

2.5. Experiment 3: Effects of ST36 Acupuncture Blood Circulation to the Eye and through the SMA. This study was also employed to clarify the hemodynamic changes by acupuncture in two different organs (intestine and eye) with simultaneous evaluation by ultrasound. The participants were recruited by the poster recruitment in Tohoku University. Thirteen subjects (mean age: 36 ± 8 years; 10 males and 3 females) were enrolled in this study. Acupuncture was performed bilaterally on ST36 with manual rotation of the disposable stainless steel needles (0.16 mm × 40 mm; Seirin Co. Ltd., Shizuoka, Japan) were kept in the same site for 15 minutes. Retrobulbar vessels and SMA circulation were measured simultaneously at rest and 15 minutes after the start of acupuncture using ultrasound.

2.6. Experiment 4: Effects of Acupuncture on Retrobulbar Circulation in OAG Patients [2]. The relation between glaucoma and retrobulbar circulation in the prognosis of the disease has been indicated [26–29], therefore we investigated the effects of acupuncture on OAG patients by CDI. The patients were recruited in the outpatient clinic of ophthalmology in Tohoku University Hospital. Eleven OAG patients (mean age: 63 ± 11 years; 1 male and 10 females; 20 eyes with OAG) were enrolled. All patients included in the study had been treated with topical antiglaucoma medications for at least 3 months prior to the study. As a control, the subjects received the measurements of retrobulbar vessel hemodynamics that were performed at rest and one hour after the first measurement. One month later, they received the same measurements before and after acupuncture treatment. Acupuncture was performed once bilaterally at acupoints BL2, EX-HN5, ST2, ST36, SP6, KI3, LR3, GB20, BL18, and BL23 for 15 minutes using disposable stainless steel needles

Table 1: Hemodynamic parameters and blood flow volume of the radial and brachial arteries by acupuncture on LR3. The values represent the mean and SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus before acupuncture. Modified from [1].

Parameters	Acupuncture on LR3				
	Before	During	30 s after	60 s after	180 s after
Systolic blood pressure (mmHg)	116.8 ± 10.1				114.5 ± 12.3
Diastolic blood pressure (mmHg)	67.3 ± 8.4				65.8 ± 7.3
Heart rate (beats/min)	67.3 ± 10.1	64.2 ± 8.8	65.8 ± 9.3	66.2 ± 9.3	66.9 ± 9.6
Blood flow volume of the radial artery (mL/min)	56.3 ± 33.5	25.4 ± 26.3	57.9 ± 47.5	67.7 ± 44.7	67.0 ± 36.5
Blood flow volume of the brachial artery (mL/min)	87.5 ± 56.4	65.7 ± 41.6	86.8 ± 53.7	90.1 ± 51.5	106.5 ± 59.8

Table 2: Hemodynamic parameters, blood flow volume of the SMA, and resistive index of retrobulbar arteries by acupuncture on LR3. The values represent the mean and SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus before acupuncture.

Parameters	Acupuncture on LR3	
	Before	After
Systolic blood pressure (mmHg)	119.6 ± 12.8	116.7 ± 11.1
Diastolic blood pressure (mmHg)	77.7 ± 9.4	76.5 ± 9.3
Heart rate (beats/min)	66.8 ± 7.1	63.3 ± 4.6**
Blood flow volume of the SMA (mL/min)	734.8 ± 312.9	704.4 ± 328.1
RI in OA	0.719 ± 0.097	0.707 ± 0.089
RI in CRA	0.661 ± 0.088	0.644 ± 0.052
RI in SPCA	0.624 ± 0.057	0.580 ± 0.037*

(0.16 mm or 0.20 mm × 40 mm; Seirin Co. Ltd., Shizuoka, Japan). Retrobulbar circulation was measured using CDI at rest prior to treatment and 1 hour later, or after acupuncture.

3. Results and Discussion

3.1. Experiment 1: Effects of LR3 Acupuncture on Upper Limb Circulation [1]. Hemodynamic parameters including blood pressure, heart rate, and blood flow volume in the radial and brachial arteries are summarized in Table 1. Figure 6 illustrates the profile of the percent changes in blood flow volume in the radial and brachial arteries. The blood flow volume in the radial artery decreased significantly during acupuncture ($P < 0.01$), but showed a significant increase at 180 seconds after acupuncture ($P < 0.05$) (Figure 6). In the brachial artery, the blood flow volume also showed a significant increase at 180 seconds after acupuncture ($P < 0.05$) (Figure 6). The physiological mechanisms of decrease and increase blood flow volume in upper limb are presumably related to a peripheral vascular resistance due to an instantaneous increase and decrease in sympathetic tone [1]. The present result suggests that LR3 located on the foot and apart from the upper limb can affect the circulation in the upper limb.

3.2. Experiment 2: Effects of LR3 Acupuncture on Blood Circulation to the Eye and through the SMA. The RI of the SPCA was significantly lower after acupuncture than before ($P < 0.05$; Table 2). However, blood flow volume in the SMA was not significantly changed after acupuncture than before (Table 2). The SPCA is the ocular branches of the OA and it supplies blood to the choroid (Figure 4) [32]. The decrease of the distal vascular resistance in the SPCA that

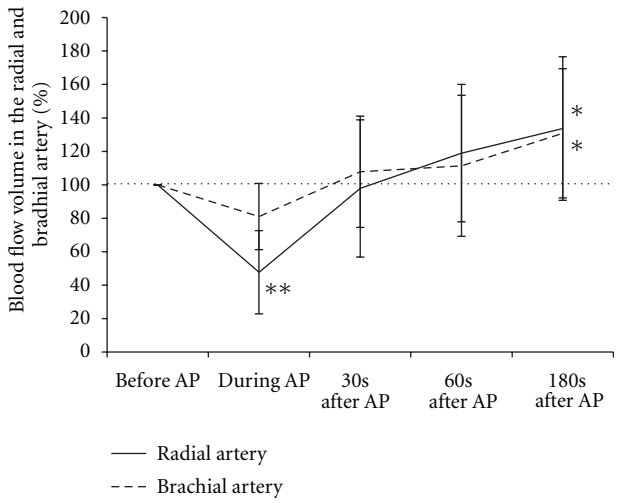


Figure 6: Percent changes in blood flow volume in the radial and brachial arteries before, during, and after acupuncture treatment. Values are presented as a percentage of the pretreatment blood flow. Values represent the mean and SD. AP: acupuncture. $^{*}P < 0.05$, $^{**}P < 0.01$ versus before acupuncture. Modified from [1].

we observed indicates that acupuncture on LR3 results in an increase of the blood flow to the choroid. It has been reported that the blood flow in the eye is controlled by sympathetic and parasympathetic nerves, and it is related with the release of nitric oxide or calcitonin gene-related peptide [33, 34]; it has also been reported that the regulation of regional blood flow by somatic afferent stimulation is based on somatoautonomic reflex mechanisms in the choroidal blood flow of the eyeball [34]. The hemodynamic changes in the SPCA by acupuncture may be related with these

Table 3: Hemodynamic parameters, blood flow volume of the SMA, and resistive index of retrobulbar arteries by acupuncture on ST36. The values represent the mean and SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus before acupuncture.

Parameters	Acupuncture on ST36	
	Before	After
Systolic blood pressure (mmHg)	121.7 ± 11.8	120.7 ± 10.9
Diastolic blood pressure (mmHg)	77.8 ± 9.4	77.6 ± 7.6
Heart rate (beats/min)	61.9 ± 6.6	61.5 ± 7.4
Blood flow volume of the SMA (mL/min)	549.8 ± 192.2	620.2 ± 188.1*
RI in OA	0.736 ± 0.07	0.728 ± 0.070
RI in CRA	0.617 ± 0.065	0.631 ± 0.043
RI in SPCA	0.600 ± 0.030	0.580 ± 0.06

Table 4: Hemodynamic parameters and resistive index of retrobulbar arteries in control and acupuncture therapy. The values represent the mean and SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus rest or before acupuncture. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus control. Modified from [2].

Parameters	Control		Acupuncture	
	Rest	After 1 hour	Before	After
Systolic blood pressure (mmHg)	116.4 ± 10.0	119.8 ± 7.6	124.5 ± 12.9	122.6 ± 9.7
Diastolic blood pressure (mmHg)	69.8 ± 6.5	68.6 ± 3.9	74.5 ± 5.4	72.0 ± 2.9
Heart rate (beats/min)	61.5 ± 7.3	60.1 ± 8.1	61.7 ± 8.5	60.3 ± 10.4
RI in OA	0.74 ± 0.04	0.75 ± 0.05	0.74 ± 0.04	0.74 ± 0.04
RI in CRA	0.75 ± 0.09	0.72 ± 0.03	0.72 ± 0.05	0.68 ± 0.04*
RI in SPCA	0.68 ± 0.05	0.68 ± 0.04	0.67 ± 0.04	0.64 ± 0.06*††

mechanisms. The present result suggests that LR3 located on the foot and apart from the eye can affect the circulation in the retrobulbar arteries.

3.3. Experiment 3: Effects of ST36 Acupuncture on Blood Circulation to the Eye and through the SMA. RI in the retrobulbar vessels was not changed by ST36 acupuncture treatment. However, the blood flow volume in the SMA was significantly greater after acupuncture than before ($P < 0.05$; Table 3). Acupuncture on the limbs was also demonstrated to elicit systemic visceral responses via the supraspinal reflexes in animal models [9, 36, 37]. According to several reports, blood flow volume in the SMA increased significantly after stimulation of the lower limbs [9, 36–38]. We speculate that this increase is caused by excitation of the parasympathetic system and inhibition of the sympathetic system via supraspinal reflexes. The present result suggests that ST36 located on the lower limb and apart from the abdomen can affect the circulation in the SMA.

3.4. Experiment 4: Effects of Acupuncture on Retrobulbar Circulation in OAG Patients. RI in the CRA and SPCA were significantly lower after acupuncture than it was before acupuncture treatment (CRA; $P < 0.05$, SPCA; $P < 0.05$; Table 4). RI in the SPCA was also significantly lower after acupuncture than when no treatment was given (SPCA; $P < 0.01$; Table 4). The CRA supplies blood to the retina and SPCA, to the choroid (Figure 4). The decrease of the distal vascular resistance in the CRA and SPCA that we observed indicates that acupuncture results in an increase of the blood flow to the retina and choroid. The possible physiological mechanisms of increase blood flow in eye has already described in the discussion of Experiment 2. The present result suggests that acupuncture can improve the retrobulbar circulation in the patients of OAG with standard medication.

4. Ultrasound and CDI

4.1. Advantage. We focused on the evaluation of CDI by ultrasound. Noninvasive and real-time measure of CDI was applied to assess circulation in organs after acupuncture. The continuous method of CDI was used to assess the brief effects of circulation in the arm (Experiment 1). The simultaneous evaluation by CDI was applied to assess the circulation in two different organs (Experiments 2 and 3). Resistive index measured by CDI is measured in the small vessels as retrobulbar arteries (Experiment 4). Acupuncture affects the autonomic nervous system via the somatic nerves. Invasive evaluation also affects these systems and reflex. Therefore, invasive evaluation might not correctly evaluate the physiological effects of acupuncture therapy. We suggest that real-time and noninvasive hemodynamic measurement as CDI is suitable to measure the physiological effects in humans.

4.2. Limitation. While CDI provides detailed images of blood vessels in real-time, there are limits to the hemodynamic measurements that can be made using this technique. In addition, while CDI is useful for the measurement of blood flow in various vessels in real time, it does not have sufficient resolution to determine the diameter of very small retrobulbar vessels such as OA, CRA, and SPCA. Therefore,

CDI cannot be used to measure blood flow volume in these vessels. However, it can provide an index of vascular resistance such as RI. A decrease in the distal vascular resistance in the small vessels indicates an increase in the blood flow in the distal part of the vessels. Additionally, care must be taken to avoid compression of the eyeball during ultrasound examination. Such compression is likely to cause intraocular pressure elevation and trigger the vagal reflex. Measurement of blood flow in the retrobulbar arteries requires attention to probe maintenance and careful avoidance of pressure on the eyeball [23]. Expert technique is required to obtain reproducible results using CDI. In addition to the limits of CDI resolution, ultrasound waves that strike blood vessels at angles greater than 60° relative to the direction of blood flow result in a large margin of error for CDI measurements. Therefore, it is important to measure blood flow at a Doppler angle of less than 60 degrees [34, 35].

4.3. Further Study. The other methods to assess the physiological changes by acupuncture noninvasively are impedance cardiography and spectral analysis of heart rate variability. Impedance cardiography is a noninvasive monitoring method that allows measurement of the cardiac index based on the changes in thoracic resistance that results from variations in intrathoracic blood flow volume [39, 40]. Spectral analysis of heart rate variability is useful to evaluate the autonomic nervous balance noninvasively [41, 42]. Combined with these measurements, we can clarify the mechanism of increased blood flow volume in several organs in humans. In the future, we would like to explore the efficacy of acupuncture as treatment for various diseases by using diagnostic tools, such as CDI.

5. Conclusion

CDI can noninvasively depict blood vessels in the human body, and can quantitatively evaluate blood flow in real time. Our studies showed the changes of blood flow in the peripheral, mesenteric, and retrobulbar arteries by acupuncture estimated by CDI. This technique is suitable as an evaluation method to consider physiological changes due to acupuncture as blood flow changes.

Conflict of Interests

The authors have no conflict of interests.

Acknowledgments

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Review Article

Development and Clinical Application of a Precise Temperature-Control Device as an Alternate for Conventional Moxibustion Therapy

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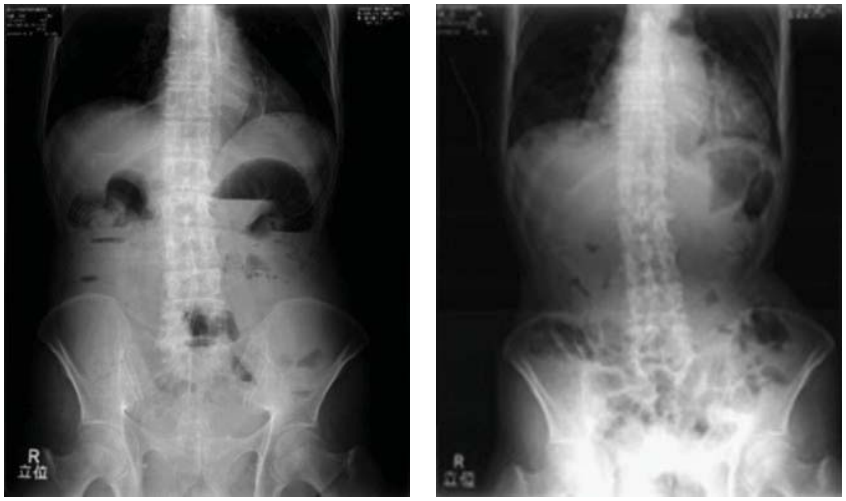
Moxibustion therapy has been used in East Asian medicine for more than a thousand years. However, there are some problems associated with this therapy in clinical practice. These problems include lack of control over the treatment temperature, emission of smoke, and uneven temperature distribution over the treatment region. In order to resolve these problems, we developed a precise temperature-control device for use as an alternate for conventional moxibustion therapy. In this paper, we describe the treatment of a single patient with paralytic ileus that was treated with moxibustion. We also describe an evaluation of temperature distribution on the skin surface after moxibustion therapy, the development of a heat-transfer control device (HTCD), an evaluation of the HTCD, and the clinical effects of treatment using the HTCD. The HTCD we developed can heat the skin of the treatment region uniformly, and its effect may be equivalent to conventional moxibustion, without the emission of smoke and smell. This device can be used to treat ileus, abdominal pain, and coldness of abdomen in place of conventional moxibustion in modern hospitals.

1. Introduction

In East Asian medicine, a local thermal therapy known as moxibustion is widely used to treat several conditions, including gastrointestinal disorders, neurodegenerative diseases, cerebrovascular diseases, and cardiovascular diseases [1–3]. In moxibustion therapy, skin is heated by burning moxa. Because the direct burning of moxa on the skin can be dangerous, materials like salt, ginger, or garlic may be used as a buffer between the skin and the moxa [4–7]. There have been several studies reporting the effects of moxibustion therapy [4–7]. However, there are some problems associated with the application of this treatment in clinical practice. Moxibustion treatment temperature is dependent upon the expertise of the treating doctor or practitioner. Thermal damage to tissue begins at temperatures above 44°C [8]. Therefore, patients who have lost temperature sensitivity are at risk for burns during moxibustion therapy. A uniform temperature distribution is important when conducting thermal therapy in order to avoid burning the patient and to heat the treatment region to the desired therapeutic temperature. Additionally, the temperature distribution in the treatment region is nonuniform. Another problem associated with moxibustion therapy is the emission of



(a) (b)

Figure 1: Abdominal X-ray radiographs of a 95-year-old paralytic ileus patient. (a) Radiograph taken at the time of hospital admission, (b) radiograph taken 18 days after moxibustion therapy.

smoke. In order to resolve these problems, we developed a precise temperature-control device for use as an alternate for conventional moxibustion therapy.

In this paper, we describe a case of repetitive ileus that was treated by moxibustion. We also describe an evaluation of temperature distribution on the skin surface after conventional moxibustion therapy, the development of a heat-transfer control device (HTCD) for use as an alternate for conventional moxibustion therapy, an evaluation of the HTCD, and the clinical effects of the HTCD.

2. Clinical Usage of Moxibustion to Treat Ileus: A Case Report

A 95-year-old man was referred to Tohoku University Hospital from a local clinic for the treatment of nausea and abdominal pain. The patient was admitted to the hospital and diagnosed with paralytic ileus. He underwent an operation for inguinal herniation 15 years before seeking treatment at our hospital and had experienced ileus twice in the preceding year. The patient indicated he had been experiencing symptoms of constipation and abdominal pain for 5 days. After hospitalization, he was prescribed aperients. An abdominal X-ray radiograph indicated gas in the intestine and air-fluid level (Figure 1(a)). There was no indication of tumor or strangulation in either the small or large intestine, and the diagnosis of paralytic ileus was confirmed. Because the patient experienced repeated ileus during treatment with medication, we added moxibustion therapy to his treatment regimen (Figure 2). Following moxibustion therapy around umbilical region for 10 days, the constipation was relieved. The patient no longer experienced nausea and abdominal pain and he was able to resume eating meals. An abdominal radiograph also indicated improved ileus (Figure 1(b)). Moxibustion therapy was repeated daily, and the patient was able to defecate without anticonstipation drugs. After his discharge, the patient continued to receive regular weekly moxibustion therapy in the outpatient clinic.

Figure 2: A type of conventional moxibustion therapy. Salt was used as a buffer between the skin and the moxa.

3. Temperature Distribution on the Skin by Conventional Moxibustion (Modified from [9])

The temperature distribution on the skin by moxibustion should be assessed to avoid the skin burning. Therefore, we investigated temperature distribution on the skin after conventional moxibustion therapy in human. Figure 3 shows the variation over time in the temperature distribution on the skin surface measured using thermography (Advanced Thermo TVS-500®; Nippon Avionics Co., Ltd., Tokyo, Japan) just after conventional moxibustion therapy (Figure 2). In this experiment, the abdominal area of 34-year-old healthy male volunteer was heated by moxibustion with a salt buffer between the skin and moxa. As shown in Figure 3, the temperature on the central part of the abdominal area reached 44°C, indicating the risk of burns from heating the

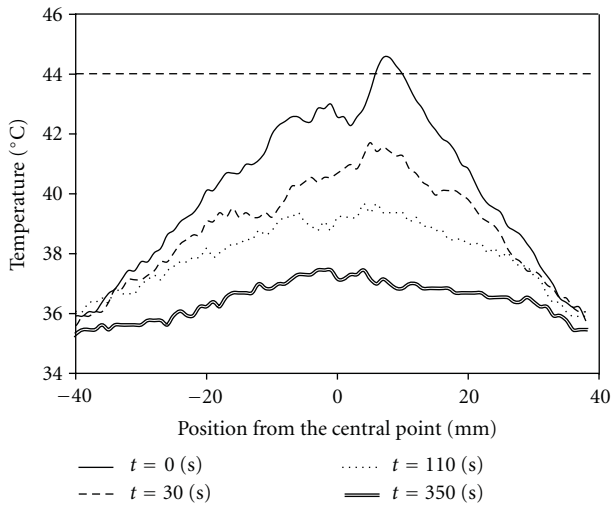


Figure 3: Spatial distribution of abdominal skin temperature from the central point at 0, 30, 110, and 350 s after completion of moxibustion therapy. The way of moxibustion is shown in Figure 2 (modified from [9]).

skin for long periods of time, because the denaturation of protein occurs at temperatures greater than 44°C [8]. In moxibustion therapy, the treatment temperature is adjusted based on the expertise of doctors and feedback from patients. Therefore, patients who have lost heat sensitivity like aged person are at increased risk of burns during moxibustion therapy. It is safer to control temperature precisely when the treatment is applied to human.

4. Development of an HTCD (Modified from [9])

According to the data of temperature distribution on the skin and the style of conventional moxibustion, we have developed a precise temperature control devise. A schematic diagram of the HTCD is provided in Figure 4. This device consists of heating disk and a control apparatus. The copper heating disk is 100 mm in diameter. Copper was used to form the heating disk because the high thermal conductivity of this material permitted uniform heating of the disk. A thermistor was implanted into the heating disk in order to monitor its temperature within 0.1°C. This device reduces the risk of burns because the temperature of the heating disk can be controlled to avoid exceeding the target temperature. This device is convenient for medical doctors because it does not emit smoke, control of device temperature is easy, and there is no risk of burns to patients. Therefore, this devise as a modern "moxibustion" therapy can be used in hospitals. Furthermore, we have demonstrated the therapeutic effects of this device on many patients.

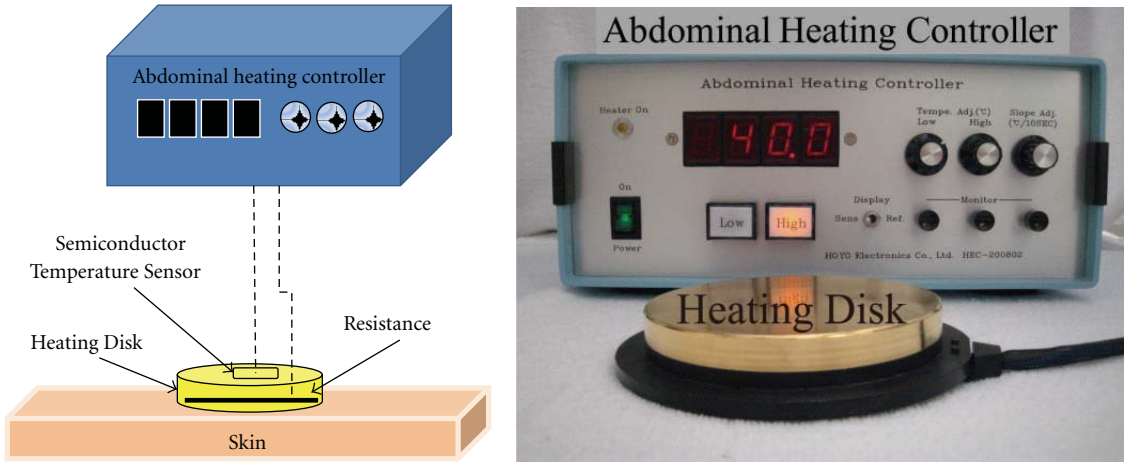
5. Temperature Distribution on the Skin by HTCD

5.1. Methods (Modified from [9]). To confirm the performance of the abdominal heating controller, we evaluated the controllability of the temperature, heating rate, and uniformity of the temperature distribution on the skin surface. A 24-year-old healthy male volunteer lay down on his back on a bed in the experiment room. The temperature of the experiment room was maintained at 25°C. The HTCD was placed directly on the skin of the subject's abdominal area (Figure 5). The temperature of heating disk was set to 42°C and the abdominal area was heated for about 12 minutes. Skin surface temperature was recorded by temperature sensor in the disk. The experiment was repeated 4 times with the interval of 2 hours and in each experiment, the incremental rate of temperature increase was altered. After heating for 12 minutes, the heating disk was removed from the subject's abdomen and the temperature distribution of the skin surface was measured using thermography.

5.2. Results. Figure 6 shows the result in the variation of the incremental rate of temperature on the skin surface of the subject's abdominal area. In all 4 repetitions of the experiment, the temperature of the heating disk increased to the target temperature, but did not exceed it. This confirms that there is no risk of skin burns to patients from high temperatures. Moreover, it was confirmed that the temperature of the disk is controllable. The temperature distribution on the skin at different time points after the removal of a heating disk set to 42°C is shown in Figure 7. Skin temperature decreased almost uniformly. This confirms that the temperature distribution of the heating disk and that of the subject's abdominal area were almost uniform during the procedure. Therefore, the HTCD is able to uniformly heat the abdominal area of patients to a precise target temperature.

6. Clinical Application [10, 11]

6.1. Methods. To confirm the effects and safety of HTCD, the thermal therapy was conducted on 26 healthy volunteers (male; 24 and female; 2) with a mean age of 30.8 ± 6.8 years (range, 21–44 years). The HTCD was placed on the skin of the paraumbilical region for 20 minutes. We measured blood flow through the superior mesenteric artery (SMA) continuously from rest to 40 minutes after removal of the HTCD. (Figure 5). There are several acupoints located at the paraumbilical region. In particular, the acupoint of CV8 and ST25 is considered to influence the stomach, spleen, and intestines in traditional East Asian medicine [12]. This size of the heating disk can cover these important acupoints for the treatment of digestive diseases. The SMA supplies blood to the whole small intestine, except the superior part of the duodenum, as well as the cecum, the ascending colon, and most of the transverse colon. Therefore, we used SMA blood flow to investigate the changes in intestinal blood flow resulting from thermal stimulation. We measured circulation in the SMA using an ultrasound system (Prosound α10®; Aloka Co., Ltd, Tokyo, Japan). Pulsed Doppler signals were used to acquire SMA measurements within 2 to 3 cm of the origin of the artery [13, 14]. To ensure accurate measurement, we employed a Doppler angle of 60° or less



(a) (b)

Figure 4: (a) Schematic diagram of an abdominal heating controller, (b) picture of heat-transfer control device.

[15, 16]. Blood flow volume was recorded 3 times during 3 different cardiac cycles and averaged for each subject in an effort to minimize errors [13].

Subjects rested in the supine position in a quiet, air-conditioned room (temperature, 25-26°C) during the entire experiment. After positioning the ultrasound system, subjects rested for 10 minutes. The HTCD was set to 40°C and placed on the skin of the paraumbilical region (Figure 5). The HTCD was left in place for 20 minutes. After 5 minutes, if tolerated by subjects, the temperature was increased to 41°C. The target temperature of the disk was set at 40-41°C for safety to avoid a skin burn. The device was removed after 20 min of thermal stimulation.

Statistical analysis was performed with SPSS software (version 16.0, SPSS Japan Inc., Tokyo, Japan). Repeated measures analysis of variance with a Tukey post hoc test was used for statistical comparison with baseline. The % change of blood flow volume in the SMA at each time was calculated in relation to the baseline value. Results are presented as the means and SD. $P < 0.05$ was used to indicate significance in all statistical tests.

6.2. Results. Figure 8 shows the result in the % change of blood flow volume in the SMA. The blood flow volume in the SMA was significantly higher during thermal stimulation ($P < 0.01$), 10 and 20 minutes after the end of thermal stimulation ($P < 0.01$), and 30 minutes after the end of thermal stimulation ($P < 0.05$), as compared to the volume before placement of the HTCD (Figure 8). There were no complications such as local burns, pain, discomfort or other problems that required treatment.

7. Discussion

In this report, we described a case of ileus that was successfully treated with moxibustion, reported the distribution of skin temperature following conventional moxibustion, described the development of a precise temperature-control

Figure 5: Abdominal thermal therapy on the abdomen by using a heat-transfer control device.

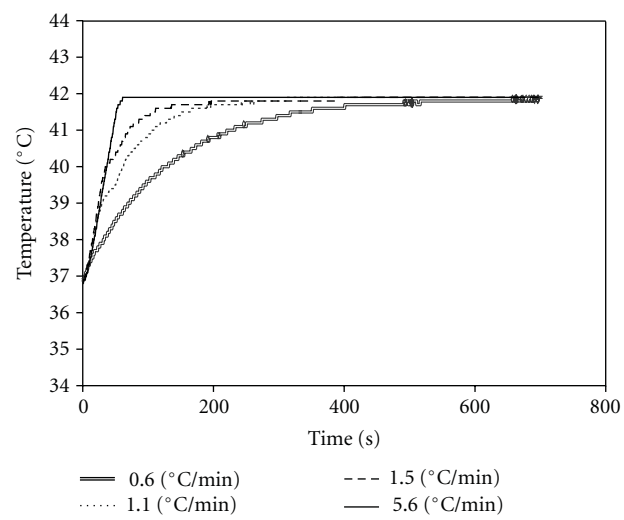


Figure 6: Skin surface temperature of a single subject during 12 minutes of stimulation by a heat-transfer control device set to 42°C. The device was set to increase from body temperature to the target temperature at the following rates: 0.6°C/min, 1.1°C/min, 1.5°C/min, and 5.6°C/min (modified from [9]).

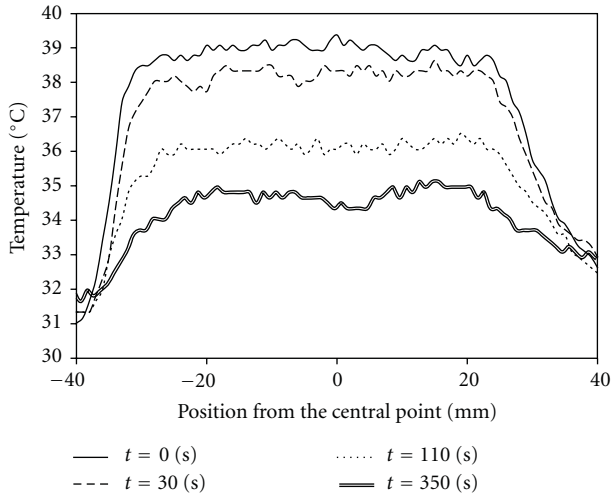


Figure 7: Spatial distribution of abdominal skin temperature after stimulation with a heat-transfer control device. The target temperature was set at 42°C. Measurements were taken 0, 30, 110, and 350 seconds after device removal. (Modified from [9]).

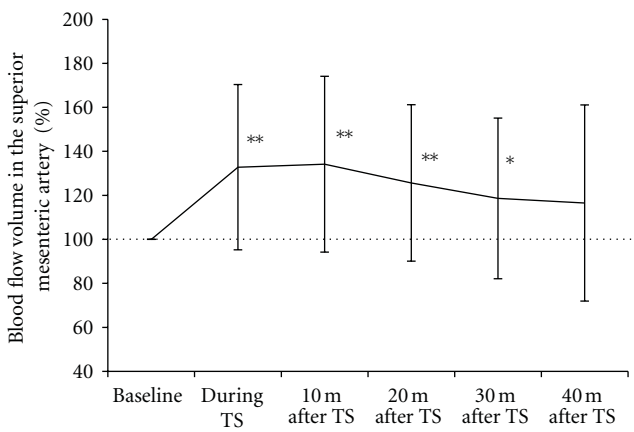


Figure 8: The % change of blood flow volume in the superior mesenteric artery during and after thermal stimulation of the abdomen. Data are presented as means and SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus baseline. TS, thermal stimulation.

device, and reported the results of an experiment demonstrating the efficacy of the devise.

Traditional East Asian medicine has a long history and the administration of herbal medicine, acupuncture, and moxibustion therapies depends on the experience of the treating doctors or practitioners. The problems associated with moxibustion therapy, such as difficulty of temperature control, smoke, and smell make it difficult to use in modern hospitals. These problems pose real risks to patients. In general clinical practice, there is a possibility of skin burning of a patient with low temperature sensitivity during moxibustion. Another patient suffered an attack of bronchial asthma during moxibustion therapy that was induced by the smoke.

The effects of moxibustion result from the heat, smell, and smoke generated by the procedure. The temperature-control device that we described in this study simulates the heat of moxibustion, but does not produce either the smoke or smell associated with this treatment. As this device can be used to control skin surface temperature to within 0.1°C, it can be used in modern hospitals as a safer and more generally useful device than conventional moxibustion.

Mesenteric ischemia results from decreased blood flow to the bowels and causes several symptoms such as pain, nausea, and vomiting. Nonocclusive mesenteric ischemia is an acute mesenteric circulatory disorder, which is induced by vasospasm [17]. Chronic mesenteric ischemia is usually caused by atherosclerosis [18]. In these conditions, the pathophysiology is same as the mesenteric ischemia. The treatment of mesenteric ischemia involves reperfusion through drug therapy or vessel reconstruction. Sometimes vasodilative drugs are selected as a conservative treatment modality [17–19]. In the current study, we demonstrated that SMA blood flow volume increases with local thermal therapy. The treatment of local thermal therapy with HTCD might be useful for increasing blood flow volume in the cases of mesenteric ischemia. During thermal stimulation, intestinal peristalsis accelerated along with the increase in blood flow volume (data not shown). Thus, thermal stimulation not only increases the blood flow volume, but also improves intestinal motility. Abdominal thermal therapy might be useful for patients with low SMA blood flow, paralytic ileus, or chronic constipation. Future study of the effect of thermal stimulation on patients with such disorders is warranted.

HTCDs have been used to treat the abdominal disorders, pain, and coldness in about a total of 600 patients at Tohoku University Hospital. Over the past 3 years, there have been no reported side effects associated with the use of this device. We conduct clinical trials to investigate its clinical effects in the patients of old cerebral infarction with chronic constipation.

8. Conclusion

The HTCD described in this paper was developed based on conventional moxibustion therapy. The temperature of this device can be controlled precisely, and it is safer than conventional moxibustion. Our studies suggest the usefulness of this device for the treatment of adnominal disorders, constipation, pain syndromes, and coldness in place of moxibustion in hospital settings.

Acknowledgments

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Research Article

Temperature and Safety Profiles of Needle-Warming Techniques in Acupuncture and Moxibustion

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The needle-warming technique combines acupuncture and moxibustion, and it is commonly practised in China to relieve pain conditions. However, burning of moxa has many disadvantages. This study examined the temperature and safety profiles of such technique. First, skin temperature changes during needle-warming were examined in anesthetized animals to determine the safe distance for needle-warming moxibustion in human subjects. Then, the practical distance for needle-warming in human subjects were verified. Finally, the temperature profiles of the needle during needle-warming moxibustion were examined using an infrared camera. Our results show that during needle-warming moxibustion there is little heat being conducted into deep tissue via the shaft of the needle, and that the effective heating time to the acupoint is rather short compared to the period of moxibustion. These findings suggest that the needle-warming technique is an inefficient way of acupoint thermal stimulation and should be modified and improved using new technologies.

1. Introduction

Acupuncture and moxibustion (Zhenjiu), which is an ancient Chinese therapy that uses physical stimulation on body surface to alleviate diseases, is gaining popularity in many countries because it is free from side effects of chemical drugs [1–5]. Still, much remains unknown about the characteristics of the therapy, its efficacy, and the underlying mechanisms of actions, especially for moxibustion. A better understanding of the physical characteristics of the therapy is important for future research, as it will help to improve the reproducibility of the intervention, standardize the intervention through development of new devices, and determine the adequate controls in clinical trials [6, 7].

The needle-warming technique involves stimulating acupuncture points by needle penetration followed by burning of a piece of moxa attached to the needle. It is often used for treatment of pain conditions such as arthritis, sciatica, cervical spondylopathy, intervertebral disk herniation, and osteoarthritis [8–12]. It is generally believed that heat from burning of moxa (moxibustion) is transmitted to the acupoint by radiation as well as by direction conduction via the shaft of the needle, thereby stimulating deep tissue within the acupoint, besides warming the acupoint on the surface [13]. The physiological basis of such practice is not well understood. Nevertheless, it has been reported that moxibustion can lead to the release of anti-inflammatory agents, such as heat shock proteins [14]. However, burning of moxa has several disadvantages. In particular, smoke produced by combustion of moxa may trigger the onset of an asthmatic attack [15, 16]. It is also time consuming to apply the technique and difficult to control the heat to prevent burn injury to the skin [17–20]. Therefore, a better understanding of the thermal characteristics of the needle-warming technique can facilitate the development of new therapeutic devices and techniques that have the same characteristics of the technique without the drawbacks. To this end, we investigated the profiles of heat transmission during needle-warming moxibustion.

2. Material and Methods

Experimental procedures were approved by the committee on the use of human and animal subjects in teaching and research of Hong Kong Baptist University.

2.1. Measurements in Anesthetized Animals. Three rabbits weighing between 1.8 kg to 2.05 kg were used. They were anaesthetized with an intravenous injection of pentobarbitone (30 mg/kg) with supplementary doses added when necessary, and euthanized using over dose of pentobarbital at the end of the experiment. The skin around the acupoint Huan Tiao (GB30) was shaved to expose an area approximately 6 × 6 cm^2, and the animal was placed on a thermostatic electric heating blanket to keep the body temperature at around 37.5 ± 0.5°C. The room temperature was kept at 23°C. A small temperature probe (MLT409/D ADInstruments) was placed on the skin surface immediately adjacent to the point of needle insertion. Signals from the temperature probes were processed by a data acquisition system (ADInstruments).

2.2. Measurements in Human Subjects. Six healthy volunteers were invited to participate in the experiment on separate days. The room temperature was maintained at 23°C. To measure skin temperature, a skin probe (ADInstruments, model MLT409/D; length × diameter: 2.8 × 9.6 mm) was placed immediately adjacent to the point of needle insertion, and the signal recorded from the probe was processed as described above.

2.3. Acupuncture and Moxibustion Procedures. Two different diameters (0.30 mm × 40 mm and 0.35 × 40 mm) of stainless steel acupuncture needles (Carbo, Suzhou, China) with a handle size of 1.0 mm × 33.6 mm were tested at acupoint Huan Tiao (GB30) in animal experiments and at acupoint Zusanli (ST36) in human subjects. A 15 mm long moxa cylinder block weighing 1.70 ± 0.05 g, which was cut from moxa sticks (Wu She Pure Moxa Roll, Suzhou, China), was attached to the handle of the needle by inserting the handle into the center of the block. The distance from the bottom of the moxa block to the junction of handle and shaft of the needle was approximately 10 mm. In a few experiments, commercially available moxa cylinders (12 mm × 15 mm; Wushe, Shuzhou, China) were tried, but were not studied further because their effective heating time on the skin surface was very brief. That is, the duration of skin temperature above 40°C during burning of these moxa cylinders was less than 30 seconds when the cylinder was at 10 mm above the skin. In some human experiments, a thin piece of cardboard (approximately 50 mm × 50 mm × 0.2 mm) was placed above the skin to mimic clinical practice procedures, which aimed to prevent possible burn injury caused by falling of moxa ash. The moxa was ignited at the top in each of the trial.

2.4. Measurement of Temperature on Acupuncture Needles. An acupuncture needles were painted black with a permanent marker (ZEBRA) and held vertically by a clamp at the end of the handle, with a moxa block attached to the handle and ignited from the top. To measure temperature of the acupuncture needle, a thermal tracer (NEC, model TH9100PMV) equipped with a close-up lens (TH-386) was used. The emissivity index of the thermal tracer was set to 0.99. The accuracy of the tracer measurement was checked by placing the needle in an enclosed thermostatic box, with the needle attached to the tip of an alcohol-filled thermometer (Hisamatsu, Japan). It was found that the difference between the thermometer reading and the tracer reading was less than 0.5°C in the range of 30–45°C, which was acceptable for the purpose of the current experiments.

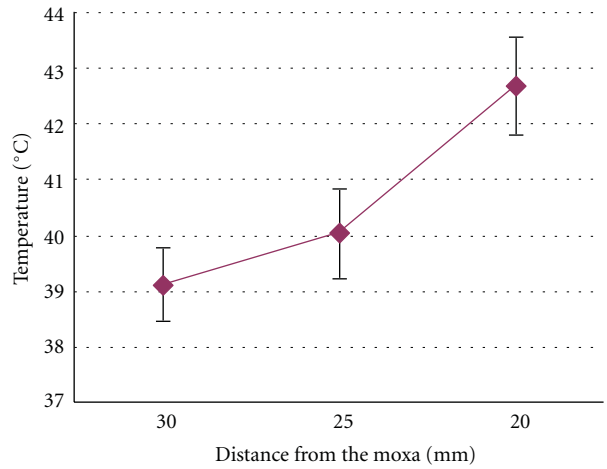

Figure 1: Line chart showing the relationship between the distance of moxibustion and the temperature on the skin surface of acupoint GB30 in anaesthetized rabbits during needle-warming (mean ± SD, $n = 6$). X-axis indicates the distance between the moxa block and skin surface.

3. Results

3.1. Safe Distance between the Moxa Block and the Skin Surface in Needle-Warming Moxibustion. We first investigated the safe distance between the moxa block and the skin surface in needle-warming moxibustion using anesthetized rabbits. With a moxa block (1.70 ± 0.05 g) attached to an inserted acupuncture needle, the maximum mean temperature (±SD) on the skin surface directly under the ignited moxa block was 39.1 ± 1.2°C, 40.0 ± 2.3°C, or 42.6 ± 2.0°C when the distance between the moxa block and the skin surface was 30 mm, 25 mm, or 20 mm, respectively (Figure 1). No significant difference was seen in the maximum mean temperature on the skin surface between two different diameters of needles. In one animal, the moxa block was placed at 15 mm above the skin, and the temperature of the skin surface exceeded 46°C, which induced a superficial burn, at which point the moxa block was quickly removed. These results indicated that for moxa block of this size, the safe distance between the moxa block and the skin surface must be over 20 mm, and burning the moxa block at a distance less than 20 mm above the skin would be unsafe in human subjects.

3.2. Skin Temperature Changes during Needle-Warming in Human Subjects. The temperature on the skin surface was studied in 6 human subjects, either with or without cardboard covering the acupuncture area. As seen in Figure 2, without cardboard covering the acupuncture area, the skin temperature was 38.4 ± 1.3°C and 40.8 ± 0.9°C when the distance between the moxa and the skin surface was 35 mm and 30 mm, respectively. The maximum skin temperature reached was 41.9°C at 30 mm distance in one occasion, at which point the volunteer reported intense heat sensation in the area surrounding the needle, but it was not painful. Moxibustion distance less than 30 mm without cardboard covering was not tested, as it might cause severe pain and even skin burn injuries.

With cardboard covering the acupuncture point and the surrounding area, the skin temperature was 36.2 ± 1°C, 37.8 ± 0.6°C, and 39.1 ± 0.9°C, when the distance between the moxa and the skin surface was 35, 30, and 25 mm, respectively (Figure 3).

For a given distance, the average maximum skin temperature reached in the group without cardboard cover was higher than that with cardboard cover, with the mean difference ranges from 1.2°C to 2.9°C ($P < 0.01$, t test).

The time course of the skin temperature change during needle-warming is illustrated in Figure 3. It can be seen that there was no increase in skin temperature after ignition of the moxa in the first 12 minutes also. Then, there was a gradual increase and a gradual decrease in temperature between 12–24 minutes. It was observed that the skin temperature did not increase significantly until the lower part of moxa started to burn, and the effective heating period (i.e., >37°C) lasted only 2-3 minutes.

3.3. Temperature Distribution along Needle Shaft during Needle-Warming Moxibustion. The temperature of the needle shaft was measured with the needle suspended in the air. As seen in Figures 4(a), 4(b), and 4(c), the temperature decreased exponentially along the shaft during needle-warming, dropping below 35°C at distance over 20 mm away from the burning moxa block. However, with a piece of cardboard placed 25 mm under the moxa block, mimicking the clinical situation in which the skin below the moxa block was covered, the temperature distribution along the needle shaft was bimodal (Figures 4(d), 4(e), and 4(f)). The lowest temperature point was found around 18 mm from the moxa block, where the maximal temperature reached was 35°C. After this point, the shaft temperature increased gradually towards the cardboard, reaching a maximum of 48°C at the juncture of the needle and the cardboard.

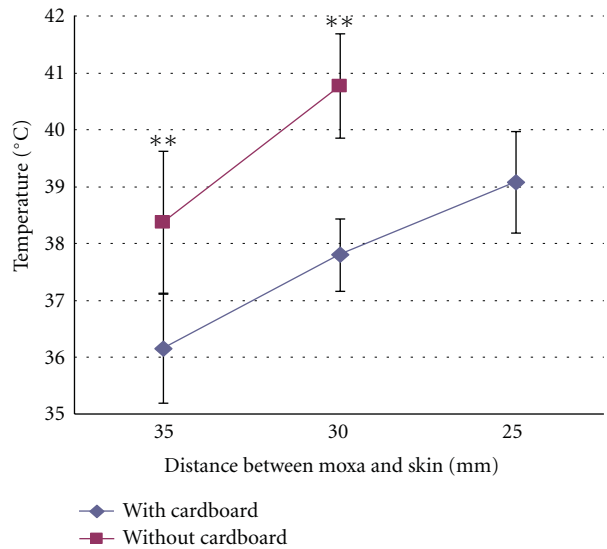

Figure 2: Line chart showing the relationship between the distance of moxibustion and the temperature on the skin surface of acupoint ST36 of human subjects during needle-warming (mean ± SD, $n = 6$). X-axis indicates the distance between the moxa block and skin surface. Measurements were taken either with cardboard overlying the acupoint (with cardboard) or without (without cardboard). $^{**}P < 0.01$.

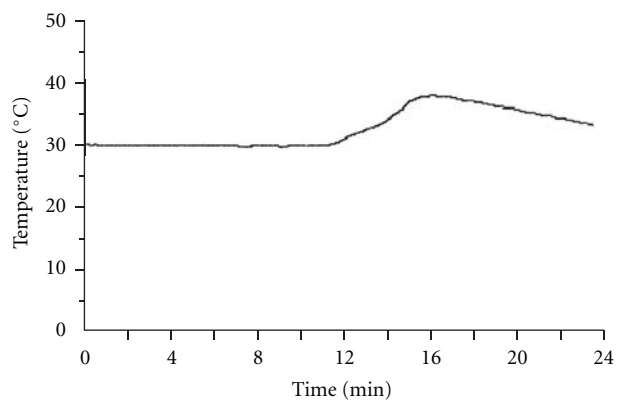

Figure 3: Computer chart record showing temperature changes during needle-warming in a human subject. The distance between the moxa block and the skin of acupoint ST36 was 35 mm. No cardboard cover was placed on the surface of the acupoint.

4. Discussion

This is the first study that measures the temperature of acupuncture needle shaft during needle-warming technique using an infrared camera. We found that the effective heating distance of the needle shaft was limited to less than 20 mm from the moxibustion site. On the other hand, at least 25 mm was required between the moxibustion site and the skin surface for safe needle-warming practice. Thus, there is no effective direct heat transmission from the needle to the skin tissue and below in needle-warming. We also found that the overall heating time of the acupoint in needle-warming was rather brief, lasting only 2-3 minutes, comparing with the 25 minutes that were required to burn the whole block of moxa. The implications of these findings are discussed below.

It has been shown that temperature over 40°C is required to produce physiological effects, but thermal pain sensation occurs at 44.4 ± 2.1°C, with some variations between individual [21–26], and tissue injury occurs if temperature is

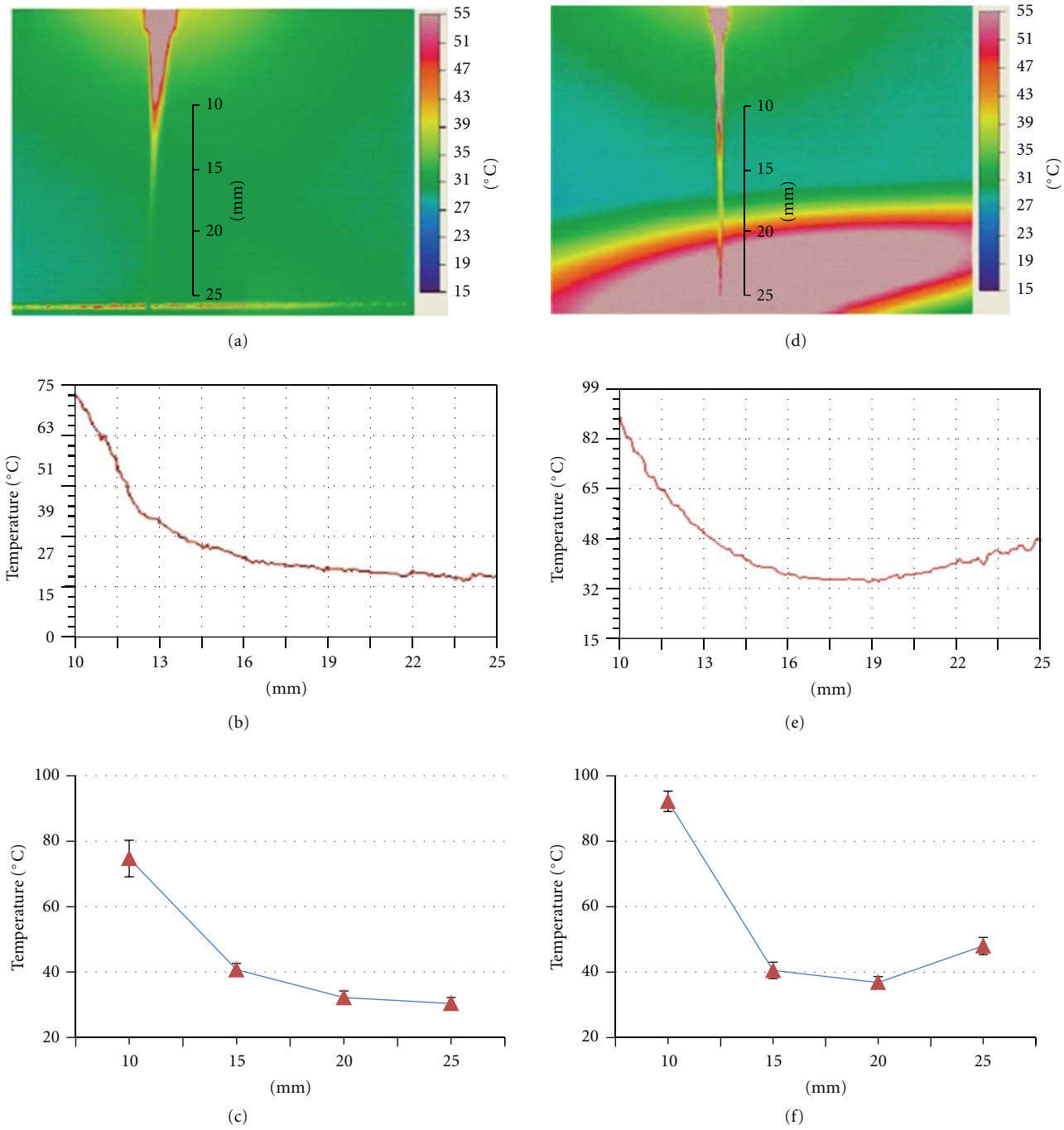

Figure 4: (a) and (d) thermographic images showing temperature along the needle during the hottest period of needle-warming. (b) and (e) temperature reading of the needles as shown in (a) and (d), respectively. The X-axis indicates the distance from the moxa block. (c) and (f) line graphs showing mean temperature reading of the needles at fix distances from the moxa block during the hottest period of needle-warming ($n = 9$ experiments). In (a), (b), and (c), a cotton thread was placed 25 mm below the moxa block perpendicularly near the needle to facilitate measurements. In (d), (e), and (f), a piece of cardboard was placed 25 mm below the moxa block with the needle piercing through it.

maintained at over 45°C [27]. In other words, the temperature window for therapeutic heat treatment without causing pain is relatively narrow, between 40–42°C. Our study showed that in heat treatment with needle-warming technique, the skin temperature varied a lot, depending on the distance between the skin and the moxa block, as well as the size of the moxa block. For smaller moxa blocks, such as those commercially available ones that had been tested, the effective heating time might be too short to be effective. For larger moxa blocks, such as those used in the current study,

the distance between the skin and the moxa block must be at least 25 mm even with cardboard protection or 30 mm without protection.

It is interesting to find that at sites over 20 mm below the moxa block, before the needle touching the skin, the needle shaft temperature was below 40°C if no object was placed underneath. This needle temperature would have little therapeutic effect. On the other hand, if a piece of cardboard was placed 25 mm below the moxa block, after dropping below 40°C at about 20 mm below the moxa block the temperature of the needle shaft increased gradually again as it approached the cardboard underneath, and reached about 48°C at the contact point between the needle and the cardboard. The second increase in shaft temperature was presumably due to reflection of heat radiation by the cardboard underneath. However, cautions must be taken when interpreting the temperature reading of the tracer because we have not controlled for the effect of stray IR, which might came from any sources other than the needle. Two factors may influence the effect of stray IR on the reading of the tracer: (i) the ratio of the intensity of the stray IR to the true IR signal from the needle and (ii) the state of the stray IR. The stray IR in the current experiment is from stationary objects, and by using averaged measurements, the variation in stray IR had been minimized. However, because the temperature of the needle shaft 20 mm below the moxa block was below 40°C, and the needle shaft was very thin, the intensity of the signal might be very low. Thus, the stray IR might distort the reading of the tracer on the part of the needle near the cardboard, resulting in higher reading than the true temperature of the needle. Such influence has only a short distance and therefore only affected less than 4 mm of the needle near the cardboard. Nevertheless, such potential measurement error has no impact on the interpretation of the main findings.

Until now, it is generally believed that heat from burning of moxa can be transmitted to the acupoint via the shaft of the needle, in addition to heat radiation [13]. Therefore, the needle-warming technique allows heat being conducted into deeper part of the acupoint hence resulting in thermal stimulation of deep tissue [28], whereas moxibustion with a moxa stick only has superficial effects on the skin [29]. Our findings suggest that at the current setting, which is similar to clinical practice, there is only radian heat stimulation to the acupuncture point, as heat from the needle is lost at a distance over 20 mm, before the needle penetrates the skin. This finding is consistent with the recent report by Cheng et al. [13], in which a maximum of 41.71 ± 1.39°C was reported on the contact point between the skin and the stainless steel needle.

5. Conclusion

The present findings indicate that the needle-warming practice is an inefficient heat stimulation by combustion of moxa because there is no direct heat transmission into the tissue via the acupuncture needle. For radian heat stimulation of the acupoint surface, other techniques of moxibustion, such as using a moxa box, may be safer and more efficient. New technologies, such as electrical heating devices, can be used in the future to improve the needle-warming technique. We also show that the heat conduction process can be visualized with an infrared camera, and such method will be useful in developing new instruments that can replace the needle-warming technique.

Author's Contribution

X. Y. Gao and C. Y. Chong contributed equally to this work.

Aknowledgment

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Research Article

Mathematical Reflections on Acupoint Combinations in the Traditional Meridian Systems

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The meridian system is a systematic order of empirical knowledge functioning as a rational ground for a balanced treatment by combining meridians. In TCM theory, a continuous circulation of Qi through 12 meridians is postulated, described as the Chinese clock (CC). On this basis, combinations of meridians and acupoints had been described in historical writings. The most common is the interior/exterior system beside the neighbouring system, the opposite clock system, and three systems, developed out of the theory of the six stages. All of these represent symmetrical combinations, which were defined by the steps in the CC. We calculated the possible combinations that fit into the systematics of the historical descriptions, leading to 19 systems. Merging the data of the 19 systems, possible steps in the CC clock for balancing a meridian are 1, 2, 3, and 6. Step 4 is not possible. Step 5 is a combinatory possibility but has no widespread tradition except for activating the yin extraordinary vessels. These possibilities can be plotted on the CC as a powerful tool for daily practice. Only two meridians might be excluded as potentially balancing meridians, so it seems almost impossible to define noneffective acupuncture points as controls in clinical trials.

1. Introduction

The theory of Chinese medicine has its basis in the old Chinese tradition dividing one into two (yin and yang) or dividing one into three (Tai Yang, Yang Ming, Shao Yang or Tai Yin, Jue Yin, Shao Yin or front, back, side) [1]. The development of the Chinese meridian system has the same basis, putting empirical knowledge into an organized system, dividing the body surface into areas in an absolutely symmetrical way. It is very likely that the system was organized for numerical and symmetrical reasons to find a paired cyclic pedant to the odd cycle of the five phases [1]. Old manuscripts only describe 11 meridians [2–4], while later sources describe 12 meridians [4]. In Chinese Medicine the human organism is understood as being linked in an endless circulation of Qi throughout the meridians at the latest since the publication of the Nan Jing [5]. Most acupuncture textbooks describe the Qi of the body as flowing in a systematical circular and continuous way through 12 meridians. Detailed diagrams of the circulatory courses of the 12 meridians were described in the Song dynasty. The book Pictures of Circulatory Courses of Meridians and the Internal Organs *(Cunzhen Huanzhong Tu)* by Yang Jie had an important influence on later descriptions of the Chinese clock [6]. Putting the systematics of the meridian system into diagrams became more popular in the Ming dynasty and was described in detail in the Lei Jing Tu Yi in 1624 [7]. Under the influence of daoism, chronobiological treatments in relation to the Chinese clock like *Ziwu liuzhu fa* were developed [8].

Balancing Yin and Yang is a basic concept in Chinese medical theory [9, 10]. Throughout the whole tradition of Chinese medicine combinations or connections of meridians (or their corresponding organs) were postulated, leading to acupoint combinations [11]. Meridians are classified as yin

or yang meridians. Lung (LU), spleen (SP), heart (HT), kidney (KI), pericardium (PC), and liver (LR) are defined as yin meridians, and large intestines (LI), stomach (ST), small intestines (SI), bladder (BL), triple energizer (TE), and gallbladder (GB) are defined as yang meridians [12–17]. The aim of acupuncture treatment is considered to maintain a balance of yin and yang organs and meridians. Disease is understood as a loss of balance between the yin and yang energies, but a relative dysbalance can occur between yin meridians or between yang meridians as well [10, 18].

This circle of meridians in the Chinese clock is divided into three cycles, cycle 1 (LU, LI, ST, SP), cycle 2 (HT, SI, BL, KI), and cycle 3 (PC, TE, GB, LR) [19, 20]. A logical explanation for dividing the circle into three cycles is hard to find. We think there is a connection to the three human body surfaces by using an anatomical model of an upright standing person with the arms hanging down on the side, with the thumbs pointed frontwards and the 5th finger pointed toward the back. This is exactly the stance of most bronze models used for training acupuncture, especially since the redefinition and compilation of Wang Wei-yi, which was described in the Illustrated Manual on the Points for Acupuncture and Moxibustion on the New Bronze Model in about 1026 [21]. Cycle 1 can be connected to the frontal part of the body, cycle 2 can be connected to the dorsal part of the body, and cycle 3 can be connected to the lateral/medial side of the body. By dividing the Qi-flow into three cycles, circulation of Qi through three body surfaces, the frontal, dorsal, and lateral/medial parts of the human body, makes most sense to us (Figure 1).

2. Material

2.1. Literature Research for the Historical Systems. To understand the logic of combination of meridians we investigated modern textbooks and historical writings of Chinese medicine for the description of the known systems for combination of acupuncture points [1, 5, 7, 9, 12–17, 21–33].

2.2. Analysis of the Historical Systems

(1) First we described the steps taken in the Chinese clock in the different historical systems by counting clockwise and counterclockwise (Figure 2).

(2) Second we analysed the graphical pattern in the Chinese clock. Combining meridians in the Chinese clock leads to graphical patterns. We analysed whether these patterns have a certain rotational symmetry. An object has a rotational symmetry if it looks exactly the same at least once during a complete rotation through 360°. Rotation may be clockwise or counter clockwise. The angle at which an object looks exactly the same during rotation is called the angle of rotation. During the rotation, the object rotates around a fixed point, the centre of rotation, while its shape and size do not change. For example a full turn refers to a rotation of 360, a half turn refers to a rotation of 180, and a quarter turn refers to a rotation of 90°.

(3) Third we checked whether yin meridians were combined with yin meridians, yang with yang meridians, or yin with yang meridians.

2.3. Mathematical Analysis. To find out whether there are more systems than historically described and whether any meridians can be excluded as potentially balancing meridians, we calculated all symmetrical combinatorial possibilities.

Since there are 12 points in the Chinese clock, the smallest angle of rotation is 30°. It is not hard to imagine that 60° is the second possible angle of rotation since 60° is two times 30°. Of course, 90° is the third possible angle (a quarter of 360°). Next is 120° (one-third of 360°). But 150° is not possible since 360°cannot be exactly divided by 150°. The last one is 180°.

In summary, all possible rotation angles are 30°, 60°, 90°, 120°, and 180°.

3. Results

3.1. Description of the Historical Systems

3.1.1. Interior/exterior (a Single-Step System). The most common system is the interior-exterior connection. It originates from Chapter 2, Volume 1 of the Lingshu [29] and was described in detail in The Systematic Classic of Acupuncture and Moxibustion (Zhen jiu jia yi jing, Book 9) [23]. It is used in almost every ancient or modern school of Chinese medicine [23, 34–36]. It combines one meridian with the following one in the Chinese clock: LU with LI, ST with SP, HT with SI, BL with KI, PC with TE, and GB with LR. The meridians are next to each other on one extremity, connecting the exterior and the interior. There is always a yang (exterior)-yin (interior) combination (Figure 2(a)).

A typical example of combining interior and exterior points in a systematic way are the luo connecting points, as described in Chapter 10 of Lingshu, which can be combined with the Yuan-source points, following the interior/exterior combination system [9, 29].

Historical point combinations fitting into the interior/exterior system are LU-3 (Tianfu) and LI-4 (Hegu) for nose bleeding [28] or SP-1 (Yinbai) and ST-45 (Lidui) for nightmares [32], Jingman (GB 25) and Xingjian (LR-2) for lumbar pain, being not able to stand upright for a long time and bending back and forth [23].

3.1.2. Neighbouring Channels (A Single-Step System). This is the second option of combining channels in a single-step system, combining LR with LU, LI with ST, SP with HT, SI with BL, KI with PC, and TE with GB. It leads to arm-leg combinations of two yin or two yang channels [36–38].

Historical point combinations fitting in the neighbouring system are ST-36 (Zusanli) and LI-4 (Hegu) for dysenteric disorder [26] or PC-6 (Neiguan) and KI-6 (Zhaohai) for quick treating of abdominal disease [27].

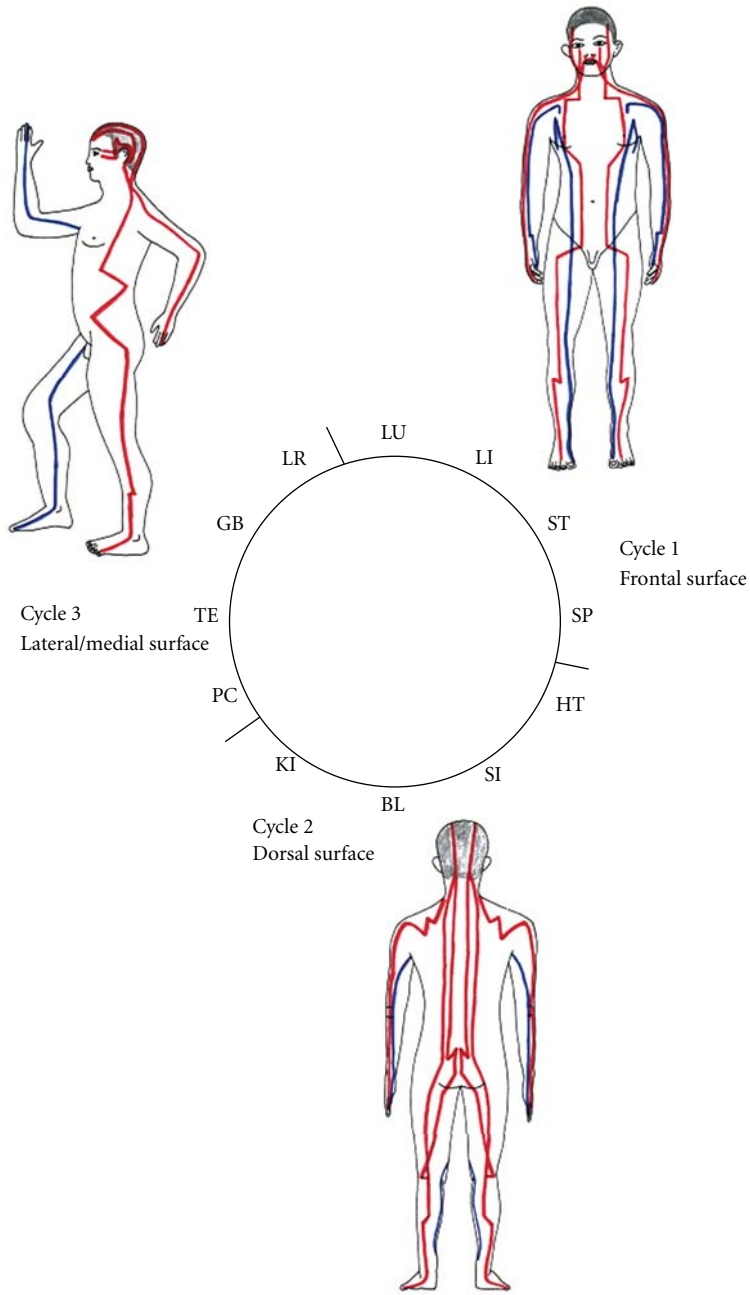


Figure 1: Connection of the three cycles in the Chinese clock with the body surface. Red: yang meridians; blue: yin meridians.

3.1.3. 6-Stage System I (a 1-Step–3-Step Alternating System). This system of channel combination refers to the tradition dividing of a unit into 3 segments (Yang into Tai Yang, Yang Ming, and Shao Yang as well as Yin into Tai Yin, Jue Yin, and Shao Yin). It originates from the Suwen (Chapter 6, (77 + 79)) and Lingshu (Chapter 5, (948 + 949)) and describes 6 stages: Tai Yang, Yang Ming, Shao Yang, Tai Yin, Jue Yin, and Shao Yin [9, 29]. The sequence of the stages is in discussion. In the Suwen Chapter 6 the order described above can already be found as well as two other possibilities. Other authors of high influence like Zhang Zhongjing used an alternative order to the version described above in the Shanghanlun in the 2nd century [21], changing the order of Jue Yin and Shao Yin. The same order was used by Huang-Fu Mi in The Systematic Classic of Acupuncture and Moxibustion in the late 3rd century [23]. Since this time, meridians are named by the stages, Hand Tai-Yang (SI), Foot Tai-Yang (BL), Hand Yang Ming (LI), Foot Yang Ming (ST), Hand Shao Yang (TE), Foot Shao Yang (GB), Hand Tai Yin (LU), Foot Tai Yin (SP), Hand Jue Yin (PC), Foot Jue Yin (LR), Hand Shao Yin (HT) and Foot Shao Yin (KI). To our understanding the order described in Suwen (Chapter 6, (77+79)) and LingShu

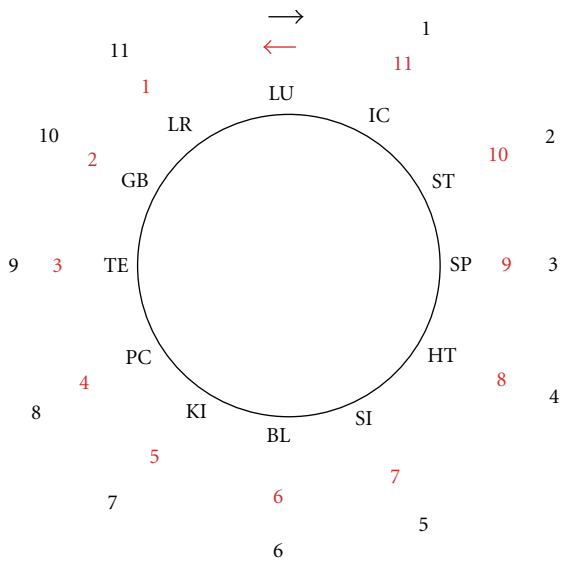


Figure 2: Counting of the steps in the Chinese clock, clockwise and counterclockwise.

Chapter 5 (948 + 949)) is the most useful for daily practice, so we number the stages as described above. For understanding our theory of the development of the meridian system the numbering of the stages is of secondary importance and irrelevant for the central message of this paper.

Using the 6-stage theories for point combination is very common. The main system uses points connected to the same stage as the starting point. For this reason and because of the similar name (e.g. Foot and Hand Yang Ming) it is called "anatomical system" in some schools [39]. It combines Hand Tai Yang (SI) and Foot Tai Yang (BL), Hand Yang Ming (LI) and Foot Yang Ming (ST), Hand Shao Yang (TE) and Foot Shao Yang (GB), Hand Tai Yin (LU) and Foot Tai Yin (SP), Hand Jue Yin (PC) and Foot Jue Yin (LR), and Hand Shao Yin (HT) and Foot Shao Yin (KI). This system represents a 1-step–3-step alternating system. We call it the 6-stage system I.

Historical point combinations fitting into the 6-stage system I are PC-3 (Quze) and LR-13 (Zhangmen) for a dry mouth [32] or TE-5 (Waiguan) and GB-2 (Tinghui) for treating impaired hearing and deafness [29].

3.1.4. 6-Stage System II (a 2-Step System). The next system we call 6-stage system II. It can be seen as a development of 6-stage system I, because it combines Hand Tai Yin (LU) with Foot Yang Ming (ST) and Hand Yang Ming (LI) with Foot Tai Yin (SP), Hand Shao Yin (HT) with Foot Tai Yang (BL) and Hand Tai Yang (SI) with Foot Shao Yin (KI), Hand Jue Yin (PC) with Foot Shao Yang (GB), and Hand Shao Yang (TE) with Foot Jue Yin (LR). This system has a highly pragmatic relevance because it combines stages I and VI, stages II and IV, and stages III and V. It is widely used in modern schools [24, 37, 38] especially when diseases do not follow in the order of stages, but if there is stage hopping [35]. It uses two-step combinations, so we always get yin-yang or yang-yin combinations.

Table 1: Intrinsic rules of the historical systems.

Every meridian pairs with only one other
Rotation symmetry of 30°, 60°, or 120°
6 pairs of meridians
Maximum of 2 alternating steps
6 yin/yang *or* 3 yin/yin and 3 yang/yang combinations

Historical point combinations fitting into this system are LU-7 (Lieque) and ST-36 (Zusanli) for acute dyspnoea [27] or LR-13 (Zhangmen) and TE-8 (Zhigou) plus TE-5 (Waiguan) for pain in the lateral costal region [30].

3.1.5. 6-Stage System III (2-Step–6-Step Alternating System). In the Ming-Dynasty Li Yan first described his "5-Zang extra relationship theory" [25]. Foot Tai Yang (BL) and Hand Tai Yin (LU), Hand Tai Yang (SI) and Foot Tai Yin (SP), Hand Jue Yin (PC) and Foot Yang Ming (ST), Foot Jue Yin (LR) and Hand Yang Ming (LI), Foot Shao Yang (GB) and Hand Shao Yin (HT), and Hand Shao Yang (TE) and Foot Shao Yin (KI) are combined. This system combines the yang meridians of stage I with the yin meridians of stage IV, the yin meridians of stage V with the yang meridians of stage II, and the yang meridians of stage III with the yin meridians of stage VI.

Historical examples using this system for point combinations are TE-6 (Zhigou) and KI-6 for constipation [26] or LI-4 (Hegu) and LR-3 (Taichong) for nasal polyps, nasal congestion, and discharge [27].

3.1.6. Opposite Clock (6-Step System). Cross needling in general was first described in Chapter 63 of Suwen. Applying this to the meridian circle is very popular and is called the opposite clock needling in one school [36] and is a basis for special techniques in Japanese acupuncture [24]. This system combines LU and BL, LI and KI, ST and PC, SP and TE, HT and GB, and SI and LR. Anatomically very distant areas of the human body are connected in a Yin-Yang combination.

Historical point combinations fitting into the 6-step system are LU-6 (Kongzui) and BL-31 (Shangliao) for febrile disease with an absence of sweating [23] or ST-8 (Touwei) and PC-7 (Dailing) for a splitting headache with severe eye pain [32].

3.1.7. Common Features of the Historical Systems. All historically described systems have in common that they build a symmetrical combination in the 12-meridian circle with a rotation symmetry of 30°, 60°, or 120°. Every meridian pair with only one other and no meridian is left over, so there are always 6 pairs of meridians. A maximum of two alternating steps are used, leading to yin-yang/yang-yin or to yin-yin/yang-yang combinations. They can be described as intrinsic rules of the historical systems, summarized in Table 1. A graphical plotting of all historical systems is shown in Figure 3.

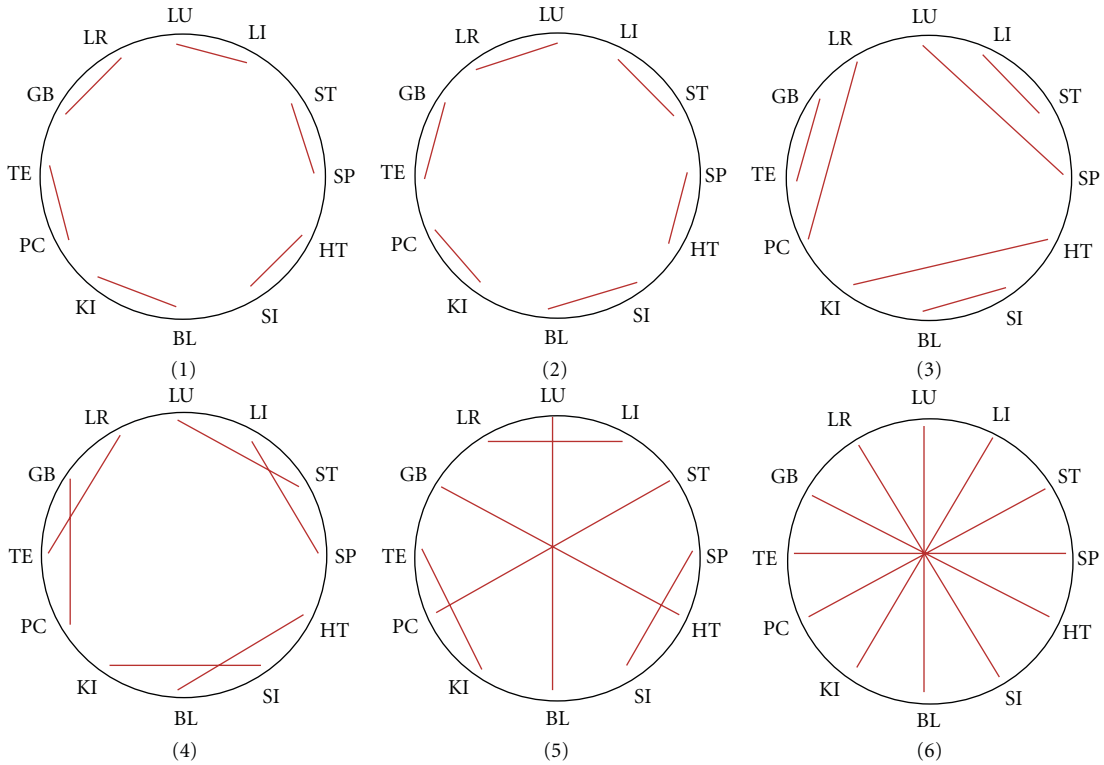


Figure 3: Graphical plotting of the historical systems: (1) interior/exterior; (2) Neighbouring channels; (3) 6-stage I; (4) 6-stage II; (5) 6-stage III; (6) opposite clock.

3.2. Graph Traversal Search for Further Systems. The 12 meridians in the circle were labelled 1, 2, 3,..., 12. They were divided into six groups. Every meridian pair with only one other and no meridian is left over. The possible combinatorial number is

$$\frac{C_{12}^2 \cdot C_{10}^2 \cdot C_8^2 \cdot C_6^2 \cdot C_4^2}{(6!)} = \frac{7484400}{720} = 10395, \qquad (1)$$

where C_n^k is the number of combinations of n things taken k at a time: $n!/((n-k)!\,k!)k!$ means the product of all the integers from 1 to k, that is, factorial.

Then, the steps of each group are calculated.

There are 6 possible steps in the Chinese clock: 1, 2, 3, 4, 5, and 6. (e.g., step 1 is a connection of two neighbouring meridians, step 3 skips one, combining the first meridian with the third one, etc.)

The number of possible combinations was counted. If the classification is rotationally symmetrical, it must satisfy the following requirements: the number of the steps is not 1, 3 or 5. The possible number is 1355. (We listed all possibilities by plotting the combinations using MATLAB.)

At this stage we removed the third step combinations. In fact, we removed all the odd step numbers, that is, steps 3 and 3. But this situation satisfies the rotationally symmetrical rule, so in the next stage we will add it to the final results.

If the number of the step is 3 and 3, for example, all the steps are 1, 3, 1, 3, 1, 3, the number of step 1 is 3 and the number of step 3 is also 3. The possible combinatorial number satisfying the above situation is

$$C_6^2 \cdot C_6^3 = 15 * 20 = 300. \qquad (2)$$

All combinations were plotted by MATLAB, and the symmetry was manually validated. 350 combinations showed a symmetrical pattern, of those one had 30°, 6 had 60°, 12 had 90°, 20 had 120°, and 311 had 180° rotation symmetry.

19 of the symmetrical patterns followed the intrinsic rules of the historical systems. One showed a rotation angle of 30°, 6 of 60°, none of 90°, 12 of 120°, and none of 180°. The steps in the Chinese clock were 1 step in 2 patterns, 1 step-3 steps alternating in 4 patterns, 2 steps in 4 patterns, 2 steps-6 steps alternating in 4 patterns, 3 steps in 2 patterns, 5 steps-7 steps alternating in 2 patterns, and 6 steps in one pattern (Table 2).

3.3. Additional Systems Found by Mathematical Calculation

3.3.1. Step Systems. There was no additional system found beside the historical systems, interior/exterior and neighbouring channel systems.

3.3.2. 1-Step-3-Step Alternating Systems. There are three more 1-step–3-step alternating systems. The first combines LU and LI, LR and ST, HT and SI, SP and BL, PC and TE, and KI and GB. The second combines LR and LU, GB and LI, SP and HT, ST and SI, KI and PC, and BL and TE. The

Table 2: Combinations that follow the intrinsic rules of the historical systems, listed according to steps in the Chinese clock.

1 step		2
1 step	3 steps alternating	4
2 steps		4
2 steps	6 steps alternating	4
3 steps		2
5 steps	7 steps alternating	2
6 steps		1
Total number		19

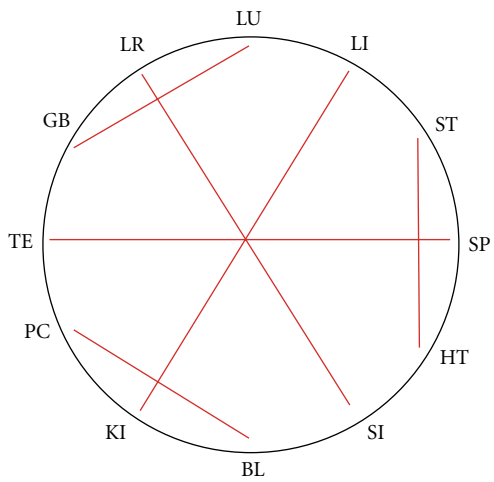


Figure 4: 6 stage system IV.

third combines GB and LR, TE and LU, ST and SP, LI and HT, BL and KI, and SI and PC. They lead to additional point combinations not covered by those historically described.

3.3.3. 2-Step Systems. There are three more 2-step systems. The first combines LU and GB, LI and LR, ST and HT, SP and SI, BL and PC, and KI and TE. The second combines LU and GB, LI and SP, ST and HT, SI and Kidney, BL and PC, and TE and LR. The third combines LU and ST, LI and LR, SP and SI, HT and BL, KI and TE, and PC and GB. They do not offer additional combinations of meridians.

3.3.4. 2-Step–6-Step Alternating Systems. There are three more 2-step–6-step alternating systems. The first is combining Hand Tai Yin (LU) and Foot Shao Yang (GB), Foot Tai Yang (BL) and Hand Jue Yin (PC), Foot Tai Yin (SP) and Hand Shao Yang (TE), Hand Yang Ming (LI) and Foot Shao Yin (KI), Hand Tai Yang (SI) and Foot Jue Yin (LR), and Foot Yang Ming (ST) and Hand Shao Yin (HT). It combines stages I and V, II and VI, and III and IV. We call it 6-stage system IV. Historical combinations fitting into the 6-stage system IV are ST-36 (Zusanli) and HT-8 (Shaofu) for difficult urination or retention of urine [32] or LU-7 (Lieque) and GB-12 (Wangu) for deviations concerning the mouth and face [31] (Figure 4).

The second 2-step–6-step alternating system combines LU and ST, LI and KI, SP and TE, HT and BL, SI and LR, and PC and GB. The third combines LU and BL, LI and SP, ST and PC, HT and GB, SI and KI as well as TE and LR. The second and the third 2-step–6-step alternating systems do not offer additional combinations.

3.3.5. 3-Step Systems. There are two 3-step systems that follow the systematics of the intrinsic historical systems. They do not offer new combinations of meridians, because they are already covered in the 1-step–3 step alternating systems as shown above.

3.3.6. 5-Step–7-Step Systems. Step 5 is a combinatorial possibility but has no large tradition in Chinese medicine except for concepts connected to the extraordinary vessels. The extraordinary vessels were not described in the Suwen or Lingshu as a system, but references can be found to the ren mai, chong mai, and qiao mai [9]. In the Nan jing the first written description of the extraordinary vessels in an organized summary can be found [39]. Treatment of the extraordinary vessels became more popular in the Jin-Yuan dynasty [22] and in the Ming dynasty [40]. The selection of four pairs of points, namely SP-4 and PC-6, LU-7 and KI-6, SI-3 and BL-62, and GB-41 and TE-5 refers to the *Zhen Jing Zhi Nan* written about 1295, which does not clearly mention the extraordinary vessels in the context of these four pairs of points [22].

In fact the first mention of therapy for the eight extraordinary vessels using the above described eight so called master points appeared in 1439 in the Complete Collection of Acupuncture and Moxibustion (Zhen Jiu Da Quan) by Xu Feng [40].

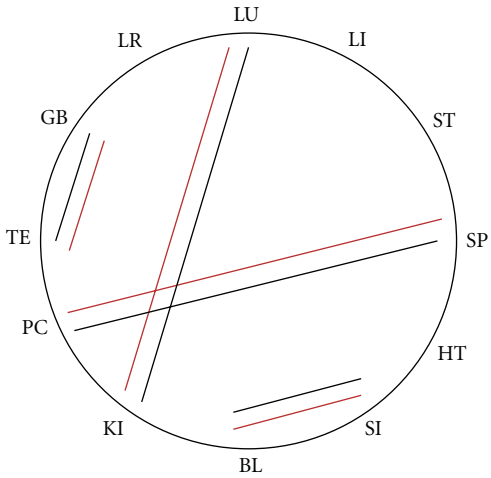
The eight master points are clearly described and connected to the extraordinary vessels and ordered in pairs: Chong mai (SP-4) and Yin Wei Mai (PC-6), Ren Mai (LU-7) and Yi Qiao Mai (KI-6), Du Mai (SI-3) and Yang Xiao mai (BL-62), and Dai Mai (GB-41) and Yang Wei Mai (TE-5) [41].

One often used technique to treat a disease connected to one of the extraordinary vessels is to combine the master points of the paired extraordinary vessels. Similar instructions are given in the Zhen Jiu Ju Ying [42], and, in Zhe Jiu Da Cheng [30], the master point of the treated extraordinary vessel is needled first, then the master point of the paired extraordinary vessel, called the coupled point, is needled second.

All pairs of Yang extraordinary vessels are opened by a 1-step combination, already familiar from the 6-stage system I (see above), the Yin extraordinary vessels are opened by a 5-step (5-step–7-step, resp., if you consider a one directional flow in the Chinese clock) combination (Figure 5).

3.3.7. 6-Step System. There is no additional system beside the opposite clock system.

3.4. Summary of the Results. The possibilities for finding a balanced treatment strategy can be described by the steps



Extraordinary vessel	Master point	Coupled point
Du Mai	SI-3	BL-62
Yang Qiao Mai	BL-62	SI-3
Yang Wei Mai	TE-5	GB-41
Dai Mai	GB-41	TE-5
Ren Mai	LU-7	KI-6
Yin Qiao Mai	KI-6	LU-7
Yin Wei Mai	PC-6	SP-4
Chong mai	SP-4	PC-6

Figure 5: Activation of the extraordinary vessels (Qi jing ba mai) by combination of the master point and the coupled point.

Table 3: Possible steps in the Chinese clock for balancing a meridian.

1, 2, and 3 are possible
4 is not possible
5 is possible but has no tradition in TCM, except in the theory of the extraordinary vessels
6 is possible

that have to be taken in the Chinese clock to combine acupuncture points. Merging the data of all systems, the steps in the Chinese clock showing a possibility for balancing are the following. Step 1, 2, and 3 are possible as well as Step 6. Step 4 is not a combinatorial possibility. Step 5 is a combinatorial possibility but has no tradition in TCM, except in the theory of the extraordinary vessel. A summary is given in Table 3. In addition, the affected meridian itself can be treated. In practice, when the affected (painful) area has been identified, (sensitive) ashi points on one or several of the chosen associated meridians can be found in correlated regions to the affected area. Pain can be reduced instantly by needling these ashi points [36].

Plotting the merged data of all 19 systems on the Chinese Clock, we found a tool for quick memorization. An example for the possible balancing meridians for the meridians of the lung is shown in Figure 6.

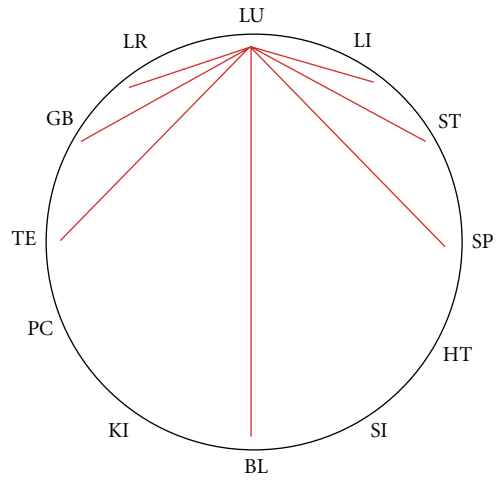


Figure 6: Merging of the combinatorial possibilities. (This can be done with every meridian in a similar way.)

4. Discussion

Our work is based on an analysis of the historically described balancing systems. Seeing the theory of Chinese medicine as an inherently logical system, we used a mathematical approach to calculate the theoretical options based on the historical systems. These findings suggest that there are many more treatment options than normally expected.

The historically established systems for combining meridians cover many, but not all, of our calculated combinations of meridians. Our findings imply that every meridian can be balanced by possibly at least seven other meridians (and itself). Possible steps of balancing one meridian with another are the steps 1, 2, 3, and 6 in the Chinese clock. Step 4 does not offer a symmetric combinatorial option. Additionally step 5 is a mathematical possibility but has historically only a tradition in the theory of the extraordinary vessels (qi jing ba mai). For the opening of the yin extraordinary vessels a combination of step 5 and 7 is postulated [40]. The historical physicians might have thought that combining two odd steps in the Chinese clock and using a combination of anatomically very distant points is something exceptional. This might be one explanation why the historical physicians saw the necessity of the development of an extra theory [22, 39, 40].

Some schools of Chinese medicine devote more attention to describing systems developed from the systematics of the 6 stages [21], some schools combine mainly the opposite meridians of the 12-meridian circle [24], and some schools concentrate on combining all known systems [36–38]. These historically described systems must either be results of daily

experience or have the advantage of fitting other theoretical approaches better. All these systems have been proved effective in daily practice by acupuncturists all over the world and are used with success.

Nevertheless, all these approaches are based on theoretical considerations from historical writings, supported by empirical knowledge obtained over the centuries as well as personal experience of the members of the schools. There are no controlled studies with hard data on the effect of point or meridian combinations so far.

The approach of this work, a mathematical search for further combinatorial possibilities, is as well a theoretical approach in the first line. It is based on the historical systems and a distinct order of the meridians in the Chinese clock.

As every meridian has the same power, a change of the order of the meridians will not change the number of mathematical possibilities for combination. While the order in the Chinese clock is not only accidental, but most likely selected for anatomical reasons (Figure 1), a different order in the Chinese clock presumably will not lead to successful treatment strategies.

Even though the historical systems have been used for centuries, from the theoretical point of view the newly discovered possibilities we found must have a similar power, because there are no superior meridians in the Chinese clock.

One of the newly discovered systems has to be emphasised. Like the above described three historical combinations, the 6-stage systems I, II, and III, it offers an extra opportunity to connect meridians of the 6 stages. It combines the meridians of stage I and V, II and VI, and III and IV in a combination of Yin-Yang and Yang-Yin, respectively. It might increase the view of how stages can follow and influence one another. We call it 6-stage system IV.

Broad research failed to uncover any mention in the literature on Chinese medicine so far. It might be a missing piece of the puzzle and could complete the view on the theory of the 6 stages.

While the authors of this paper have started to transfer their consideration into treatment strategies with remarkable success, their reflections still have only a theoretical basis and have to be approved by clinical studies.

Most controlled acupuncture trials used minimal, superficial, sham, or "placebo" acupuncture as control. Often points at locations distant from acupuncture points described in the textbook were used [43]. Only step 4 in the Chinese clock does not open any combinatorial possibility, so these meridians might function as theoretical areas for placebo treatment. But these meridians are anatomically extremely close to the affected or the unbalanced meridian. For example, if you look at the LU meridian, the meridian of the HT and PC is step 4 in the Chinese clock (the LU, PC, and HT meridians are direct neighbours on the arm). And if the ST meridian is chosen, GB and BL are step 4 in the Chinese clock (ST, GB, and BL are direct neighbours on the leg). This leads to the problem, considering that the peripheral nerves are involved in the effects of acupuncture, that anatomical variants of the peripheral nerves have an influence on the location of acupuncture points [44]. Tao showed that meridians are not clearly described in historical texts and that the localisation of acupuncture points in historical writings differs in some points extremely from descriptions in modern acupuncture textbooks [45].

In some patients painful affections can be found not only on a defined meridian area but also in regions between meridians. In these cases, pain-releasing ashi points are often needled between associated meridians, which was already described and discussed in the Systematic Classic of Acupuncture and Moxibustion [23] and is consistently used in some modern schools [36, 46, 47]. Additionally we can learn from acupuncture microsystems and related techniques, for example, Chinese and French ear acupuncture, Korean hand acupuncture, hand and foot reflexology, that other somatotopies exist which partly overlap with the classical meridian system. In consequence, defining anatomical regions without any influence on other regions seems extremely difficult. The use of needle insertions at locations distant from acupuncture points defined in acupuncture textbooks as controls in clinical trials is not suitable as an inert placebo [48].

Increasing the number of treatment options does not mean that all treatment strategies have similar power, but it shows that manipulation in multiple areas of the body surface can be potentially effective in acupuncture treatment. Generally a case can be solved successfully by different treatment strategies, but it is good to know which treatment is not very likely to solve a problem or could do some harm to the patient. Knowing different treatment possibilities is especially helpful in pain management, where usually a fast effect is needed for quick pain relief. There might be an area of repletion (fullness) on the body surface which helps to choose the right meridian. But the search should not be limited to the painful meridian and its interior/exterior partner as practised by acupuncturists still at the beginning of their careers. The search for an effective treatment should be more extended to find all treatment possibilities. Further research is necessary to distinguish which system might be most useful for different clinical conditions.

This systematic description of the combinatorial possibilities offers a powerful tool for daily practice. Knowledge of all systems and combination systems helps preserve the balance of the treatment, especially if more than two needles are used in a treatment.

5. Conclusion

Following these considerations only two meridians might be excluded as potentially balancing meridians on the basis of TCM theory. For application in treatment further appraisal of the results is necessary. For acupuncture trials most meridians and acupuncture points must be considered as potentially effective, so it seems almost impossible to define noneffective acupuncture points as controls.

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Research Article

Role of AC-cAMP-PKA Cascade in Antidepressant Action of Electroacupuncture Treatment in Rats

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Adenylyl cyclase (AC)-cyclic adenosine monophosphate (cAMP)-cAMP-dependent protein kinase A (PKA) cascade is considered to be associated with the pathogenesis and treatment of depression. The present study was conducted to explore the role of the cAMP cascade in antidepressant action of electroacupuncture (EA) treatment for chronic mild stress (CMS)-induced depression model rats. The results showed that EA improved significantly behavior symptoms in depression and dysfunction of AC-cAMP-PKA signal transduction pathway induced by CMS, which was as effective as fluoxetine. Moreover, the antidepressant effects of EA rather than Fluoxetine were completely abolished by H89, a specific PKA inhibitor. Consequently, EA has a significant antidepressant treatment in CMS-induced depression model rats, and AC-cAMP-PKA signal transduction pathway is crucial for it.

1. Introduction

Depression is a common and disabling illness affecting a rising percentage of the world's population. Among the most prevalent forms of mental illness, depression is a severe, recurrent, and life-threatening illness, with about 15% of depressed patients committing suicide [1]. Although significant progress has been made in pharmacological treatment for depression over the past several decades, currently available chemical antidepressants, which exhibit 60–70% effective rate only [2], have severe side effects [3] and call for alternative treatment [4]. Acupuncture has been applied in the clinic to treat depression for a long time, and Siguan acupoints, including LI4 (*Hegu*) and LR3 (*Taichong*) acupoints, are classic acupoints in the antidepressant treatment of acupuncture. Recently, evidence from clinical and experimental studies have indicated that acupuncture may alleviate the symptoms of depression [5–7]. However, the underlying mechanism remains unknown.

Cyclic adenosine monophosphate (cAMP) cascade, as the second messenger cascade, is considered to be associated with the pathophysiology and treatment of depression, which is a common target for several types of antidepressants. Dysfunction of the AC-cAMP-PKA cascade, including decreased G protein and cAMP level, reduced AC and PKA activity and altered PKA-mediated phosphorylation, has been observed in depressed patients [8–10]. Simultaneously, several lines of evidence clearly indicate that chronic antidepressant treatment upregulate the cAMP postreceptor signal transduction pathway at several levels. Antidepressant treatment enhanced AC/G protein coupling, expression of AC and GTP, forskolin-stimulated cAMP accumulation and levels, and activity of PKA [11–14].

In the present study, we investigate the antidepressant effect of electroacupuncture (EA) treatment in chronic mild stress (CMS)-induced depression model rats and the role of AC-cAMP-PKA postreceptor signal transduction pathway.

2. Methods

2.1. Animals. Male adult Sprague-Dawley (Experimental Animal Center, Guangdong provincial hospital of TCM, Guangzhou, China) rats, weighing 200–250 g, were used in

the experiment. Upon arrival, animals were given 1 week to adjust to the new environment (20 ± 3°C, 45–60% 60–70% humidity, white noise (40 ± 10) db and 12/12 h light/dark cycle with the light from 6:00 AM to 6:00 PM), with food and water available freely prior to experimental procedures. All experimental procedures were performed during the light cycle. For all experiments, mice were randomly assigned to experimental and control groups and tested in a counterbalanced order. The animal care procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize their suffering.

2.2. Experimental Procedure. Before stress, all rats were screened out by the openfield test in which the total scores between 70 and 120 for all experiments and then were randomly divided into the following four groups ($n = 8$ per group): (1) control group (normal control with no stress); (2) CMS group (only received stress); (3) CMS plus EA group (received stress and EA); (4) fluoxetine group (received stress and Fluoxetine). To identify the role of cAMP cascade in the antidepression effect of EA treatment, a further 24 rats (8 in each group) were divided into three groups: (5) normal saline (NS) plus EA group; (6) H89 plus EA group; (7) H89 plus Fluoxetine group. Rats in the control group did not receive any stimulation. In the CMS group, rats were exposed to chronic mild unpredictable mild stress for 6 weeks. In the EA group, rats receive CMS treatment and EA stimulation at Siguan points (bilateral *Hegu* (LI4) and *Taichong* (LR3)) once every other day for 3 weeks after CMS exposure. In the Fluoxetine group, rats receive the same treatment as the CMS group and Fluoxetine treatment everyday for 3 weeks after CMS. The groups plus H89 were conditioned with the same as the EA or Fluoxetine group except for the intracerebroventricular injection of H89 between CMS and EA/Fluoxetine delivery. The design of the experiment was shown in Figure 1.

2.3. CMS Procedure. The CMS protocol, as described by Willner et al. [15], consists of the sequential application of a variety of mild stresses: 24-h water deprivation, 24 h food deprivation, 30 min cage rotation, 5 min forced swim, reversal of the light/dark cycle, 5 min hot environment (40°C), a cage tilt of 45°C, white noise (100 dB), and wet bedding (100 mL of water per individual cage). The stressors were done in a random order to maximize the unpredictable nature of the stressors. The CMS procedure was carried out in stressed animals once per day for 3 weeks.

2.4. Open-Field Test. The open-field test was performed as previously described [15] and was carried out before stress (day 0), 3 weeks after stress (day 22), and 4, 5, and 6 weeks after stress. In the openfield test, rats were placed at the center rectangular arena side walls, which was a four-sided 100 × 100 × 40 cm^3 wooden box with the walls painted black. The floor of the box was divided into 16 squares. The room was in a dimly lit with a video camera above the center of the floor. Each animal was placed in the center of the box and

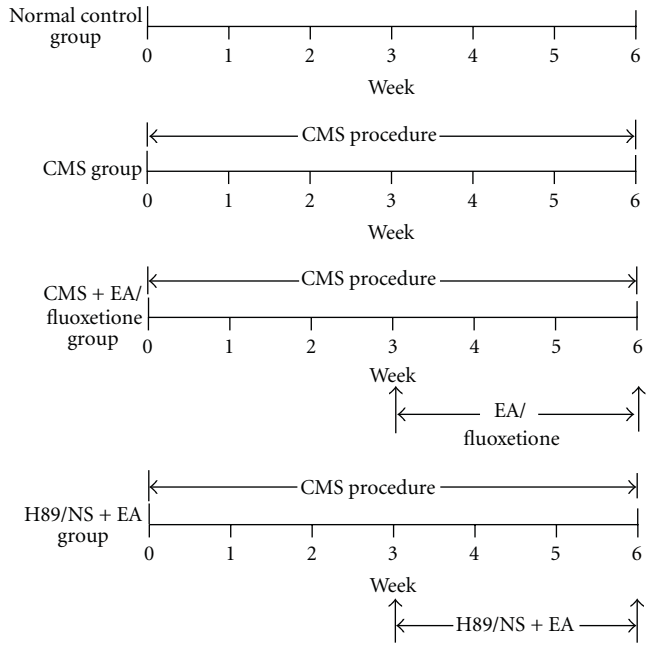

Figure 1: Experimental procedure.

was allowed to explore freely for 3 min. During the test time the number of crossings (defined as at least three paws in a quadrant) and rearings (defined as the animal standing upright on its hind legs) was measured. After the test of each animal, the test box was cleaned with a 10% ethanol solution and water to remove any olfactory cues.

2.5. Sucrose Intake Test. The sucrose intake test was performed on days 0, 22, and 43. Prior to the start of the test, animals were trained to consume 1% sucrose solution. They were habituated for 48 hours to two bottles: one with 1% sucrose (Sigma), the other with tap water, followed by a period of 24 h without any food and water available, and a 1-h exposure to the two identical bottles again for testing fluid consumption. In order to have concordance measure for all groups, each rat in control group was randomly selected out and kept housed individually at the beginning of this test. Two-bottle tests for each cage were adopted throughout the procedure. Sucrose solution consumption was recorded by calculating volume of test solution.

2.6. Measurement of Body Weight. Body weight in all rats was measured every week throughout the experiment.

2.7. EA and Drug Treatment. For EA stimulation, the rat was slightly immobilized in a small cylindrical container so that the movement of the rat's head restrained while the body could move freely. EA at Siguan acupoints, bilateral Hegu (LI4), and Taichong (LR3) were performed for 30 min once every other day. Location of the acupoints was determined by comparative anatomy. LI4 is located on the dorsum of the forelimb, between the 1st and 2nd metacarpal bones, approximately in the middle of the 2nd metacarpal bone

on the radial side and LR3 is located on the dorsum of the claw of the hindlimb, proximal to the first metatarsal space. Stainless-steel needles of 0.18 mm diameter and 15 mm length were inserted into the acupoints. The electrical stimulation was from a medical EA apparatus (model G6805-2, Shanghai, China). The stimulation parameters were of frequency 2 and 20 Hz, alternatively, strong enough to only elicit slight twitches of the limbs. In order to exclude the possibility of stress induced by animal fixation, all groups were slightly immobilized in the same container for 30 min. For the Fluoxetine treatment, animals were given Fluoxetine (Eli Lilly, USA) at a dose of 1.8 mg/kg i.g. (dissolved in sterile 0.9% physiological saline) daily prior to the immobilized period.

2.8. Intracerebroventricular Injection. Rats were anesthetized with 10% hloralhydrate (350 mg/kg, i.p.) and placed in a rat brain stereotaxic apparatus (Narishige, Japan). After a midline scalp incision, the head position was adjusted to place bregma and lambda in the same horizontal plane. A small hole (0.8 mm posterior to bregma, 1.5 mm lateral to midline) was drilled on the skull. A stainless-steel guide cannula (22 gauge, ID 0.58 mm, and OD 0.90 mm) was placed unilaterally in the lateral cerebral ventricle at a depth of 4.0 mm and fixed with dental cement onto the skull, serving as a guide for the accurate insertion of a Internals cannula (extending 0.5 mm below the tip of the guide cannula). To prevent clogging or infection of the brain tissue, a dummy cannula (OD 0.56 mm) was always placed in the guide cannula as a cap for covering the guide cannula except the duration of injection. The rats were given about 3 days to recover completely from the surgery. The animals with implanted cannula were placed in transparent plastic cages and were freely moving throughout the perfusion. The Osmotic mini pump filled with either H89 or normal saline (NS) was connected through a PE tube (150 mm length) to the internal cannula. H89 (dissolved in sterile saline, 10 μM, 5 μL) or NS (0.9%, 5 μL) was microinjected into the lateral cerebral ventricle through the cannula at a flow rate of 1uL/min. EA or Fluoxetine was applied at least 30 min after the perfusion procedures.

2.9. Assay of AC Activity. The dissected hippocampus was homogenized (1 : 40 (w/v)) in ice-cold buffer (320 mM sucrose, 1.6 mM EGTA, and 2 mM Tris pH 7.4) using 10 strokes with a Teflon tissue grinder and centrifuged (1000 rpm, 10 min, 4°C), and the supernatant was centrifuged again (20, 000 rpm, 20 min, 4°C). The pellet was resuspended in ice-cold assay buffer. The AC assay was performed after 10 min preincubation at 30°C in a reaction mixture (final volume, 500 μL) containing 80 mM Tris pH 7.4, 2 mM EGTA, 3 mM MgCl2, 0.5 mM IBMX, and 5 μM forskolin. The reaction was initiated by addition of ATP to a final concentration of 200 μM and then incubated (10 min at 30°C) and stopped by boiling for 3 min. The samples were centrifuged (3000 rpm, 10 min), and cAMP accumulation was quantified in 50 μL supernatant aliquots by using the [^{3}H] cAMP assay kit (China Institute of Atomic Energy, China).

2.10. Assay of cAMP Level. Hippocampus was homogenized in 1 : 40 (w/v) in ice-cold buffer and centrifuged (1000 rpm, 10 min, and 4°C) as described above. The supernatants were incubated by boiling for 5 min and then centrifuged (15,000 rpm, 10 min). cAMP accumulation was quantified in 50 uL supernatant aliquots by using a [^{3}H] cAMP assay kit (China institute of atomic energy, China).

2.11. Assay of PKA Activity. PKA activity was assayed using radioactive PKA assay kit (Promega, USA) following the manufacturer's instructions. Protein/sample (50 mg) was used for kinase activity.

2.12. Statistical Analysis. Data were presented as mean ± standard error of the mean (SEM). Differences between groups were considered to be statistically significant for $P < 0.05$. The significance of differences was determined using the one-way ANOVA followed by least significant difference (LSD) as post hoc multiple comparisons test. When two factors were assessed, the significance of differences was determined using two-way ANOVAs.

3. Results

3.1. Openfield Test. All stress groups began to show behavior deficit in the 3rd week, indicating obvious difference with the control group. However, there was no remarkable difference among all stress groups. In the sixth week, EA (46.25 ± 7.03, $P < 0.01$) and Fluoxetine (51.62 ± 2.41, $P < 0.01$) as well as EA + NS (46.5 ± 3.86, $P < 0.01$) treatment leaded to increase in the number of crossing, indicating significant difference with CMS group (25.25 ± 4.42). Enhanced effects induced by EA or Fluoxetine were reversed by H89 pretreatment, respectively (18.0 ± 3.32 versus 46.25 ± 7.03, $P < 0.01$; 34.0 ± 4.03 versus 51.62 ± 2.41, $P < 0.01$, resp.) (Figure 2). Changes in the number of rearing were similar to the crossing (Figure 3).

3.2. Sucrose Intake. Sucrose intake in all stress groups decreased significantly in the 3rd week and was much less than the control group ($P < 0.05$ or $P < 0.01$). However, there was no remarkable difference among all stress groups. EA (18.09 ± 1.90) or Fluoxetine (18.51 ± 1.30) treatment leaded to increase in sucrose intake in the sixth week, however, not indicating significant difference with CMS group (14.93 ± 1.83). H89 pretreatment inhibited obviously increase in sucrose intake induced by EA (12.99 ± 1.45 versus 18.09 ± 1.90, $P < 0.05$), but had no effect on the Fluoxetine (17.25 ± 1.61 versus 18.51 ± 1.30, $P > 0.05$) (Figure 4).

3.3. Body Weight. Body weight in all groups increased during the whole experiment. EA (292.75 ± 10.23 versus 318.38 ± 7.38, $P < 0.05$), and Fluoxetine (295.88 ± 4.74 versus 318.38 ± 7.38, $P < 0.05$) group had less body weight than CMS group at the end of the last week. H89 + EA or H89 + Fluoxetine group had similar body weight gain to EA or Fluoxetine group (Figure 5).

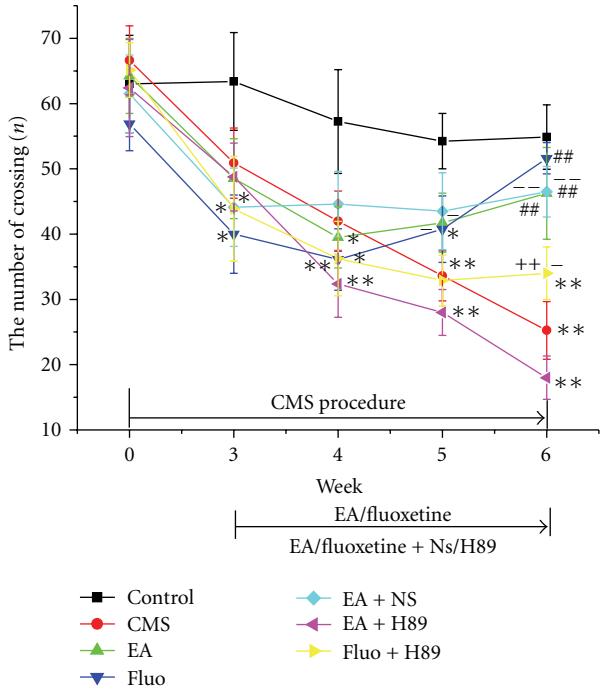

Figure 2: Effect of EA or Fluoxetine on the number of crossing in openfield test. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group, respectively; $^{\#\#}P < 0.01$ versus CMS group; $^{++}P < 0.01$ versus Fluoxetine group; $^{-}P < 0.05$ and $^{--}P < 0.01$ versus EA + H89 group, respectively.

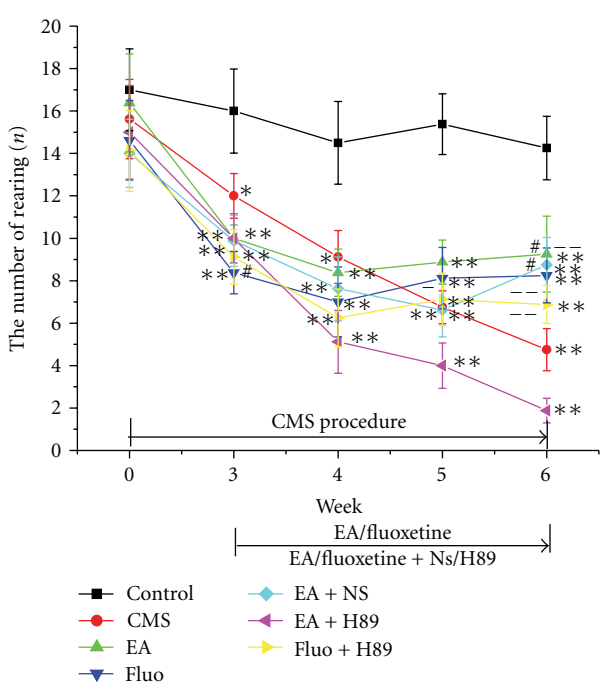

Figure 3: Effect of EA or Fluoxetine on the number of rearing in openfield test. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group, respectively, $^{\#}P < 0.05$ versus CMS group, $^{-}P < 0.05$ and $^{--}P < 0.01$ versus EA + H89 group, respectively.

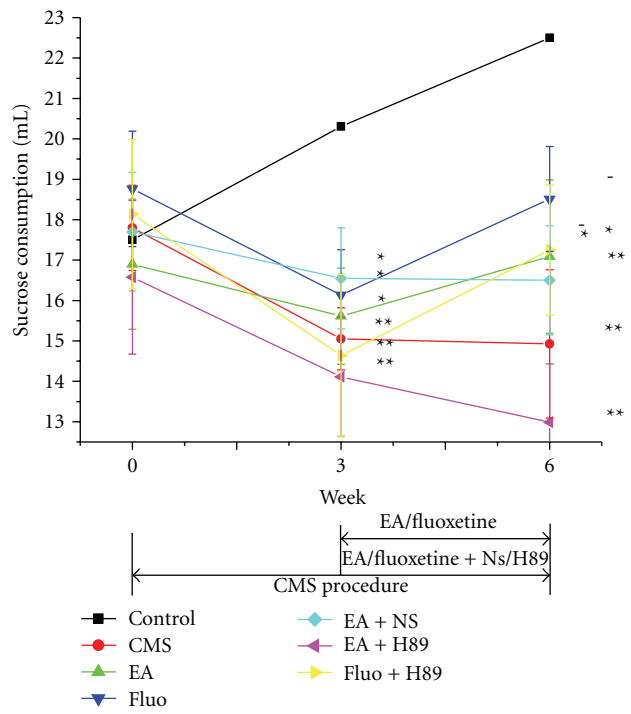

Figure 4: Effect of EA or Fluoxetine on sucrose intake. $^{*}P < 0.05$, and $^{**}P < 0.01$ versus control group, respectively; $^{-}P < 0.05$ versus EA + H89 group.

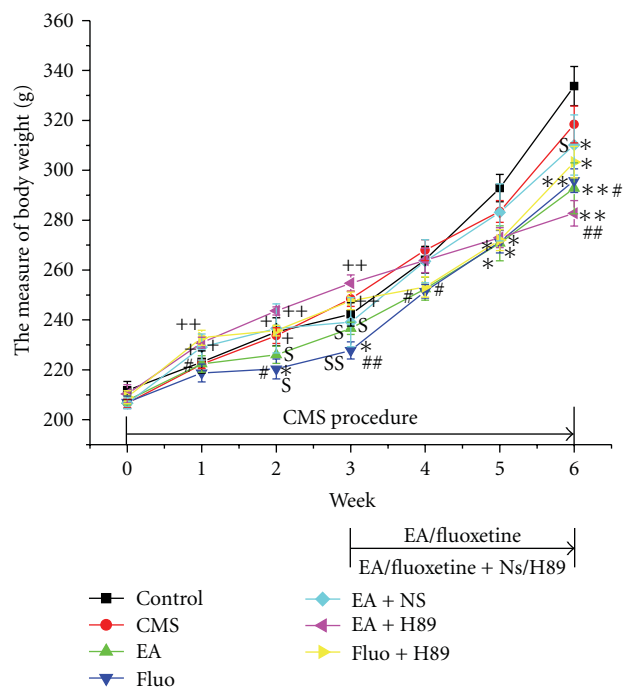

Figure 5: Effect of EA or Fluoxetine on body weight. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group, respectively; $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus CMS group, respectively; $^{+}P < 0.05$ and $^{++}P < 0.01$ versus Fluoxetine group, respectively.

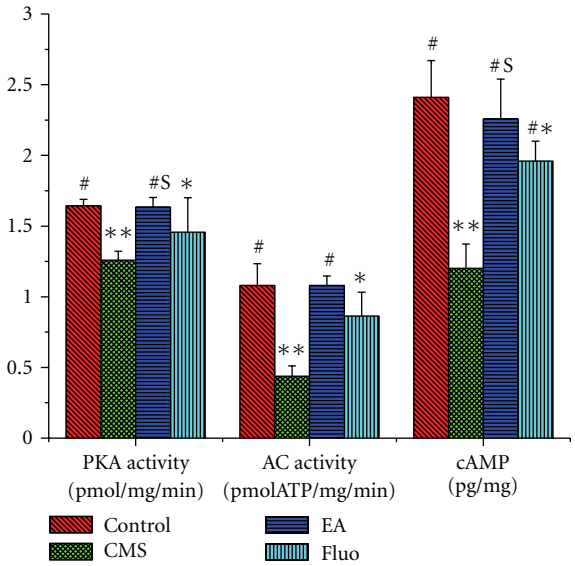

Figure 6: Effect of EA or Fluoxetine on AC transformation ratio, cAMP level, and PKA activity. $^{*}P < 0.05$ versus control group; $^{\#\#}P < 0.01$ versus CMS group; $^{S}P < 0.05$ versus Fluoxetine group.

3.4. AC-cAMP-PKA Cascade. CMS produced a significant decrease in the ratio of AC transformation compared with the control group (0.44 ± 0.07 versus 1.08 ± 0.15, $P < 0.01$), which was reversed by EA or Fluoxetine treatment (1.08 ± 0.07, 0.86 ± 0.17, $P < 0.01$, $P < 0.01$). Changes in cAMP level and PKA activity were similar to AC (Figure 6).

4. Discussion

Because of good predictive validity, face validity and construct validity, the CMS model has become the most extensively used animal model of depression [16]. In this study, the results showed that CMS induced obvious behavior deficits and decrease in sucrose intake, which were reversed by EA or fluoxetine, suggesting that EA may be as effective as antidepressants in treating depression. Simultaneously, we observed that changes in body weight were different from behavior and sucrose intake. EA and Fluoxetine treatment had less body weight than CMS and control group, which was not reversed by a specific PKA inhibitor H89, suggesting that EA or Fluoxetine had no effect on body weight in CMS-induced depression model rats. Body weight has been viewed as a marker in depression study, and CMS causes about 0–10% loss of body weight [16]. However, some researches show that CMS rats have body weight gain and antidepressants, including Fluoxetine and clomipramine, have even less body weight than CMS and normal control group [7, 17]. A recent study also shows that EA treatment or EA combined with clomipramine has similar body weight gain to the CMS and control group [7]. Michelson et al. observed changes in weight during a 1-year trial of Fluoxetine and found that acute therapy during initial 4 weeks with Fluoxetine is associated with modest weight loss and fluoxetine or placebo produced weight gain after 50-week therapy [18]. Therefore, whether body weight measurement is an important marker in depression study needs more sufficient evidence.

In the present study, the results showed that CMS induced downregulation of AC-cAMP-PKA cascade, which was reversed by EA and Fluoxetine treatment. AC-cAMP-PKA cascade, as the second messenger cascade, has been implicated in the pathophysiology of depression and antidepressant action. Receptor activation induced by ligand (hormones, neurotransmitters and growth factors, etc.) contribute to the generation of cAMP via the stimulation of AC by the G-protein subtype Gs, which then leads to the activation of PKA. PKA is responsible for regulatory effects on cellular functions through the phosphorylation of specific target proteins. Amongst the substrates of PKA is the cAMP response element binding protein (CREB), a transcription factor that mediates the actions of cAMP cascade on gene expression and exhibits an increase in its ability to modulate transcriptional activity, in the dephosphorylated form. Modulation of this transcription factor and its target genes results in the cellular adaptations underlying the antidepressant actions [19–21]. Dysfunction of the AC-cAMP-PKA cascade, including decreased G protein and cAMP level, reduced AC and PKA activity and altered PKA-mediated phosphorylation, have been observed in depressed patients [8–10]. Simultaneously, evidence clearly indicates that chronic antidepressant treatment upregulates the cAMP postreceptor signal transduction pathway at several levels. Antidepressant treatment enhanced AC/G protein coupling, which contributes to increased AC activity, and expression of AC and GTP and forskolin-stimulated cAMP accumulation [11–14]. An important evidence about the role of cAMP cascade in antidepressant action comes from rolipram, a phosphodiesterase inhibitor, which inhibits the cAMP metabolism. Rolipram has been reported to have antidepressant effects in clinical trials and is not in clinical use because of its side effects [22]. Moreover, Levels and activity of PKA are enhanced by antidepressant treatment [14]. An increase of PKA levels is observed in the crude nuclear fraction following antidepressant administration, indicating a translocation of PKA into the nucleus [12]. The nuclear translocation of PKA suggests that antidepressant treatments may recruit the cAMP cascade to regulate its target gene expression, such as brain-derived neurotrophic factor (BDNF). These results are in agreement with the present study.

Furthermore, an interesting result was observed in this study. Pretreatment of H89, a specific PKA inhibitor, abolished completely the antidepressant effect of EA, and the depressive-like behavior and sucrose intake as well as body weight in EA + H89 group were all much less than CMS group, suggesting that PKA activity is crucial for antidepressant effect of EA treatment. Furthermore, the dosage of H89 administration in this study may be sufficient to inhibit completely the PKA activity in the hippocampus. However, PKA activity in CMS may partly decrease. So H89 + EA had even more depressed sign than CMS.

At the same time, H89 did not influence the antidepressant action of Fluoxetine, suggesting other signal transduction pathway may be involved in it. Tronson et al.

find that intrahippocampal injection of PKA inhibitor Rp-cAMPS has no remarkable effect on depression-like behavior in mice [23]. Chronic Fluoxetine treatment exerts a more marked effect on phospho-CREB (pCREB) in hippocampus and prefrontal/frontal cortex. However, desipramine and reboxetine, but not Fluoxetine, increase consistently the activity of nuclear PKA, suggesting that PKA does not seem to account for increase of pCREB induced by Fluoxetine [24]. Moreover, various kinds of studies have demonstrated that, in addition to cAMP-PKA cascade, calcium/calmodulin (CaM)-dependent kinases (CaMK) and mitogen-activated protein kinases (MAPK) cascades are involved in the selective serotonin reuptake inhibitors (SSRIs)-induced antidepressant actions [24, 25]. Consequently, although Fluoxetine may upregulate the AC-cAMP-PKA cascade, dysfunction of PKA did not abolish the antidepressant actions.

In conclusion, EA has a significant antidepressant treatment in CMS-induced depression model rats, as effective as Fluoxetine, and AC-cAMP-PKA postreceptor signal transduction pathway may be crucial for it.

Authors' Contribution

Jian-hua Liu and Zhi-feng Wu contributed equally to this paper.

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Research Article

System Identification Algorithm Analysis of Acupuncture Effect on Mean Blood Flux of Contralateral Hegu Acupoint

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Background. Acupoints (belonging to 12 meridians) which have the same names are symmetrically distributed on the body. It has been proved that acupoints have certain biological specificities different from the normal parts of the body. However, there is little evidence that acupoints which have the same name and are located bilaterally and symmetrically have lateralized specificity. Thus, researching the lateralized specificity and the relationship between left-side and right-side acupuncture is of special importance. *Methodology and Principal Findings*. The mean blood flux (MBF) in both Hegu acupoints was measured by Moor full-field laser perfusion imager. With the method of system identification algorithm, the output distribution in different groups was acquired, based on different acupoint stimulation and standard signal input. It is demonstrated that after stimulation of the right Hegu acupoint by needle, the output value of MBF in contralateral Hegu acupoint was strongly amplified, while after acupuncturing the left Hegu acupoint, the output value of MBF in either side Hegu acupoint was amplified moderately. *Conclusions and Significance*. This paper indicates that the Hegu acupoint has lateralized specificity. After stimulating the ipsilateral Hegu acupoint, symmetry breaking will be produced in contrast to contralateral Hegu acupoint stimulation.

1. Introduction

Acupuncture has been widely used to reduce some symptoms or to treat diseases in clinical practice for at least 2000 years [1]. During the past 30 years, a large number of studies focused on the antinociception mechanism of acupuncture, which made it more acceptable to clinical practice and mechanism research. According to the principles of Unschuld [2], acupuncture effects might be related to the appropriate acupoints during the treatment. However, previous studies have indicated that electroacupuncture (EA) is involved in modifying a variety of brain functions and in promoting the release of endogenous opioid peptides, which might be responsible for its analgesic effects in the whole body [3, 4]. It means that acupuncture specificity contributes a little to its effect. What is more, many researchers firmly believe that placebo effect may be the best explanation for acupuncture [5, 6].

On the other hand, specificity of acupuncture points seems to be confirmed by the evidence from neuroimaging studies [7, 8]. It has been shown that acupuncture at different acupoints induced differential hemodynamic neural responses in some brain areas [9]. In contrast, there is little evidence to discern the differences of acupoints which have the same name and are located bilaterally and symmetrically. Goldman et al. reported significant analgesic effects of

ipsilateral but not contralateral acupoints in a mouse model of inflammatory pain [10]. Somers and Clemente [11] obtained opposite results: transcutaneous electric nerve stimulation on the contra- but not ipsilateral side of neuropathic pain resulted in antinociceptive effects in rats. Furthermore, a study [12] indicated that although the anti-nociceptive effect of both contralateral and ipsilateral EA was definitely confirmed, lesions of the rostral anterior cingulated cortex completely abolished the anti-nociceptive effects of contra- but not ipsilateral EA. These studies intensively suggested that there might be a difference between ipsilateral acupuncture and contralateral acupuncture.

In our lab in 1997, Zhang WB measured the transcutaneous CO_2 emission on left and right 24 source acupoints and calculated the correlations between the points. It showed a significantly higher correlative coefficient (0.814) between the left and right same name acupoints than the correlative coefficient between general acupoints (0.379) [13]. Our recent studies have also shown that thermostimulation could result in an increase of blood perfusion not only in the local area [14] but also in the same area on the contralateral side [15]. This phenomenon can be observed both in the upper limb [15] and lower limb [16]. However, the same stimulation has no effect on periumbilicus area [14], which indicated that there might be intrinsic and symmetrical correlation between contra- and ipsilateral parts. This view was supported by Kubo et al. [17]. Their work indicated that after acupuncture or thermostimulation in the ipsilateral side, the blood volume increased gradually in contralateral Achilles tendon, and the amount of increase in blood volume of the nontreated tendon (contralateral side) was significantly correlated to that of the treated tendon (ipsilateral side) during the last phase of recovery period. Recently we reported that when either side Hegu acupoint (LI4) was stimulated, there was an increase in mean blood flux at LI4 of the contralateral side. However, the intrinsic correlation between contra- and ipsilateral LI4 is not clear. The purpose of this study is to investigate the correlation of bilateral LI4 through system identification algorithm analysis.

2. Methods

2.1. Ethics Statement. This study was reviewed and approved by the Institutional Review Board at the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences. Each participant read and signed an informed consent form.

2.2. Subjects. One hundred and twenty (120) healthy volunteers were recruited in this study (as shown in Figure 1; for demographic data see Table 1). All subjects were students from the China Academy of Chinese Medical Sciences and Beijing University of Traditional Chinese Medicine. All subjects had no history of diseases and had not taken any medicine in the past six months before the experiment. Each subject had an adequate understanding of the procedure and purpose of this study.

Table 1: Subjects' demographic data.

Group	n	Gender (female/male)	Age (years, mean ± SD)
AL group	40	34/6	25.34 ± 1.77
AR group	40	25/15	25.85 ± 1.24
Control group	40	34/6	25.33 ± 1.69

AL: acupuncture left Hegu point; AR: acupuncture right Hegu point.

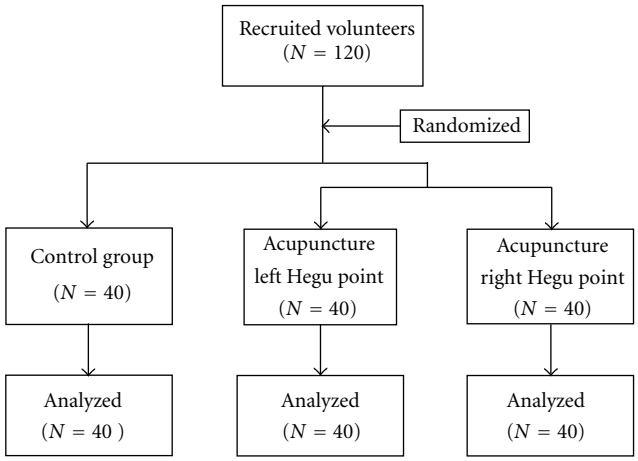

Figure 1: Flow diagram of participants in the study.

2.3. Procedures

2.3.1. Protocol for Mean Blood Flux Measurement. Before arrival to the laboratory, subjects were placed in a temperature-controlled room (24–26°C) as a resting state for 60 minutes. Measurements of skin blood perfusion were carried out using Moor full-field laser perfusion imager (moor FLPI, Moor Instruments Ltd, UK). Before recording, both hands were immobilized with a cylindrical object to ensure positioning. The measurement parameters were as follows: high resolution/250 frames; number of images = 10; exposure time = 8.3 ms; time interval = 10 s. Measurements were carried out every 30 minutes over a total of 180 minutes. During the experiment, the laboratory room was kept in dark light condition, and the protocol for measurement operation was abided strictly. The measurement process is illustrated in Figure 2.

2.3.2. Acupuncture Protocol. For acupuncture, a small acupuncture needle, 0.25 × 25 mm (100112, Zhen Huan), was gently inserted in a depth of 15 mm in the LI4. The position of LI4 was confirmed according to the previous studies [18, 19]. The needle was slowly rotated every 5 min for a total of 30 min during an acupuncture session in order to maintain the soreness and numbness sensation of De-Qi [10]. The acupuncture procedure is illustrated in Figure 2. In the left acupuncture group (Left Acup.), just left LI4 was acupunctured whereas in the right acupuncture group (Right Acup.), right LI4 was stimulated. In the control group (No Acup.), all subjects maintained still, without any intervention.

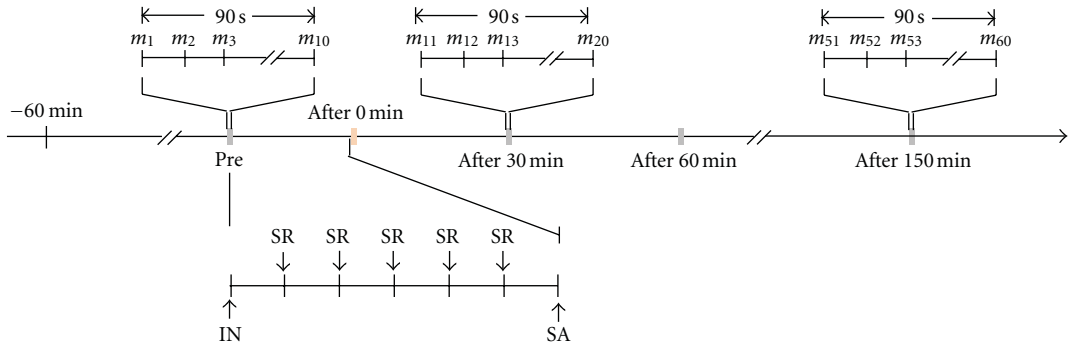

Figure 2: Procedure of acupuncture and mean blood flux measurement. Pre: pre acupuncture; Post, post acupuncture; IN: insert needle; SA: stop acupuncture; SR: slowly rotate the needle every five minutes; m_i $(i = 1, 2, 3, \ldots, 60)$: mean blood flux of Hegu acupoint at a specific time point.

2.3.3. Image Analysis Protocol. The Mean Blood Flux (MBF) of LI4 on both hands (left side abbreviated as L, right side abbreviated as R) was measured (Figure 3). In order to exclude the nonspecific effect of acupuncture practice, a total of 10 data from the post-0-minute phase were excluded from the final analysis (Figure 2). We symbolized left acupuncture group, right acupuncture group, and control group as A, B, and C, respectively. So for every person, there are 60 pairs of data acquired. For every group, there are 2400 pairs of data acquired. We denoted the mapping relationship of these data pairs, namely, mapping relationship from {AL} to {AR}, from {BL} to {BR}, from {CL}to {CR} as {AL} → {AR}, {BL} → {BR} and {CL} → {CR}, and summarized as $\left\{\begin{smallmatrix}\mathrm{A}\\ \mathrm{BL}\\ \mathrm{C}\end{smallmatrix}\right\} \rightarrow \left\{\begin{smallmatrix}\mathrm{A}\\ \mathrm{BR}\\ \mathrm{C}\end{smallmatrix}\right\}$.

2.3.4. System Identification Algorithm. Under the condition of $\left\{\begin{smallmatrix}\mathrm{A}\\ \mathrm{BL}\\ \mathrm{C}\end{smallmatrix}\right\} \rightarrow \left\{\begin{smallmatrix}\mathrm{A}\\ \mathrm{BR}\\ \mathrm{C}\end{smallmatrix}\right\}$, totally 2400×3 pairs of data were acquired and symbolized as $f_{\mathrm{AL}}(k)$, $f_{\mathrm{BL}}(k)$, $f_{\mathrm{CL}}(k)$, $f_{\mathrm{AR}}(k)$, $f_{\mathrm{BR}}(k)$, $f_{\mathrm{CR}}(k)$, $k = 1, 2, \ldots, 2400$, where A represents left acupuncture group; B represents right acupuncture group; C represents control group; L represents left Hegu acupoint; R represents right Hegu acupoint; $k = 1, 2, \ldots, 2400$. Then, we determined $f_{\mathrm{AL}}(k)$, $f_{\mathrm{BL}}(k)$ and $f_{\mathrm{CL}}(k)$, as input and $f_{\mathrm{AR}}(k)$, $f_{\mathrm{BR}}(k)$, and $f_{\mathrm{CR}}(k)$ as output, respectively. System identification algorithm was performed in the Matlab software (Version: 6.5). The flow diagram of system identification algorithm is shown in Figure 4.

3. Results

3.1. Mapping Model. Three mathematical models were obtained as follows by executing system identification algorithm, which reflects the correlation of bilateral Hegu acupoints under different intervention conditions.

Model 1:

$$\begin{aligned} f_{\mathrm{AL}}(k) &= a_1 f_{\mathrm{AL}}(k-1) + b_1\left[f_{\mathrm{AL}}(k-1)\right]^{0.3} \\ &\quad + c_1 f_{\mathrm{AR}}(k) + d_1\left[f_{\mathrm{AR}}(k)\right]^{0.3}. \end{aligned} \tag{1}$$

Model 2:

$$\begin{aligned} f_{\mathrm{BL}}(k) &= a_2 f_{\mathrm{BL}}(k-1) + b_2\left[f_{\mathrm{BL}}(k-1)\right]^{0.3} \\ &\quad + c_2 f_{\mathrm{BR}}(k) + d_2\left[f_{\mathrm{BR}}(k)\right]^{0.3}. \end{aligned} \tag{2}$$

Model 3:

$$\begin{aligned} f_{\mathrm{CL}}(k) &= a_3 f_{\mathrm{CL}}(k-1) + b_3\left[f_{\mathrm{CL}}(k-1)\right]^{0.3} \\ &\quad + c_3 f_{\mathrm{CR}}(k) + d_3\left[f_{\mathrm{BR}}(k)\right]^{0.3}. \end{aligned} \tag{3}$$

Using the values (measured on the left side) of $f_{\mathrm{AL}}(k)$, $f_{\mathrm{BL}}(k)$, and $f_{\mathrm{CL}}(k)$ as input variables of models (1), (2), and (3), the estimated value (mapping value) of the right Hegu acupoint can be obtained with help of the models, symbolized as ${f_{\mathrm{AR}}}^*(k)$, ${f_{\mathrm{BR}}}^*(k)$ and ${f_{\mathrm{CR}}}^*(k)$, following the mapping relationship $f_{\mathrm{AL}}(k) \rightarrow {f_{\mathrm{AR}}}^*(k)$, $f_{\mathrm{BL}}(k) \rightarrow {f_{\mathrm{BR}}}^*(k)$, and $f_{\mathrm{CL}}(k) \rightarrow {f_{\mathrm{CR}}}^*(k)$.

3.2. Error Evaluation and Signal-Noise Ratio. The errors between "true" value and their estimated values (symbolized as $d_i(k)$) are defined as

$$\begin{aligned} d_{\mathrm{A}}(k) &= {f_{\mathrm{AR}}}^*(k) - f_{\mathrm{AR}}(k), \quad k = 1, 2, \ldots, 2400, \\ d_{\mathrm{B}}(k) &= {f_{\mathrm{BR}}}^*(k) - f_{\mathrm{BR}}(k), \quad k = 1, 2, \ldots, 2400, \\ d_{\mathrm{C}}(k) &= {f_{\mathrm{CR}}}^*(k) - f_{\mathrm{CR}}(k), \quad k = 1, 2, \ldots, 2400. \end{aligned} \tag{4}$$

Then we defined the signal-noise ratio sn_i $(i = A, B, C)$ as

$$\begin{aligned} \mathrm{sn}_{\mathrm{A}} &= \frac{\left\{\sqrt{\sum_{k=1}^{2400}\left[{f_{\mathrm{AR}}}^*(k)\right]^2} / \sqrt{\sum_{k=1}^{2400}\left[d_{\mathrm{A}}(k)\right]^2}\right\}}{2400}, \\ \mathrm{sn}_{\mathrm{B}} &= \frac{\left\{\sqrt{\sum_{k=1}^{2400}\left[{f_{\mathrm{BR}}}^*(k)\right]^2} / \sqrt{\sum_{k=1}^{2400}\left[d_{\mathrm{B}}(k)\right]^2}\right\}}{2400}, \\ \mathrm{sn}_{\mathrm{C}} &= \frac{\left\{\sqrt{\sum_{k=1}^{2400}\left[{f_{\mathrm{CR}}}^*(k)\right]^2} / \sqrt{\sum_{k=1}^{2400}\left[d_{\mathrm{C}}(k)\right]^2}\right\}}{2400}. \end{aligned} \tag{5}$$

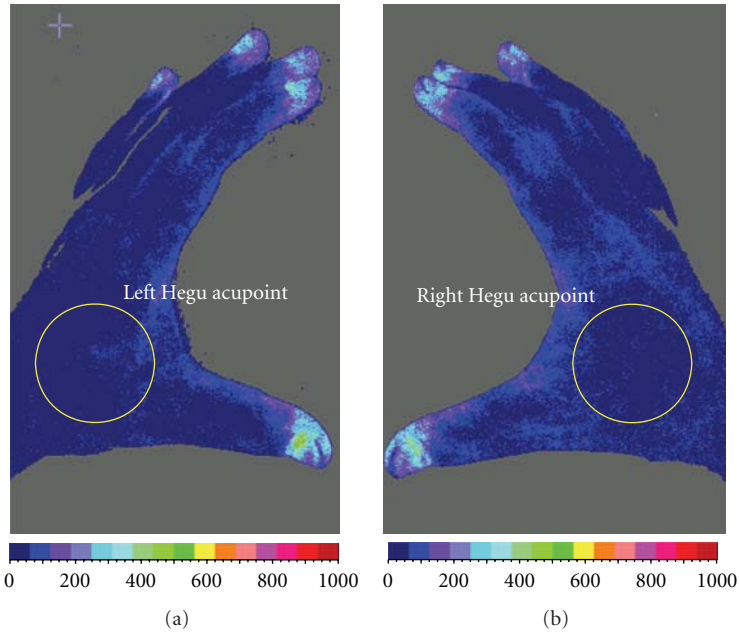

Figure 3: Confirmation of Hegu acupoint. (a) left hand, (b) right hand.

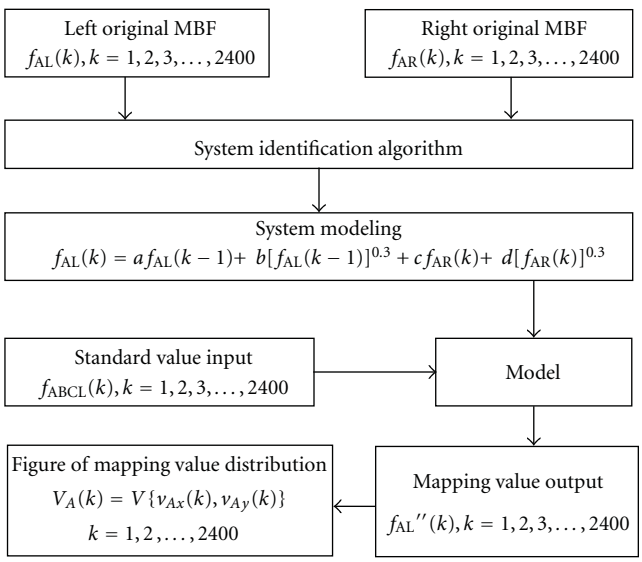

Figure 4: Flow diagram of system identification algorithm.

3.3. Standard Signal Input. Standard signal value series used as common model-input value series denoted as $f_{ABCL}(k)$ are mathematically calculated as

$$f_{ABCL}(k) = 10 \times \left\{ \sin\left(\frac{k}{100}\right) + \sin\left(\frac{k}{80}\right) + \sin\left(\frac{k}{60}\right) + \sin\left(\frac{k}{40}\right) + \cos\left(\frac{k}{90}\right) + \cos\left(\frac{k}{70}\right) + \cos\left(\frac{k}{50}\right) + \cos\left(\frac{k}{30}\right) \right\} \quad k = 1, 2, \ldots, 2400. \tag{6}$$

The standard signal input is to maintain the complexity and stability. If $f_{AL}(k)$, $f_{BL}(k)$, and $f_{CL}(k)$ were all replaced by $f_{ABCL}(k)$, the output $f_{AL}''(k)$, $f_{BL}''(k)$, and $f_{CL}''(k)$ will be produced with help of the models, instead of $f_{AR}^*(k)$, $f_{BR}^*(k)$, and $f_{CR}^*(k)$, respectively. Thus the mapping relationship will change into $f_{ABCL}(k) \rightarrow f_{AL}''(k)$, $f_{ABCL}(k) \rightarrow f_{BL}''(k)$, $f_{ABCL}(k) \rightarrow f_{CL}''(k)$.

3.4. Determination of Characteristic Vectors. In order to set up model output characteristic vectors to describe the different mapping results of different interventions, we define one subvector of the model output characteristic vector as

$$\begin{aligned} v_{AL\,y}(k) &= f_{AL}''(k), \quad k = 1, 2, 3, \ldots, 2400, \\ v_{BLy}(k) &= f_{BL}''(k), \quad k = 1, 2, 3, \ldots, 2400, \\ v_{CLy}(k) &= f_{CL}''(k), \quad k = 1, 2, 3, \ldots, 2400. \end{aligned} \tag{7}$$

The other subvector of the model output characteristic vector was determined as

$$\begin{aligned} v_{Ax}(k) &= \mathrm{sn}_A + d_A(k), \quad k = 1, 2, \ldots, 2400, \\ v_{Bx}(k) &= \mathrm{sn}_B + d_B(k), \quad k = 1, 2, \ldots, 2400, \\ v_{Cx}(k) &= \mathrm{sn}_C + d_C(k), \quad k = 1, 2, \ldots, 2400. \end{aligned} \tag{8}$$

Then the 2-dimensional diagram of mapping value distribution was produced. In the left acupuncture group, the distribution of output was determined as

$$V_A(k) = V\left\{v_{Ax}(k), v_{Ay}(k)\right\}, \quad k = 1, 2, \ldots, 2400. \tag{9}$$

In the right acupuncture group, the distribution of output was determined as

$$V_B(k) = V\left\{v_{Bx}(k), v_{By}(k)\right\}, \quad k = 1, 2, \ldots, 2400. \tag{10}$$

Table 2: Mapping value distribution center and signal-noise ratio in different groups.

Original input/output	Intervention method	Distribution center (PU)
$f_{AL}(k) \rightarrow f_{AR}(k)$	Acup. Left	255.41
$f_{BL}(k) \rightarrow f_{BR}(k)$	Acup. Right	591.01
$f_{CL}(k) \rightarrow f_{CR}(k)$	No acup.	71.58
$f_{AR}(k) \rightarrow f_{AL}(k)$	Acup. Left	222.32
$f_{BR}(k) \rightarrow f_{BL}(k)$	Acup. Right	965.81
$f_{CR}(k) \rightarrow f_{CL}(k)$	No acup.	96.33

In the control group, the distribution of output was determined as

$$V_C(k) = V\left\{v_{Cx}(k), v_{Cy}(k)\right\}, \quad k = 1, 2, \ldots, 2400. \tag{11}$$

The output distribution is shown in Figure 5(b). To exclude the possibility that these results were lateralized to one side, we defined the original $f_{AR}(k)$, $f_{BR}(k)$, $f_{CR}(k)$ as input, $f_{AL}(k)$, $f_{BL}(k)$, $f_{CL}(k)$ as output, respectively, summarized as $\left\{\begin{smallmatrix}A\\BR\\C\end{smallmatrix}\right\} \rightarrow \left\{\begin{smallmatrix}A\\BL\\C\end{smallmatrix}\right\}$. Then the system identification algorithm was carried out with MATLAB software again, and the other 3 models were produced. When the same standard signals were input into the different models, the distribution of output was produced (Figure 5(a)). Mapping value distribution center and signal-noise ratio in different groups were shown in Table 2.

From Figure 5, we can find that stimulation of right LI4 has the strong amplification effect on blood perfusion in left LI4, and this strong amplification effect is independent of the original input and output selection in the system identification algorithm analysis. In contrast, acupuncture at left LI4 just produces moderate amplification effects on blood perfusion in right LI4, and this moderate amplification is independent of the original input and output selection too. There is no amplification effect produced in the control group. These results indicated that after acupuncture, the amount of amplification effect on blood perfusion in contralateral side was just related to which lateral acupoint was acupunctured. $i = A, B, C$, $k = 1, 2, 3, \ldots, 2400$. (A) under the condition of $\left\{\begin{smallmatrix}A\\BR\\C\end{smallmatrix}\right\} \rightarrow \left\{\begin{smallmatrix}A\\BL\\C\end{smallmatrix}\right\}$. (B) under the condition of $\left\{\begin{smallmatrix}A\\BR\\C\end{smallmatrix}\right\} \rightarrow \left\{\begin{smallmatrix}A\\BL\\C\end{smallmatrix}\right\}$. A, acupuncture left Hegu acupoint; B, acupuncture right Hegu acupoint; C, no acupuncture.

4. Discussion

"In physics, symmetry means uniformity or invariance" [20], in other words, "the existence of different viewpoints from which the system appears the same" [21]. In Traditional Chinese Medicine (TCM), the principle is to maintain the body balance. Under the guidance of TCM theory, clinical practice is always in the pursuit of balance and symmetry. For example, according to the Neijing theory, if someone has disease in the left body, the treatment point is usually selected in the right side, and vice versa. However, "increasing levels of broken symmetry in many-body systems correlates with increasing complexity and functional specialization" [20]. In acupuncture theory, the symmetry breaking means there are differences between two meridians or two acupoints which have the same name and are located bilaterally and symmetrically on the body.

Recently, a system review analyzed the contralateral and ipsilateral acupuncture effect on poststroke hemiplegic patients [22]. Although this system review and meta-analysis could not come to a definitive conclusion, it indicates the importance of distinction between contralateral and ipsilateral acupuncture. According to traditional acupuncture theory, if we stimulate LI4 on one side, the function of the large intestine meridian (LI) located on the other side might also be activated. As a result, the running of Qi and blood which flow in both LI meridians were changed. So the basis of contra- or ipsilateral acupuncture is the specificity of acupoints which have the same name.

But up to now, it is difficult to evaluate the activation of acupoints, and, as a result, it is also difficult to analyse the specificity of acupoints after meridians are stimulated. Recently, more and more attention has been focused on the relationship of acupuncture and circulation [23–25]. In TCM theory, one of the definitive causes of acupuncture effect is the special sensation in local acupoints after stimulation, which might be related to the blood perfusion changes in acupoints or meridians [18]. According to the previous study, the mean blood flux (MBF) was larger at the acupoints than in their surrounding tissues, which indicates that the MBF can be used as an index for discriminating differences in the microcirculatory conditions between acupoints and their surrounding tissues [26]. It has also been shown that acupuncture can not only increase general circulation [27] and circulation in specific organs [28] but also change the skin microcirculation as well [19, 24, 29, 30]. When an acupoint was stimulated adequately, the blood perfusion of this point continued to increase whereas the blood perfusion of nonacupoint only changed slightly by the same acupuncture stimulation [31]. These results indicated that the blood perfusion in acupoints can be recommended as candidate for acupuncture effect evaluation.

Our previous study has shown that ipsilateral acupoint stimulation could result in an increase of blood perfusion in contralateral side. But the lateralized characteristic is still not clear. This study indicated that the stimulation effect was different in different intervention groups. After stimulation of right LI4, the amplification effect on blood perfusion in contralateral is better than that in other two groups. It means under resting condition, the mean blood flux in both Hegu acupoints is symmetrical; after stimulating either side Hegu acupoint, this symmetry is broken. As a result, the MBF in the contralateral acupoint was amplified. But this amplification effect is different in different groups, which might be another phenomenon of symmetry breaking on a high level.

According to our previous study [32], under anesthesia condition, thermostimulation has no effect on the blood perfusion in the contralateral side foot. These results indicated that this asymmetry phenomenon was strengthened by the

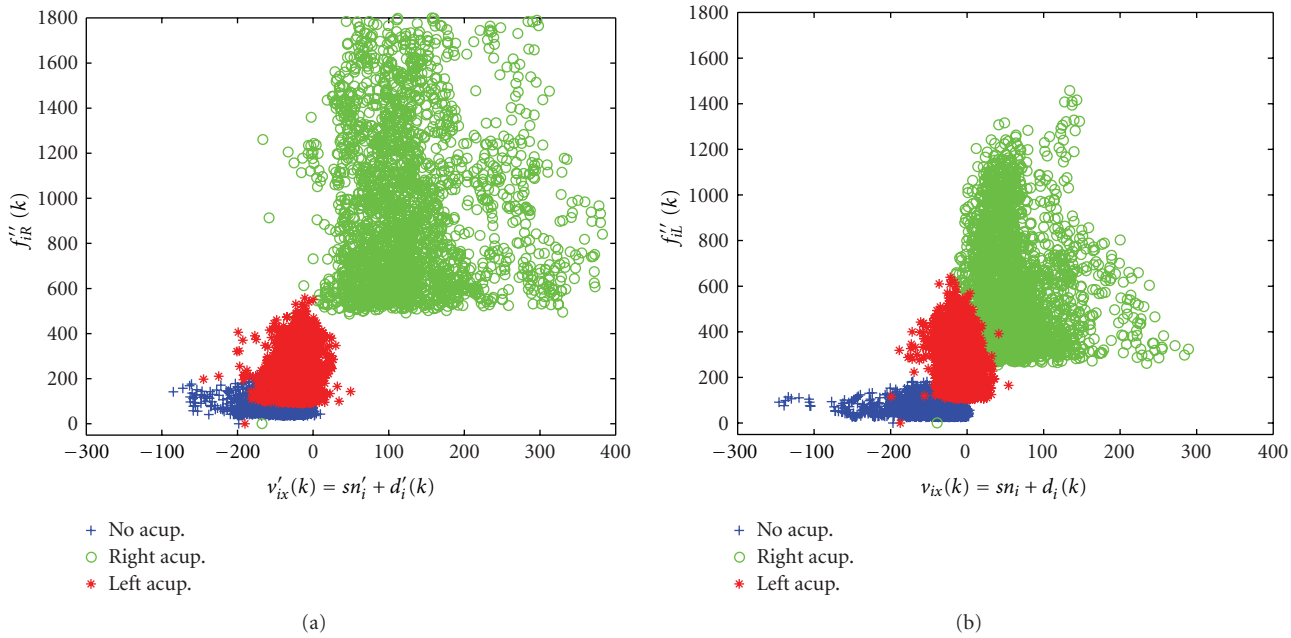

Figure 5: Mapping value distribution of standard input in different models.

anesthesia. In other words, synchronous changes of bilateral blood perfusion might be related to the wakefulness status. Although it is difficult to explore the reasons, we think it might be related to the asymmetry of brain.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Research Article

Exploration of New Electroacupuncture Needle Material

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Background. Electro Acupuncture (EA) uses the acupuncture needle as an electrode to apply low-frequency stimulation. For its safe operation, it is essential to prevent any corrosion of the acupuncture needle. *Objective*. The aim of this study is to find an available material and determine the possibility of producing a standard EA needle that is biocompatible. *Methods*. Biocompatibility was tested by an MTT assay and cytotoxicity testing. Corrosion was observed with a scanning electron microscope (SEM) after 0.5 mA, 60 min stimulation. The straightness was measured using a gap length of 100 mm, and tensile testing was performed by imposing a maximum tensile load. *Results*. Phosphor bronze, Ni coated SS304, were deemed inappropriate materials because of mild-to-moderate cytotoxicity and corrosion. Ti-6Al-4V and SS316 showed no cytotoxicity or corrosion. Ti-6Al-4V has a 70 times higher cost and 2.5 times lower conductivity than SS316. The results of both straightness and tensile testing confirmed that SS316 can be manufactured as a standard product. *Conclusion*. As a result, we confirmed that SS316 can be used a new EA electrode material. We hope that a further study of the maximum capacity of low-frequency stimulation using an SS316 for safe operation.

1. Introduction

Electroacupuncture is a combination of acupuncture from oriental medicine and low-frequency (1–1,000 Hz) stimulation, which is a type of physical therapy used in western medicine. Low-frequency stimulation was first proposed in 1816 by Louis Berlioz of France, who suggested that electrical stimulation combined with acupuncture treatment could enhance the effectiveness of the treatment. Later, in 1825, Sarlandiere used this technique to treat gout and neurological diseases, and he published a report in which he referred to the technique as "galvanopuncture," from which the term electroacupuncture is derived [1]. Since that time, electroacupuncture has been used by many researchers who have noted a variety of effects, such as an increased pain threshold [2], increased gastrointestinal movement [3], and weight loss [4], and as a result, there has been an increase in the technique's application in clinics worldwide. However, due to the electrical properties of the current generated by a low-frequency stimulator, electro acupuncture poses safety problems that are distinct from those of traditional acupuncture. Lytle et al. [5] have identified a number of elements related to the safety of the electrical properties of the current generated by a low-frequency stimulator, such as voltage, waveforms, and the pulse frequency and width. Cummings [6] has also focused upon a number of safety elements, such as the possibility of a change in needle depth during electrical stimulation, risk of damage to internal organs due to muscle contraction and needle vibration, needle corrosion due to excessive charging, the caution required when treating areas at risk of shock, such as the area around carotid artery, as well as concerns regarding the interaction of the instrument with patients wearing pacemakers. In particular, Park [7] and Jin [8] studied several electro acupuncture devices and reported that there was no significant difference in corrosion, hardness, or cytotoxicity on the needle surface and tip before and after its operation, while Hwang [9] reported that corrosion was observed in the interface between skin and air when the current is above 0.05 mA and that this corrosion increased with an

Table 1: Allocation of experimental group and control group.

No.	Wire	Assignment
(1)	Ti-6-Al-4V 0.20 mm	Experimental group
(2)	Phosphor bronze 0.25 mm	Experimental group
(3)	SS 304 Ni 20 mm	Experimental group
(4)	SS 304 Ni 25 mm	Experimental group
(5)	SS 304 Ni 30 mm	Experimental group
(6)	SS 316 0.20	Experimental group
(7)	SS 316 0.25	Experimental group
(8)	SS 316 0.30	Experimental group
(9)	DMEM/F-12	Control group

Table 2: Chemical composition of Hank's solution.

Component	Concentration (mol dm^{-3})
NaCl	137.0
KCl	5.4
Na_2HPO_4	0.25
KH_2PO_4	0.44
$CaCl_2$	1.3
$MgSO_4$	1.0
$NaHCO_3$	4.2

increase in time or in the applied current. Of the austenitic stainless steels, SS 304 is reported to be most vulnerable to corrosion [10]. It becomes vulnerable to corrosion due to sensitization from thermal treatment, as Cr is extracted at 400~800°C, and the Cr-exhausted region causes corrosion [11]. In the investigation of the corrosion safety of SS 304 ear-acupuncture needles, the authors have confirmed that defective processing of the needle point and surface leads to more severe corrosion [12], a finding that is considered applicable to needles that undergo a similar manufacturing process. In the present study, 4 materials—2 materials with higher conductivity and 2 materials with a higher safety than current material—were tested for the possibility of being used as new material for an electroacupuncture needle. The most appropriate material was selected, and its specifications were evaluated to replace the current new acupuncture needles with a new material.

2. Materials and Methods

2.1. Experimental Materials. Among the metal wires that are commercially available, we selected phosphor bronze (hereafter referred to as PB) and Ni-coated SS 304 (hereafter referred to as SS 304 Ni), which both have superior electrical conductivity as compared to SS 304, which is currently used for electro acupuncture, along with titanium alloy (Ti-6-Al-4V) and SS 316, which have relatively low electrical conductivity but were expected to demonstrate superior stability. Due to the circumstances involved in purchasing, a thickness of 0.25 mm was used for PB and 0.2 mm for Ti-6-Al-4V, and thicknesses of 0.2, 0.25, and 0.3 mm were used for SS 304 Ni and SS 316. All materials were purchased from a company (KOS, Korea) specializing in metal wire.

2.2. Experimental Methods

2.2.1. Biocompatibility. MTT (3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) assay and cytotoxicity testing were performed in order to assess the biocompatibility of the experimental material. The procedure for each experiment conducted was as follows.

(A) MTT Assay

(a) Assay Standards and Methods. The cytotoxicity test of ISO 10993-5 for assessment of the cytotoxic potential of a test element (medical device) after direct contact was used as the assay standard.

The procedure composed with cell seeding, contact of the test element, incubation for more than 24 hours, preparation of the coloring solution of revelation, revelation of cytotoxicity, reading.

(b) Extraction Condition. The wires were segmented into 10 mm sections, and 40 sections were added to 3 mL of Dulbecco's Modified Eagle's Medium: nutrient mixture F-12 (DMEM/F-12, GIBCO). Extraction was performed at 37°C for 48 hours. The assignment of experimental and control groups was as follows (Table 1).

(c) Experimental Method and Evaluation Standards

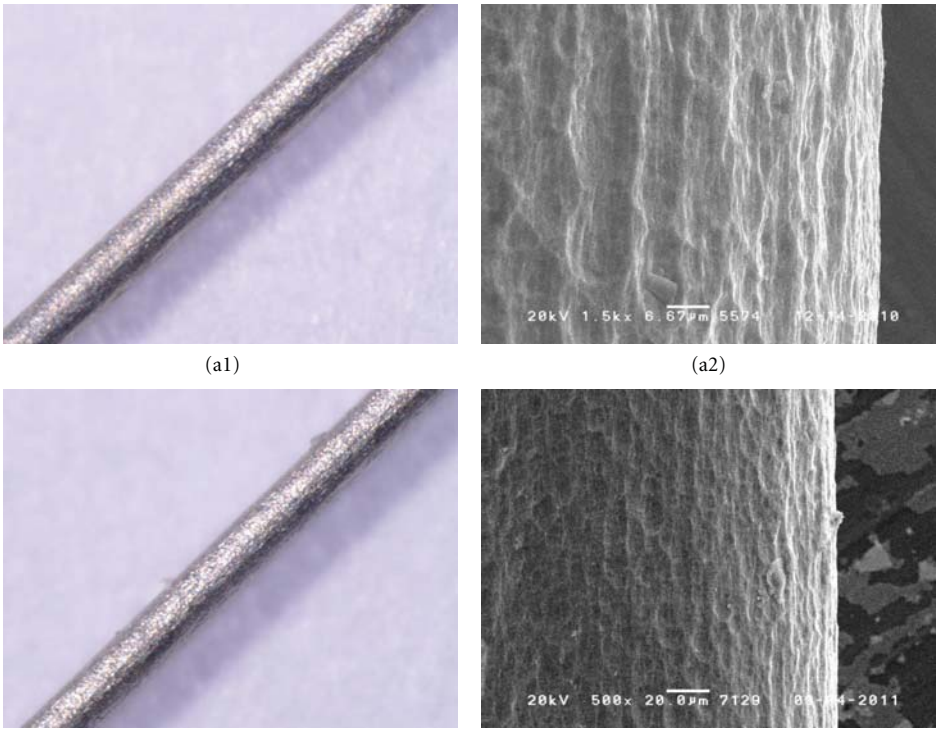
(1) Experimental Methods. Mouse osteoblast (MC3T3 cell) was cultured for 24 hours with DMEM/F-12 (5% FBS, penicillin-streptomycin added). It was inspected for contamination before use. Media of sufficiently grown cells were removed, and media extracts of the experimental group and the control group were cultured separately for 24 hours. The media were then added to a plate with formazan crystal. After the formazan had dissolved, a Dymatech MRX ELISA microplate reader (Dynatech laboratories, Chantilly, VA, USA) was used to measure the absorbance at 540 nm. The average of 3 measurements was used to calculate the percentage of cell solubility. The result of the control group was used as the negative control.

(B) Stain Test

(a) Experimental Method and Evaluation Standard. The conditions for extraction and cell culture were performed as in an MTT assay. The cultured cells were stained with 0.3% crystal violet, and a stereoscopic microscope (Leica microsystem DE/EZ4) was used to compare their viability.

2.2.2. Corrosion Stability

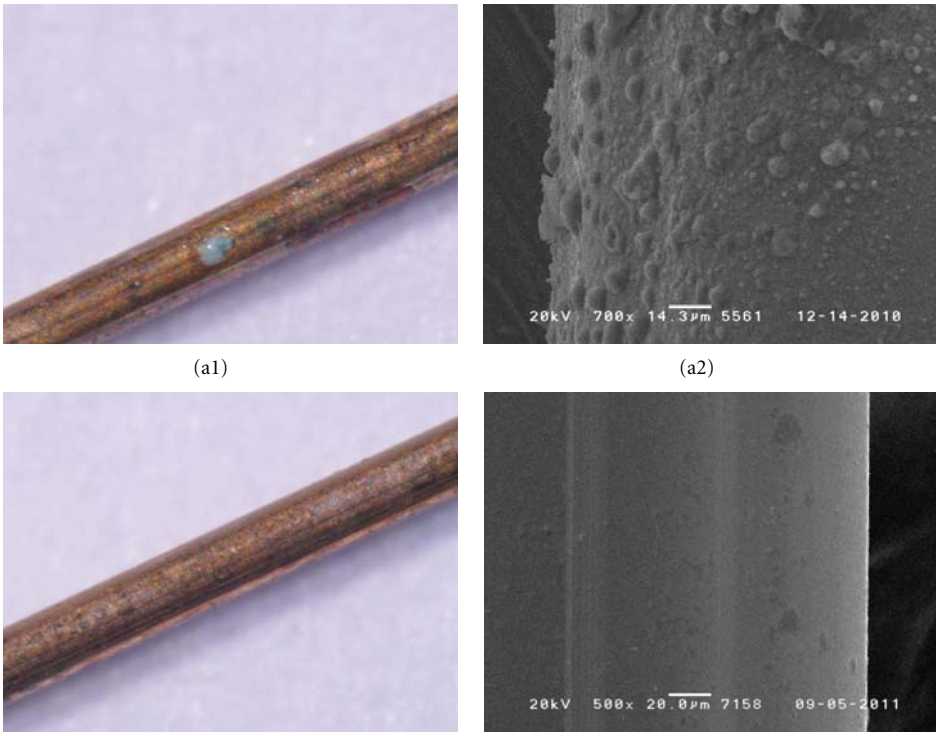
(A) Corrosion Condition. We prepared 5 cm of wire for each type. In order to consider body fluid conditions, 1 cm of each wire was dipped separately into 50 mL of Hank's solution (Table 2), which is a simulated body fluid, and current was applied at 0.5 mA for 60 minutes as a continuous wave, step response, 1 ms duration, and single-phase current. Current



(a1) (a2)

(b1) (b2)

Figure 1: Corrosion test of Ti-6Al-4V: no corrosion was identified on the thickness of 0.20 mm (B is the control).



(a1) (a2)

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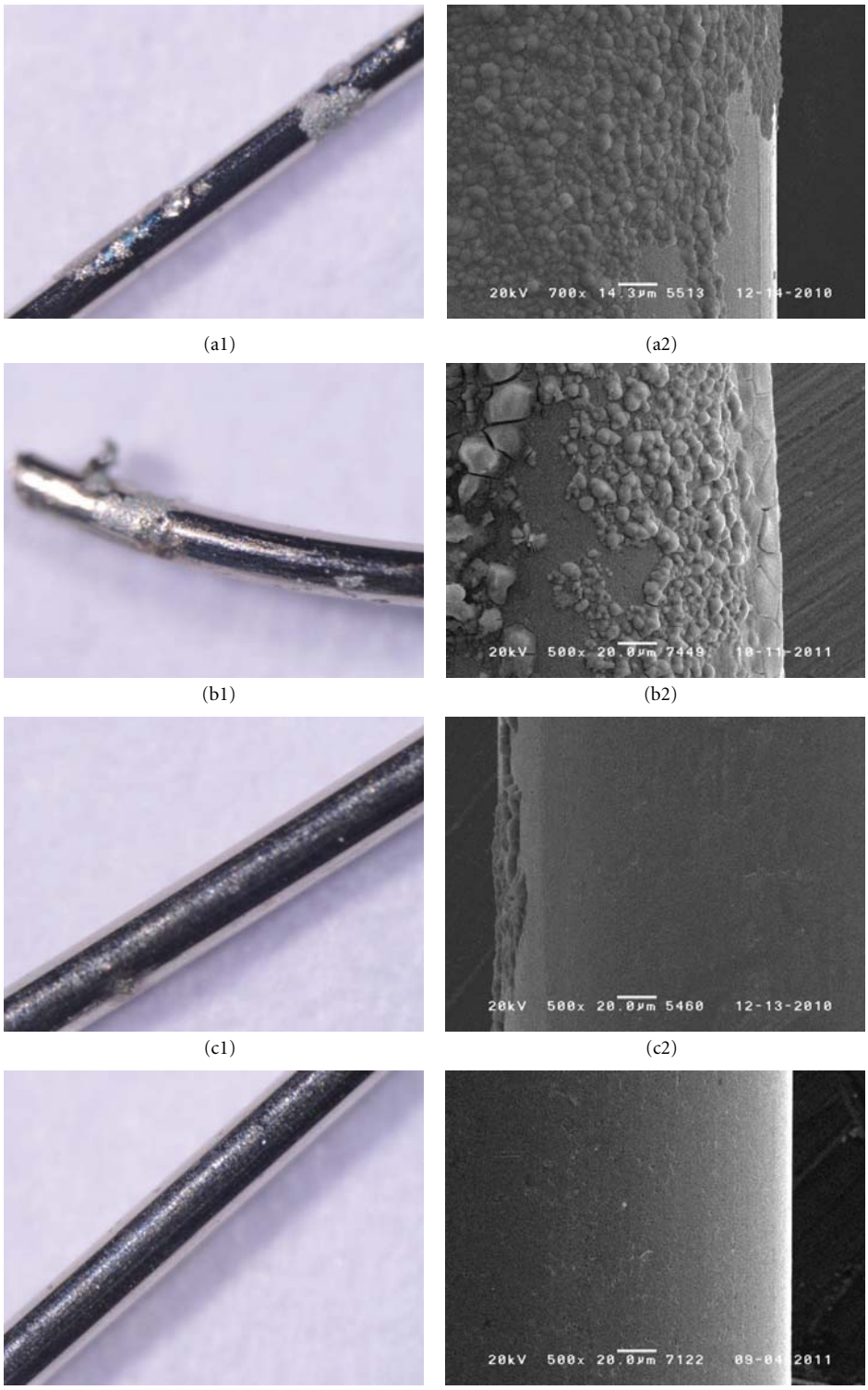
Figure 2: Corrosion test of PB: corrosion was identified on the thickness of 0.25 mm (B is the control).



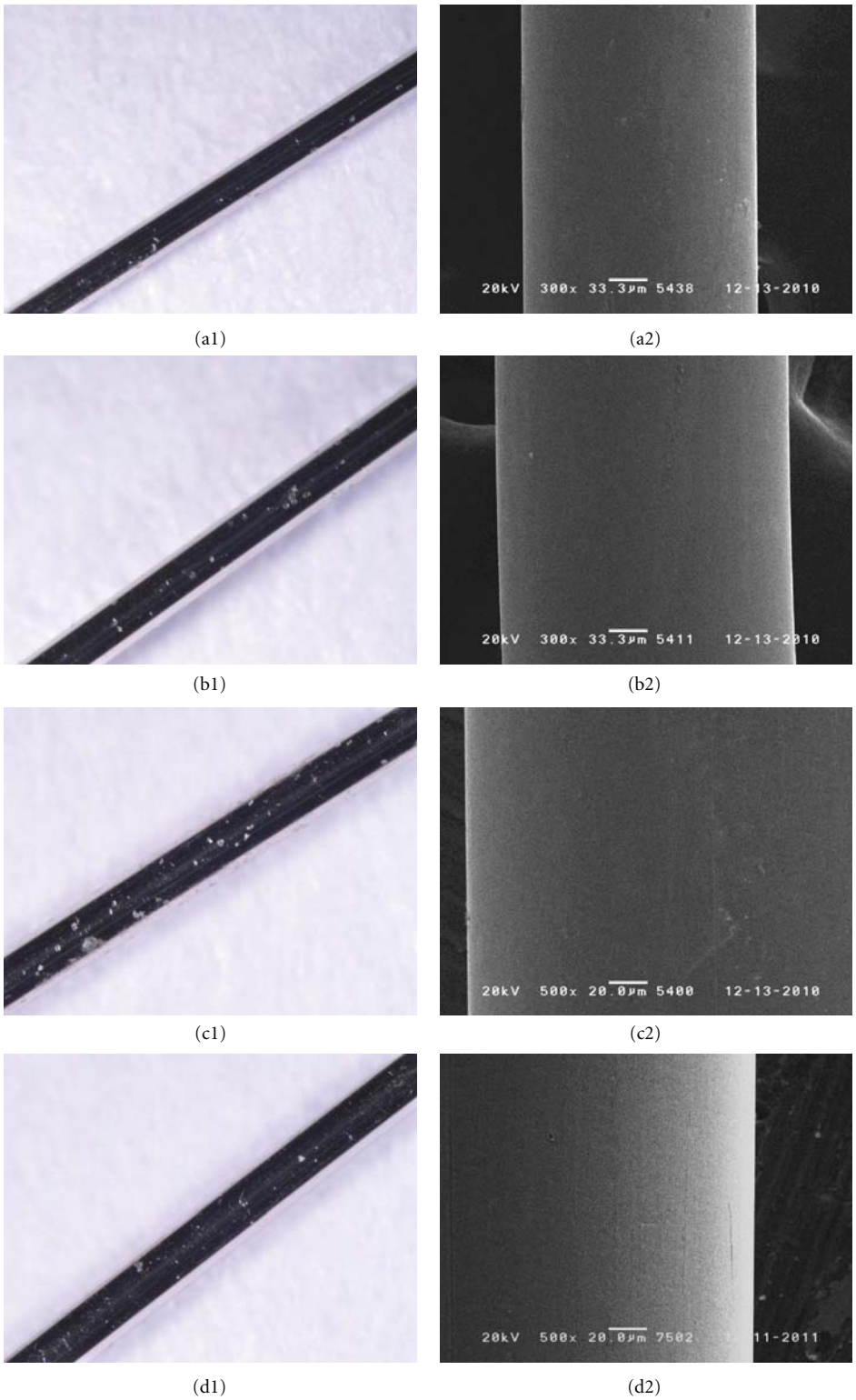
(a1) (a2)

(b1) (b2)

(c1) (c2)

(d1) (d2)

Figure 3: Corrosion test of STS 304 Ni: corrosion was identified on all thicknesses (A: 0.20 mm, B: 0.25 mm, C: 0.30 mm, D: control).



(a1) (a2)

(b1) (b2)

(c1) (c2)

(d1) (d2)

Figure 4: Corrosion test of STS 316: no corrosion was identified on any of the thicknesses (A: 0.20 mm, B: 0.25 mm, C: 0.30 mm, D: control).

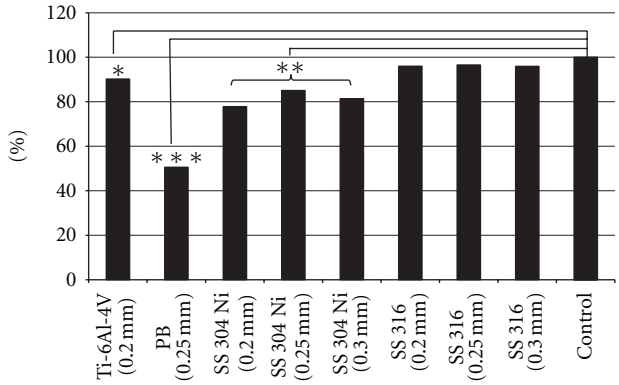


Figure 5: MTT assay result (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ versus control group).

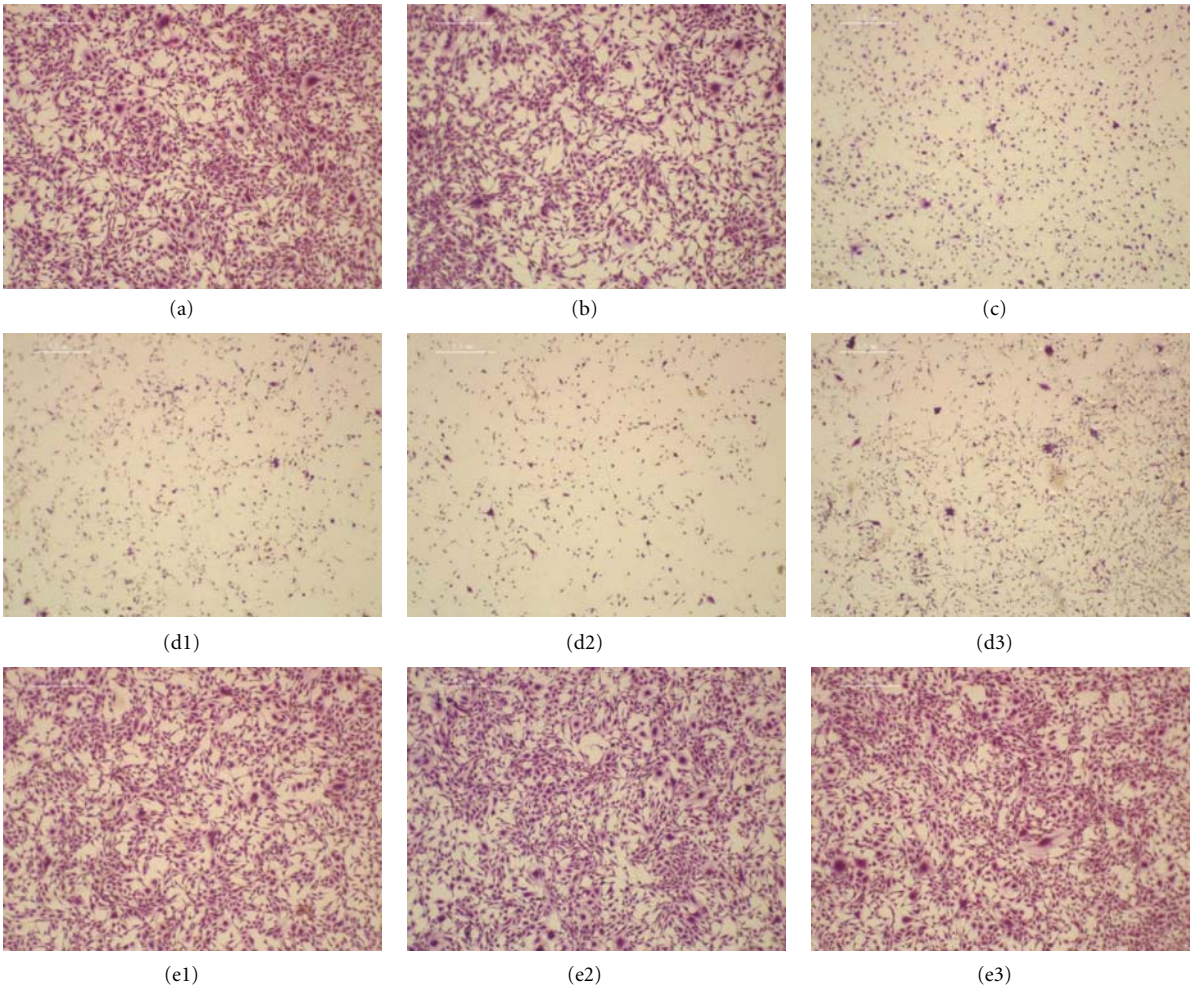


Figure 6: Stain test of each material. B (Ti-6Al-4V) and E (SS 316) show higher rate of survival, while (c) (PB) and (d) (SS 304 Ni) show a lower rate ((a) is the control).

was supplied by a S88 stimulator (GRASS; USA: 0.01~0.5 mA).

(B) Measurement of Corrosion. A stereoscopic microscope (SMZ 1500, Nikon, Japan) and scanning electron microscope (SEM) were used to observe corrosion. The stereoscopic microscope was used to observe changes in color and shape, and SEM was used to observe surface changes due to corrosion.

2.3. Evaluation of Tensile Strength and Straightness. Wires selected for corrosion stability and biocompatibility were

Table 3: Comparison of cost, conductivity, and tensile strength of wire material (1st of July, 2010).

Material	Diameter (mm)	Material	Tensile strength (N/mm^2)	Electrical conductivity (% IACS)	Unit cost (1000 won/Kg)
Ti-6Al-4V	0.25	C 0.08%, Al 5.5~6.5%, Ni 0.05%, O 0.13%, Ti 88~90.08%, V 3.5~4.5%, Fe 0.25%, H 0.013%	2005	1.01	1720
Phosphor bronze	0.25	P 0.03–0.35%, Sn 4.5–9.0%	2131	15	16
SS 304 Ni	0.2	C 0.075%, Si 0.45%, Mn 1.25%, P 0.004%, Ni 8.47%	1685	25	22.5
SS 316	0.25	C below 0.08%, Si below 1.0%, Mn below 2.0%, Cr 16~18%, Ni 10~14%, Mo 2.0~3%	999	2.5	24.5

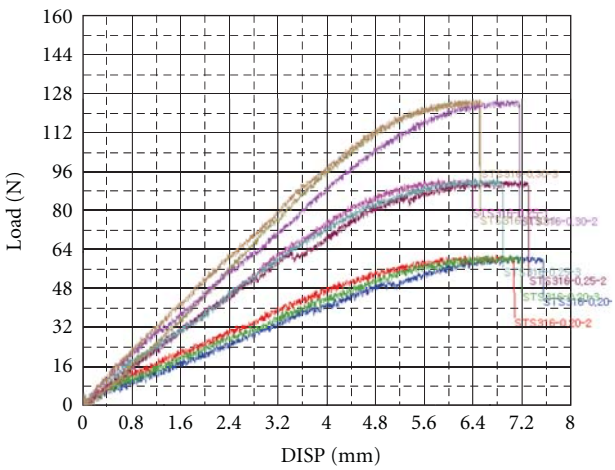


Figure 7: Tensile graph of SS 316(0.20, 0.25, 0.30 mm); result of tensile strength was 183~210 kgf/mm2, which is superior to the range of tensile strength of currently used SS 304.

Table 4: Corrosion assessment of wire material in Hank's solution (x: corrosion was not observed. o: corrosion was observed).

Diameter (mm)	Ti-6Al-4V	Phosphor bronze	SS 304 Ni	SS 316
0.2	x	—	o	x
0.25	—	o	o	x
0.3	—	—	o	x

evaluated for travel speed and straightness with respect to thermal treatment conditions, and tensile strength was calculated with respect to the evaluated straight line. For the evaluation of straightness, the gap length was defined as the deviation from a reference line at a distance of 100 mm, to measure the extent of bending. The tensile strength was calculated by dividing the maximum tensile load before material breakdown by that of an original cross-sectional area of the sample.

3. Results

3.1. Properties of Electroacupuncture Needle Materials. Ti-6Al-4V is a Ti alloy with excellent biocompatibility that is used as a material for dental implants. Its electrical conductivity, however, is low, at 1.01% (IACS—International Annealed Copper Standard), and it is also expensive. SS 304 Ni is an existing SS 304 wire that is coated with Ni, whose high electrical conductivity, at 25% (IACS), improves the wire's conductivity. It is expected that this higher electrical conductivity can improve the effectiveness of electro acupuncture. SS 316 is the most commonly used stainless steel, along with SS 314. Compared to SS 314, SS 316 has a lower Cr- and a higher Ni-content, and Mo is added to it. It has a higher resistance to corrosion and creep but has inferior electrical conductivity, at 2.5% (IACS), as compared to 3.0% for SS 304 (IACS) (Table 3).

3.2. Biological Safety

3.2.1. Corrosion Stability of Electroacupuncture Needle Material. The results of the corrosion stability tests of new acupuncture needle materials in simulated body fluid are as follows (Table 4). Examination with a stereoscopic microscope showed that there was no difference before and after electrical stimulus in Ti alloy (Figure 1). For PB, a stereoscopic microscope showed discoloring and SEM showed corrosion (Figure 2). SS 304 Ni showed corrosion with both a stereoscopic microscope and SEM, for all thicknesses (Figure 3). SS 316 showed a corrosion-like appearance under a stereoscopic microscope, but no corrosion using SEM (Figure 4 and Table 4).

3.2.2. MTT Assay

(A) 5.1 Microplate Reader Absorbance Analysis (540 μm). When cell viability was expressed as a percentage with respect to the negative control, PB showed the lowest viability (50.5%) and SS 316 (0.25 mm) showed the highest viability (Table 5), (Figure 5).

Because there is no separate standard of cell viability for oriental medicine equipment, the standards of the Federation Dentaire Internationale (FDI) were used for our evaluation (Tables 6 and 7). In our results, Ti-6Al-4V, SS 316, and SS 304 Ni (0.25 mm and 0.3 mm) showed mild cytotoxicity, while SS 304 Ni 0.2 mm and phosphor bronze showed moderate cytotoxicity.

3.2.3. Stain Test Results. In the MTT assay, the degree of cell viability was high, except for PB and SS 304 Ni 0.2 mm, but

Table 5: The absorbance (540 nm) from MTT assay and viability.

	Absorbance					
	1st	2nd	3rd	Average	S.D.	Viability (average/control)
Ti-6Al-4V (0.20 mm)	0.337	0.34	0.336	0.338	0.002	*90.2%
PB (0.25 mm)	0.188	0.189	0.191	0.189	0.002	***50.58%
SS 304 Ni (0.20 mm)	0.289	0.295	0.29	0.291	0.003	**77.83%
SS 304 Ni (0.25 mm)	0.321	0.315	0.319	0.318	0.003	**85.049%
SS 304 Ni (0.30 mm)	0.304	0.306	0.304	0.305	0.001	**81.39%
SS 316 (0.20 mm)	0.362	0.361	0.355	0.36	0.004	95.99%
SS 316 (0.25 mm)	0.36	0.359	0.365	0.362	0.003	96.52%
SS 316 (0.30 mm)	0.358	0.357	0.362	0.359	0.003	95.9%
Control	0.376	0.374	0.373	0.374	0.002	100%

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control).

Table 6: Definition of index values.

0	No observable lysis
1	Up to 20 percent
2	20–40 percent
3	40–60 percent
4	60–80 percent
5	Over 80 percent

Table 7: Response index and cytotoxicity.

Response index	Cytotoxicity
0	None
1	Mild
2-3	Moderate
4-5	Severe

Table 8: Results of safety and economic evaluation according to the material (x: corrosion was not observed. o: corrosion was observed).

Material	Thickness	Corrosion	Viability (MTT assay)	Viability (stain test)	Cost effectiveness
Ti-6Al-4V	0.20 mm	x	90.20%	High	Low
PB	0.25 mm	o	50.58%	Low	High
Ni co	0.20 mm	o	77.83%	Low	Normal
Ni co	0.25 mm	o	85.04%	Low	Normal
Ni co	0.30 mm	o	81.39%	Low	Normal
SS 316	0.20 mm	x	95.99%	High	Normal
SS 316	0.25 mm	x	96.52%	High	Normal
SS 316	0.30 mm	x	95.90%	High	Normal

in the cell stain testing, PB and SS 314 Ni for all thicknesses showed low cell viability compared to the control group (Figure 6(a)), except for Ti-6Al-4V (Figure 6(b)) and SS 316 (Figure 6(e1), 6(e2), 6(e3)).

3.3. Straightness and Tensile Strength. In our evaluation of corrosion, only Ti-6Al-4V and SS 316 were not corroded.

Table 9: Process in accordance with the conditions of the wire straightness evaluation.

Classification	Temperature (°C)	Travel speed (m/s)	Result (mm)	Note
0.20	670	0.50	2.2	Fail
0.20	670	0.58	2.4	Fail
0.20	670	0.66	2.5	Fail
0.20	700	0.50	1.2	Pass
0.20	700	0.58	0.8	Pass
0.20	700	0.66	1.0	Pass
0.20	720	0.50	2.6	Fail
0.20	720	0.58	2.4	Fail
0.20	720	0.66	2.3	Fail
0.25	670	0.50	2.0	Pass
0.25	670	0.58	2.1	Fail
0.25	670	0.66	2.3	Fail
0.25	700	0.50	1.2	Pass
0.25	700	0.58	1.0	Pass
0.25	700	0.66	1.2	Pass
0.25	720	0.50	2.0	Pass
0.25	720	0.58	1.9	Pass
0.25	720	0.66	1.6	Pass
0.30	670	0.50	2.1	Fail
0.30	670	0.58	2.3	Fail
0.30	670	0.66	2.4	Fail
0.30	700	0.50	1.7	Pass
0.30	700	0.58	1.4	Pass
0.30	700	0.66	1.5	Pass
0.30	720	0.50	2.5	Fail
0.30	720	0.58	2.2	Fail
0.30	720	0.66	2.1	Fail

In the MTT assay, Ti-6Al-4V, SS 316, and SS 314 Ni showed excellent cell viability of grade 1 or higher. However, only Ti-6Al-4V and SS 316 showed moderate cell viability in the stain test. In terms of cost, Ti alloy (1720 KRW/kg) is about 70 times more expensive than SS 316 (24.5 KRW/kg) (Table 8).

Table 10: Result of tensile test.

No.	Sample size	Sectional area	Maximum load	Tensile strength	Maximum displacement	Required time
SS 316-0.20-1	0.1997	0.03	6.28	209.333	7.540	00:00:45
SS 316-0.20-2	0.1997	0.03	6.32	210.667	7.070	00:00:42
SS 316-0.20-3	0.1997	0.03	6.32	210.657	7.200	00:00:43
SS 316-0.25-1	0.248	0.05	9.54	190.880	6.390	00:00:38
SS 316-0.25-2	0.248	0.05	9.46	189.200	7.310	00:00:43
SS 316-0.25-3	0.248	0.05	9.56	191.200	6.890	00:00:41
SS 316-0.30-1	0.297	0.07	12.86	183.714	6.510	00:00:39
SS 316-0.30-2	0.297	0.07	12.86	183.714	7.170	00:00:43
SS 316-0.30-3	0.297	0.07	12.90	184.285	6.530	00:00:39

In terms of electrical conductivity, the conductivity of Ti-6Al-4V with 1.01% (IACS) was about 2.5 times lower than that of SS 316 with 2.5% (IACS). After consideration of the above results, SS 316 was selected as a candidate material for an electro acupuncture needle. After transforming the material into a needle shape, straightness and tensile strength tests were performed to check its conformity to standard specifications.

(1) Manufacture of Straight Wire. As a result of the linearization of SS 316 wire, wires with thicknesses of 0.20, 0.25, and 0.30 mm were all appropriate at a temperature condition of 700°C ± 3°C. The most appropriate straight product under 2.0 mm was obtained at a travel speed of 0.58 m/s ± 0.01 m/s (Table 9).

(2) Evaluation of Straight Wire Tensile Strength. Measurements of the tensile strength of the qualified wire showed that the range of tensile strength was 183∼210 kgf/mm^2, which is superior to the range of tensile strength of currently used SS 304, which is 170∼190 kgf/mm^2 (Table 10, Figure 7).

4. Discussion

Electro acupuncture is a technique that applies an electrical stimulus to an inserted needle, and it is currently applied to a variety of illnesses in clinics worldwide [13]. However, the needles used in electro acupuncture, which correspond to electrodes for the low-frequency stimulation of meridian acupuncture points, are the same disposable needles that are used in conventional acupuncture. This has created a controversy regarding the corrosion of the needle in the course of the electrical stimulus treatment [5–7]. In order to resolve this controversy by discovering a new material that can replace the existing electrode and satisfy conditions for a disposable needle, for this study we selected 2 types of wires that have excellent electrical conductivity and 2 types of wires with a high degree of stability, both of which were commercially available. Phosphor bronze is a widely used contact terminal of the electronic device and known as a stable and a good conductor material. However, biological safety has not been confirmed. For this reason, we choose the material as a lowest reference of candidate. SS304 is conventional acupuncture needle material used in EA also. Ni-coated SS 304 was evaluated as composite material combined high conductive material with a conventional EA needle material, for batter electrical conductivity. Ti-6Al-4V is the most widely used titanium alloy in medical implant and known very safe. For this reason, we choose the material as a highest reference candidate. SS 316 has also been evaluated as a good candidate for the reason wide use in medical implant also, and other various invasive device material. A biocompatibility study, economic analysis, and corrosion testing after the application of electrical current showed that PB was unusable due to the severe cytotoxicity it displayed and that SS 304 Ni was also unsuitable, as it showed low cell viability in stain testing and showed corrosion after the application of current. SS 316 and Ti alloy performed well in terms of cell viability and cytotoxicity and did not exhibit corrosion under 0.5 mA continuous wave, step response, 1 ms duration, and single-phase current for 60 minutes. The results of economic analysis and electrical conductivity testing showed that Ti-6Al-4V (1720 KRW/kg) is about 70 times more expensive than SS 316 (24.5 KRW/kg) and that the electrical conductivity of Ti-6Al-4V, at 1.01% (IACS), was about 2.5 times lower than that of SS 316, at 2.5% (IACS). In addition, even in biological safety SS 316 showed better results than Ti-6Al-4V. As a result, SS 316 was selected as a candidate material for an electro acupuncture needle and was then tested for straightness and tensile strength in order to confirm its successful transformation into needle form and its conformity to standard—KS, JIS and GB (Korea Standard— KS, Japanese Industrial Standard—JIS, Guojia Biaozhun/National Standard/China—(GB) specifications. SS 316 satisfied straightness test under a condition of 0.58 m/s ± 0.01 m/s, and its range of tensile strength was 183∼210 kgf/mm^2, which is higher than the range of the currently used SS 304, which is 170∼190 kgf/mm^2. Based upon these overall results, it was confirmed that SS 316 is appropriate for use as a material for an acupuncture needle. This result is further supported by a report from Tang et al. [14] which states that SS 316 demonstrates superior resistance to electrochemical corrosion compared to SS 304 in both body fluid and cell growth environments. Clinical conditions, however, are much more complex than this, and a simple corrosion test in body fluid does not constitute a guarantee of safety. Therefore, wide-ranging research on the safety of an

SS 316 needle with applied current under various conditions must be performed, and, based upon such researches, guidelines for safe usage should be developed for different treatment conditions.

5. Conclusion

In order to develop new material for an acupuncture needle that is safe for electrical current stimulus, 4 types of commercially available materials were tested for their biological safety and risk of corrosion caused by applied current. Based upon our results, the following conclusions were reached.

(1) SS 316 showed best biological safety and cost effectiveness as an electro acupuncture needle material.

(2) Testing for straightness and tensile strength of SS 316 showed that it is suitable as an acupuncture needle under the condition of 0.58 m/s ± 0.01 m/s.

In summary, it was confirmed that a disposable needle capable of transmitting electrical stimulus can be manufactured using SS 316. If an animal study using an SS 316 needle is performed in the future to study the degree of corrosion under various electrical stimulus conditions and to research the materials capacity to provide safe treatment, this will facilitate the development of safer and more effective acupuncture treatment.

Acknowledgments

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Research Article

Heterogeneity of Skin Surface Oxygen Level of Wrist in Relation to Acupuncture Point

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The distribution of partial oxygen pressure (pO_2) is analyzed for the anterior aspect of the left wrist with an amperometric oxygen microsensor composed of a small planar Pt disk-sensing area (diameter = 25 μm). The pO_2 levels vary depending on the measurement location over the wrist skin, and they are systematically monitored in the analysis for both one-dimensional single line (along the wrist transverse crease) and two-dimensional square area of the wrist region. Relatively higher pO_2 values are observed at certain area in close proximity to the position of acupuncture points with statistical significance, indicating strong relationship between oxygen and acupuncture point. The used oxygen microsensor is sensitive enough to detect the pO_2 variation depending on the location. This study may provide information helpful to understand possible physiological roles of the acupuncture points.

1. Introduction

Acupuncture is a method of medical treatments, based on inserting small needles on the specified body skin locations called acupuncture points. The practice of acupuncture as a healing treatment dates back over 2500 years in traditional eastern medicine. In the late 20th century, acupuncture became to be accepted as an alternative and complementary therapy even in western countries including the United States [1]. In fact, the National Institute of Health (NIH) published a consensus on the use acupuncture in the treatment of pain symptom in 1997 [2].

According to acupuncture meridian theory, a network of 12 main meridians passes through body internal organs and links acupuncture points on skin together. Through the meridian channels, a vital energy, called Qi, circulates the body to regulate body functions. Acupuncture is considered to stimulate the Qi circulation to attain the balance of Qi. Although acupuncture practice is widely used for chronic illness, the efficacy and mechanism of the acupuncture action in mediating analgesia still remain controversial. Indeed, the lack of anatomical and scientific evidence supporting the existence of meridians, acupuncture points, and Qi makes more difficult for the acupuncture treatment to be generally accepted in modern science. Some research efforts for anatomical studies of meridians and acupuncture points have been reported [3–11]. However, there is still controversy since the experimental results could not provide direct/obvious evidence, and moreover they often show a lack of reproducibility.

In acupuncture studies, three kinds of Qi are described to be obtained from air, food, and inheritance, suggesting the close relationship between Qi and air (i.e., oxygen) [1]. Oxygen is essential for energy metabolism in most living organisms. Meanwhile, higher expression of nitric oxide (NO) synthase enzyme, producing endogenous NO, was reported around skin acupuncture points and meridians than other areas [12]. NO is a well-known vasodilator increasing

blood flow and volume and therefore relates to oxygen transport in body [13]. From these separate reports, we inferred that acupuncture points were possibly associated with body oxygen supply and, therefore, recently reported the real-time quantitative measurements of oxygen levels on acupuncture points using a highly sensitive electrochemical oxygen microsensor [14]. The localized oxygen levels at small acupuncture points and at nearby nonacupuncture points were measured successfully, because of the small planar dimension of the sensor (sensing diameter = 25 μm). In fact, the oxygen levels measured at two acupuncture points (LI4 (Hegu) and PC8 (Laogong)) were observed to be higher than those at the corresponding nonacupuncture points, providing an evidence of the physical existence of acupuncture points which may be functionally connected with the oxygen supply [14]. Advanced from the previous work, this paper reports the blind measurements of oxygen levels within confined areas of wrist skin surface and the relationship between the oxygen levels with acupuncture points.

2. Materials and Methods

2.1. Electrochemical Oxygen Microsensor. A clark-type amperometric microsensor for selective oxygen measurement was fabricated as described previously [14]. The oxygen microsensor consists of a glass-sealed Pt disk cathode (Pt diameter = 25 μm, Good Fellow) and a coiled Ag/AgCl wire anode (127-μm diameter, A-M Systems) covered with PTFE gas-permeable membrane (W. L. Gore & Associates, thickness < 19 μm, porosity 50%, pore size 0.05 μm). The composition of an internal solution, both the cathode and anode, is 30 mM NaCl and 0.3 mM HCl in deionized water. The surface of the Pt disk cathode was electrochemically platinized using platinizing solution (YSI Inc., Yellow Springs, OH) to increase the real active surface of the electrode and eventually to enhance the sensor sensitivity to oxygen [15]. A potential of −0.6 V (versus Ag/AgCl anode) was applied to the Pt cathode where the electrochemical reduction of oxygen occurs favorably at this potential. The current between the cathode and anode, induced by the oxygen reduction, was monitored as a function of time using CHI1000A electrochemical analyzer (CH Instruments Inc., USA). As-prepared oxygen microsensor was calibrated before and after oxygen measurements by recording the sensor current at −0.6 V with successive several injections of a given amount of phosphate-buffered saline (PBS, pH 7.4, Fisher Scientific) solution saturated with oxygen into deaerated PBS (pH 7.4) solution to alter the oxygen concentrations.

2.2. Oxygen Measurements on Wrist Skin. The experimental details for the measurements of oxygen levels on skin are described previously [14]. Briefly, the prepared oxygen microsensor was positioned above the first wrist skin point of interest, which was wetted with a drop of PBS (pH = 7.4) solution (15 μL). A micromanipulator (World Precision Instrumentation Inc., Sarasota, FL, USA) was used to position the sensor and maintain the separation between the sensor and skin surface, ∼1 mm. Then, the sensor current between the cathode and anode, which is proportional to the partial oxygen pressure (pO_2), was recorded using an electrochemical analyzer. Once the measured current reached to a quite stable one, the sensor was moved horizontally to the second skin point of interest while the sensor current was monitored continuously. After the stable current was achieved at the second point, the sensor was moved to the third point to measure the pO_2 level at that location. This whole procedure was repeated until the measurements of pO_2 levels for all the projected points were finished.

The measurements of pO_2 levels were performed for (1) one-dimensional single line and (2) two-dimensional square area within the wrist independently. For the one-dimensional experiment, the sensor currents responding to pO_2 levels were measured at 15 different points along the lateral line on the anterior aspect of the left hand-wrist transverse crease. The 15 points were evenly distributed with the same separation (d = 3–3.5 mm depending on individual subject) between two adjacent points along the transverse wrist crease line. The first point and the last 15th point were positioned 5 mm apart from the left and right sides of the wrist as shown in Figure 1(a).

For the two-dimensional analysis, the first 5 points were evenly positioned with the same separation between two adjacent points (d = 10–12 mm depending on individual subject) along the lateral line on the anterior aspect of the left hand-wrist boundary crease. Again, the first point and the fifth point were positioned 5 mm apart from the left and right sides, respectively. The central five points (nos. 3, 8, 13, 18, and 23) were positioned along the centered vertical line dividing the anterior wrist evenly, with the same separation (d) as the one for the first lateral wrist line. Then, the other points could be distributed while keeping the same point-to-point separation as shown in Figure 1(b).

The measurements were carried out for five healthy volunteers (average age = 24.2) in calm and rest conditions at room temperature. None of the subjects were previously treated with acupuncture needle insertion at the skin locations investigated. The measured sensor currents were converted to the corresponding pO_2 levels using prior calibration data.

2.3. Data Analysis and Statistics. For each volunteer subject, the pO_2 levels measured twice and these two pO_2 values obtained at the same location were averaged, and the standard deviation was also calculated independently. The averaged data obtained at the same skin location of five different subjects were also averaged. The data for a few specific points showing relatively higher pO_2 values than the other region were compared with that at other points exhibiting relatively lower pO_2 values using two tailed t-test with a Bonferroni correction. P value < 0.05 was considered significantly different in statistical meaning.

3. Results and Discussion

The analytical performance of an amperometric oxygen microsensor was characterized. Figure 2(a) shows the dynamic

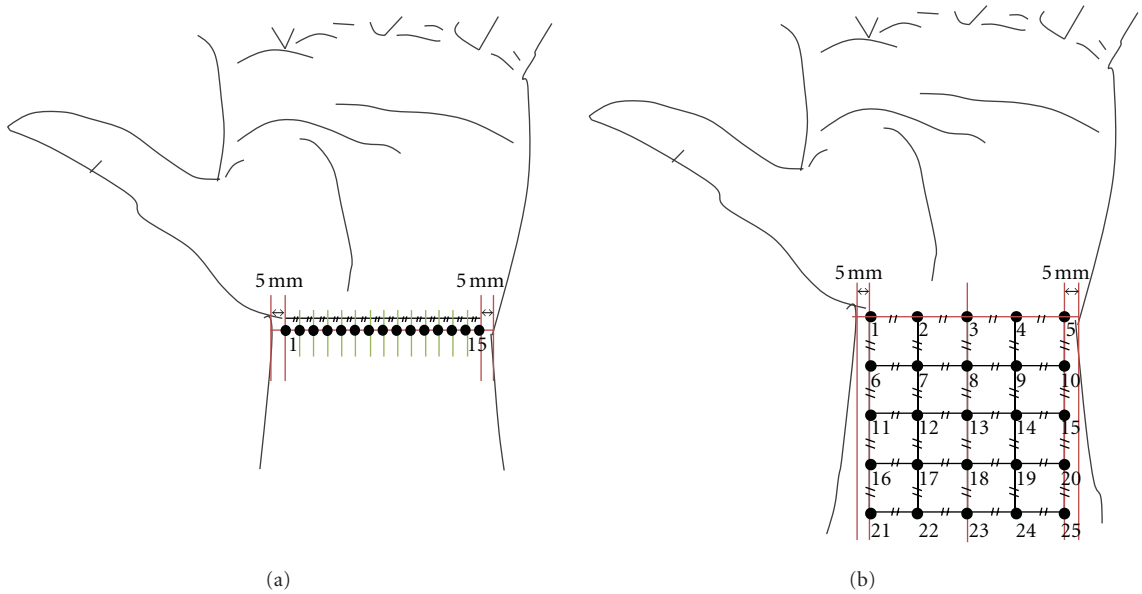

Figure 1: Schematic illustration for the points on the wrist skin where an oxygen microsensor was positioned for pO_2 analysis: (a) one-dimensional and (b) two-dimensional measurement. The points, No. 1, 15 in (a) and the points, No. 1, 5 in (b) were positioned 5 mm apart from the left and right sides of the wrist. Symbol, //, represents the same separation.

sensor response obtained by measuring the sensor current responding to the pO_2 value which was altered by successive injections of a given amount of oxygen standard solution into a deareated PBS sample solution. The sensor current increases in proportion to pO_2 value, and the corresponding calibration curve (Figure 2(b)) shows reasonable linearity and sensitivity of 523.8 ± 58.0 pA/mmHg ($n = 7$). The sensor sensitivity varied within <∼2% before and after the skin oxygen measurements and <0.5% for 10°C temperature change (25–35°C), confirming the sensor stability.

Figure 3 shows typical data showing the pO_2 values monitored at 15 different locations along the transverse wrist crease line. The sensor current measured in terms of time was converted to the pO_2 value based on the prior sensor calibration data. Gray-colored sections represent the measurements made over the projected points with the sensor-to-skin separation of ∼1 mm. Current signals observed in noncolored sections are induced by the sensor movement from one to the other points. In fact, the comparatively higher pO_2 values than the other regions are observed at the regions around the points, nos. 1, 8, 9, and 15. For all five different subjects without exception, similar patterns to Figure 3(a) were observed. Figure 3(b) displays the pO_2 value averaged for five entire subjects (with standard deviation) corresponding to each point. The pO_2 at each point was taken as the average for the data obtained for the last 60 s of the overall measurement time at that point before the sensor movement to another point. This makes sure that the fully equilibrated pO_2 value is obtained. Interestingly, the pO_2 values are observed to be closely related to the positions of acupuncture points. In fact, three acupuncture points, LU9 (Taiyuan), PC7 (Daling), and HT7 (Shenmen) from left to right side, are known along the transverse wrist crease line. Rather large standard deviations of the averaged pO_2 values could be ascribed to the interindividual variation such as wrist circumference. In the one-dimensional study, the measured pO_2 value was in the range of 125–143 mmHg with the greatest difference between the highest and lowest pO_2 that was 5.7–9.2 mmHg depending on the individual subject.

In addition, the pO_2 levels at six representative points (nos. 5, 6, 8, 11, 14, and 15) were compared with one another. A two-tailed t-test with a Bonferroni correction verifies that the relatively higher pO_2 values at the points, nos. 8, 14, and 15 are significantly different from the lower pO_2 values at the points, nos. 5, 6, and 11 (Table 1). Current study is in good agreement with our previous work reporting higher pO_2 levels at the acupuncture points (LI4 and PC8) than at nonacupuncture points.

As indicated in the Methods section, the pO_2 analysis was also carried out at 25 different points evenly distributed in two-dimensional square area over the wrist as depicted in Figure 1(b). The measured pO_2 values showed even higher interindividual variation in this two-dimensional analysis compared to the one-dimensional one. It is presumably considered that the subject body size difference induces a relatively large variance in the analysis of a wider region. Therefore, typical measurement examples are presented without the statistical analysis of the overall subjects.

For convenient comparison purpose, each measured pO_2 was normalized to the average of all 25 values. In fact, the normalized pO_2 was obtained as follows:

$$pO_{2,\mathrm{norm}} = \frac{pO_2}{pO_{2,\mathrm{avg}}}, \tag{1}$$

where $pO_{2,\mathrm{norm}}$ is the normalized pO_2; pO_2 is the measured pO_2 value at each point; $pO_{2,\mathrm{avg}}$ is the average of all the pO_2 values measured at 25 different point for each subject.

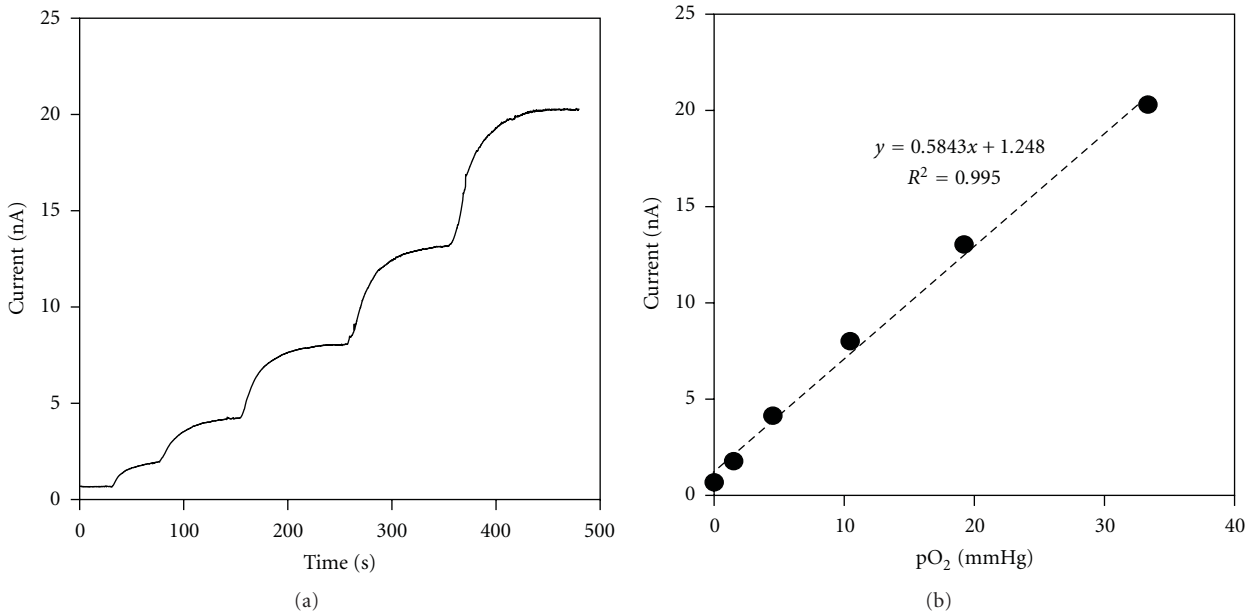

Figure 2: (a) A typical current response curve of an oxygen microsensor with the varied oxygen concentration. (b) Corresponding calibration curve in terms of pO_2.

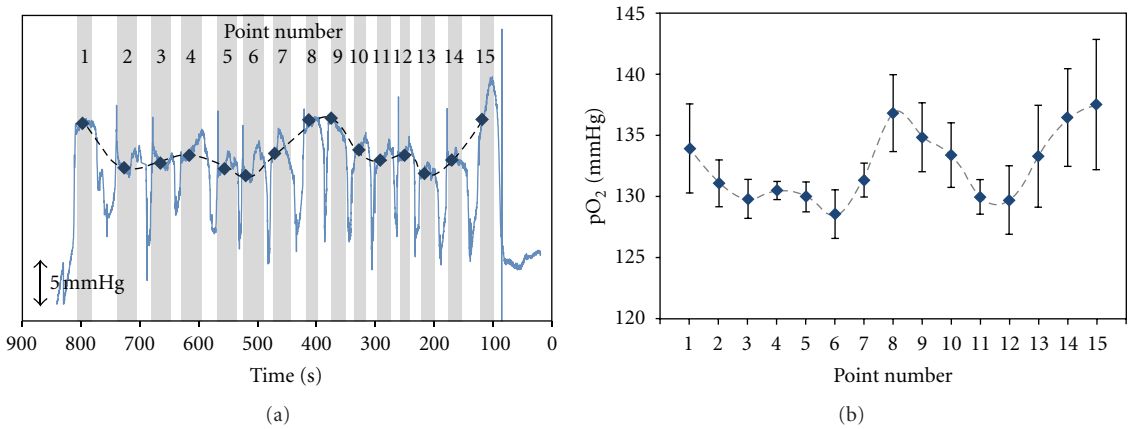

Figure 3: (a) A representative pO_2 measurement along the wrist transverse crease as shown in Figure 1(a). The pO_2 values were measured continuously at 15 different points with the one-dimensional sensor movement. Note that time increases to the left direction in x axis, since the measurement was carried out with the sensor movement from point no. 15 to point no. 1. The scattered symbols representing the pO_2 values measured at corresponding points are overlaid for clear presentation. (b) Averaged pO_2 levels ($n = 5$) for 15 different points. The sensor's measurements at each point were averaged across five subjects. A paired t-test with a Bonferroni correction was conducted for six representative points (nos. 4, 6, 8, 10, 14, and 15), and the P values are summarized in Table 1.

Thus, the points exhibiting the $pO_{2,norm}$ values less or greater than 1 represent the areas where the measured pO_2 is lower or higher than the average, respectively. Each $pO_{2,norm}$ was obtained by averaging the two $pO_{2,norm}$ values obtained from two separate measurements at the corresponding same location for each subject. The average values of 25 pO_2 values were laid within a range of 130–140 mmHg for five subjects.

Figure 4 is the color-coded contour plots of a typical two-dimensional oxygen measurement for one subject. For these contour plots, a linear change in pO_2 was assumed between two adjacent points. As in the one-dimensional experiment, the pO_2 values were varied depending on the locations, showing the heterogeneity of skin oxygen levels. The acupuncture point-high pO_2 relationship is observed more clearly in the two-dimensional analysis. According to Eastern medicine, there are eight acupuncture points known in the wrist region for which the oxygen measurements were carried out: LU9 (Taiyuan) and LU8 (Jingqu) on the lung meridian; PC7 (Daling) and PC6 (Neiguan) on the pericardium meridian; and HT7 (Shenmen), HT6 (Yinxi), HT5 (Tongli), and HT4 (Lingdao) on the heart meridian. The locations of these acupuncture points are indicated in

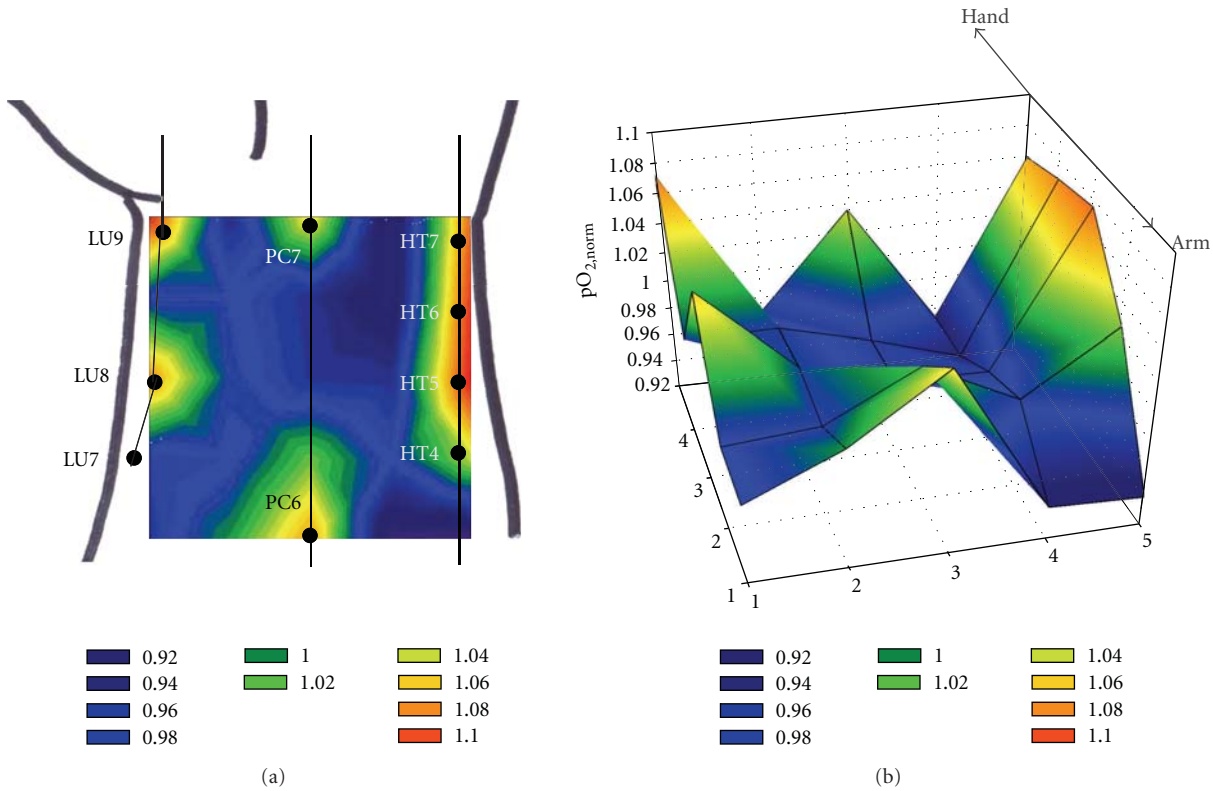

Figure 4: (a) 2-D and (b) 3-D illustration for the color-coded contour plots for a typical example of the two-dimensional oxygen measurement over the wrist skin. A linear change in the $pO_{2,norm}$ values was assumed between two adjacent points.

Table 1: Calculated P values for the paired t-test ($^*P < 0.05$).

Point no.	P6	P8	P11	P14	P15
P5	0.0561	0.0181*	0.962	0.0369*	0.0488*
P6		0.0157*	0.361	0.0267*	0.0403*
P8			0.0015*	0.856	0.677
P11				0.0165*	0.0149*
P14					0.499

Figure 4(a). In fact, the $pO_{2,norm}$ values are greater than 1 (i.e., higher pO_2 level than the average) at the points close to the area where these acupuncture points are supposed to be present. Another typical example is shown in Figure 5. In this example, heterogeneous pO_2 distribution over the wrist is also observed, obviously showing the strong correlation between the acupuncture point and high pO_2 level. Similar pO_2 distribution patterns were observed for overall five subjects.

As indicated in our previous report [14], the measured pO_2 value shows a dependence on the distance between the sensor end plane and skin. In fact, the higher pO_2 was observed when the sensor-skin separation was shorter. However, the pO_2 variation induced by a slight difference in the sensor-to-skin separation during the experimental course was relatively small. In fact, pO_2 variation < 1 mmHg was observed from 10 separate measurements at the same point of one subject, supporting the reliability of the sensor movement/reposition procedure. Since the pO_2 difference depending on the location is reasonably greater than the one induced by the sensor vertical positioning, the observed heterogeneous pO_2 distribution can be considered to be valid.

The reason for the relatively higher pO_2 levels around acupuncture points is not clearly understood yet. One possible explanation is that the oxygen supply by capillary oxygen transport is greater, and the oxygen uptake from the atmosphere is lesser around the acupuncture points than the other area. The oxygen supply to skin was demonstrated as a balance between the oxygen transport by blood and uptake from the atmosphere by Stücker et al. [16, 17]. Therefore, large blood vessels or Primo-nodes/vessels would possibly exist underneath the skin acupuncture points. Primo-vascular system was first proposed to be corresponding to acupuncture points by Kim [18] and recently rediscovered as a new circulatory system by Seoul National University group [19]. Further research, such as pO_2 analysis combined with anatomical study, needs to be performed to clarify the possible relationship between Primo-vascular system and acupuncture points.

Regarding the connection between gas and acupuncture points, higher pO_2 at the acupuncture points was previously reported for rabbits while the pO_2 was measured in the tissue ~0.5 cm below four selected skin acupuncture points (ST36, ST37, CV16, and CV17) with the sensor insertion [20]. In addition, higher transcutaneous carbon dioxide emission at

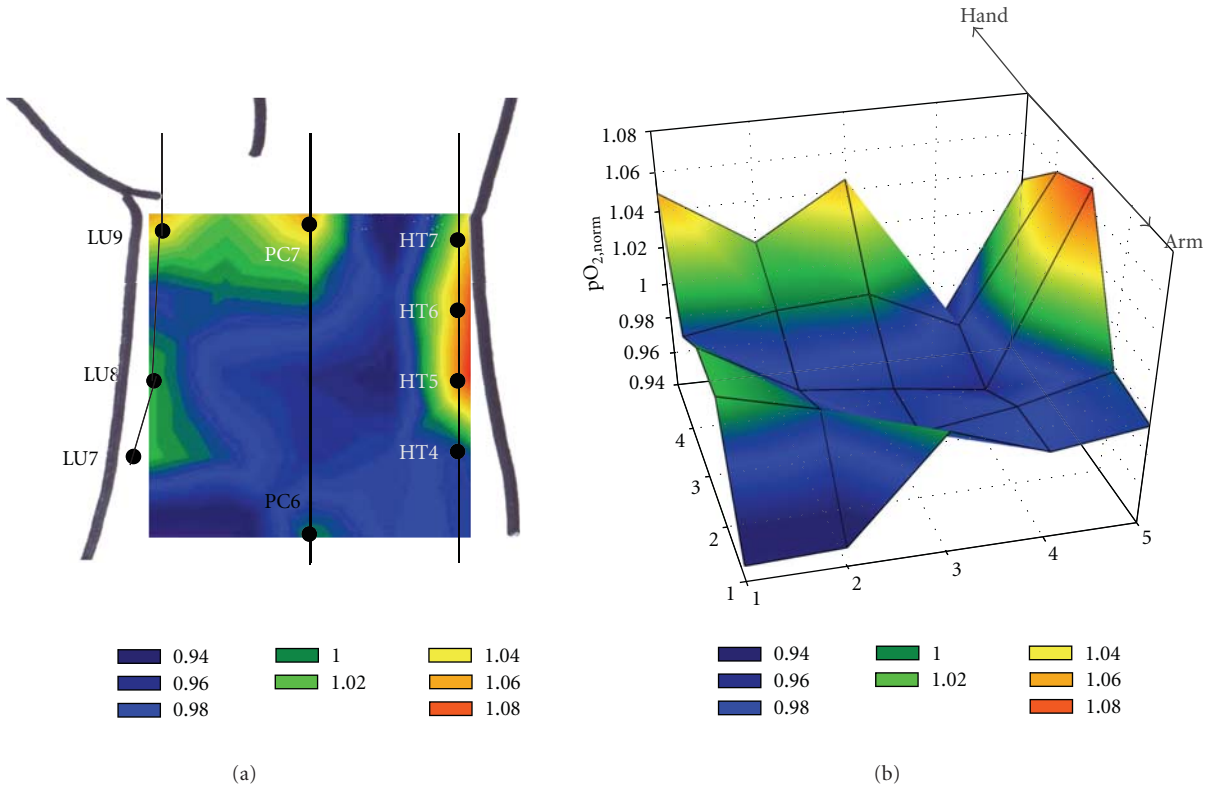

Figure 5: (a) 2-D and (b) 3-D illustration for the color-coded contour plots for another typical example of the two-dimensional oxygen measurement over the wrist skin. A linear change in the $pO_{2,norm}$ values was assumed between two adjacent points.

12 acupuncture points on the pericardium meridian was reported compared with control points beside the meridian line [21]. Including these reports, the research regarding the acupuncture points and meridian has been conducted by comparing the characteristics of acupuncture points with that of control. Our current work provides clearer evidence on the strong pO_2-acupuncture point correlation successfully by extending the analysis to the two-dimensional as well as noninvasive one. This investigation may verify the physical existence of the acupuncture points and provide helpful information to understand their physiological/biological functions believed in Eastern medicine for ages.

4. Conclusions

The skin surface pO_2 levels measured for the anterior aspect of the left wrist varied depending on the locations in both the one-dimensional and two-dimensional analyses. The regions showing relatively higher pO_2 levels compared to the other regions showed a strong correlation to the positions of acupuncture points for entire five subjects without exception. The higher pO_2 values near the acupuncture points were observed with statistical significance. The used amperometric oxygen microsensor to monitor pO_2 provided high sensitivity and small disk sensing area (diameter = 25 μm) which are sufficient to detect the pO_2 variation as a function of location. Current study provides direct and scientific evidence on the physical existence of acupuncture points and may contribute to understand their possible biological/physiological functions.

Authors' Contribution

M. Hong, S. S. Park, and Y. Ha contributed equally to this work.

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Research Article

Thermographical Measuring of the Skin Temperature Using Laser Needle Acupuncture in Preterm Neonates

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In children, laser acupuncture is used more often than needle acupuncture in Western countries, due to their aversion to needles. When applying laser acupuncture to premature babies and neonates, firstly the degree of the thermal increase to the skin has to be evaluated so as to guarantee safe application. The patients were premature neonates before their discharge from hospital. The measurements were carried out by means of a polygraphy while they were asleep shortly. The large intestine 4 acupoint (LI4, *Hegu*) was stimulated by a microlaser needle (10 mW, 685 nm) twice (5 and 10 min). Local thermographic pictures were taken with a thermal camera (Flir i5, Flir Systems Inc., Portland, USA), and the warmest point was determined and subsequently compared. The study included 10 premature neonates (7 male, 3 female). The measurements were carried out on the 33rd day of life (weight 2030 g, gestational age 36 + 3 weeks of pregnancy). In comparison to the initial temperature (32.9°C), after 5 minutes of stimulation (33.9°C) ($P = 0.025$) and also after 10 minutes of stimulation (34.0°C) ($P = 0.01$), there was found to be a significant increase in the skin temperature. The singular maximum value of 37.9°C bears a potential danger; however, compared to the local temperatures reached in transcutaneous blood gas measurements it appears not to entail any risks.

1. Introduction

Based on the aversion children show for needles, the practice of acupuncture in children did not play a great role in therapy in traditional Chinese medicine (TCM) in Western countries. Rather, the pillars of treatment here have always been massage (*Tuina*), diets based on the five elements, and medicinal therapy. If, however, acupuncture is used in children, it is usually limited to brief light needling, or acupressure. This is the reason why, in the past, we found very little in terms of publications on this subject compared to the great interest revealed in acupuncture treatment in adults [1, 2].

However, the last few years have shown an increased interest in the complementary medical treatment of children [3, 4]. Even though, to this day, there exist only a few randomized studies on their treatment by means of acupuncture, this method is seen as efficient and safe provided it is practiced under standard conditions by well-trained acupuncturists [5, 6]. The development of laser acupuncture has opened up new dimensions altogether in the treatment of children [7]. Still, to this day, there is only very limited literature on laser acupuncture in children, with a lack of clear recommendations [8, 9].

Even if only in individual cases, acupuncture in neonates [10] has been carried out as a therapy to combat infantile colic [11–13] applying a light needling technique using single use needles and giving mild stimulation. Laser acupuncture provides a noninvasive therapeutic approach, thus excluding

the risk of infection caused by needle prick injuries [14, 15]. Nevertheless, depending on the relevant laser classification, laser acupuncture cannot be seen as not being dangerous at all since it bears a potential risk for the eyes and the surface of the skin [16]. Since it is especially the skin of premature babies and neonates that exhibits various histological and physiological peculiarities and, additionally, a normalization of the sweating function only sets in over a period of time of the first 6–8 months of life [17], exposure to laser acupuncture and, going hand in hand with it, a warming of the skin would indeed subject this very sensitive patient population to a particularly high risk.

It was the aim of the study to explore whether or not acupuncture by means of laser significantly changes the surface temperature, thus representing a potential risk of application given the circumstances.

2. Materials and Methods

2.1. Probands. The probands were former premature babies of the Division of Neonatology at the Graz University Clinic Department of Pediatrics who all underwent tests in the sleep lab before being discharged from hospital. Their parents were informed about the examination and gave their prior written consent. The study itself was submitted to the Ethics Committee of the Medical University of Graz and approved.

2.2. Laser Acupuncture. The probands were comfortably placed on a Babytherm 8000 incubator (Dräger GmbH, Lübeck, Germany) in the course of the sleep lab examination. In all incidents, a time period of 10 min of waiting was respected before applying the laser needles to give the skin of the neonates a chance to stabilize temperaturewise. The laser needle used for acupuncture (Laserneedle EG GmbH, Berlin, Germany) provides continuous laser light with a wavelength of 685 nm and an output power of 10 mW per laser needle.

Then, after a waiting period of 25 min, laser needle acupuncture was performed simultaneously in both arms on the large intestine 4 (*Hegu*) point. The first stimulation carried out lasted for 5 min. After an interval of 10 min, a second stimulation was carried out in the same way, but this time lasting for 10 min.

We attempted to select an acupoint that is one of the most commonly used acupuncture points in TCM: large intestine 4 (LI4, *Hegu*). LI4 (*Hegu*) is located in the large intestine meridian in the middle of the 2nd metacarpal bone on the radial side. There are a total of 20 points on the large intestine meridian. The pathway begins on the index finger and travels along the arm, over the shoulder to end on the face just to the outside of the nose.

2.3. Thermography. Before laser acupuncture application, as well as 1 min, 5 min, and 10 min after, respectively, thermographic pictures of both the left and right hands were taken by means of a thermal camera (Flir i5, Flir Systems Inc., Portland, USA). Subsequently, the warmest spot was identified and reidentified and compared in the course of time.

Figure 1: Infrared analytic measurement setup.

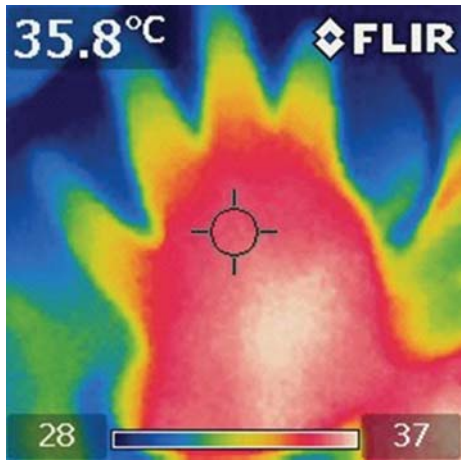

Figure 2: Thermogram of the hand after laser needle stimulation.

Figure 1 shows the use of the thermal camera during the examination, and Figure 2 gives an example of the pictures taken with the use of the thermal camera.

Additionally in the course of the examination, any parameters measurable in the context of polygraphy, such as the heart rate, oxygen saturation, end-expiratory CO_2, and breathing movements including electroencephalography (EEG), were recorded and analyzed. Throughout the examination, the ambient temperature and humidity were kept constant. Atthat point in time of the examination, no medication modifying the blood circulation was administered.

2.4. Statistics. All data was taken as a mean value ± SD (standard deviation). The statistical evaluation was done using ANOVA test for repeated measurings and the Tukey test, respectively.

3. Results

All together 10 neonates (7 male, 3 female, gestational age (GA) 31 + 5 weeks of pregnancy, birth weight 1703 g) were included in the study. On average, the measurings were carried out on the 33rd day after birth (weight at that point in time of examination 2030 g, GA 36 + 3 weeks of pregnancy).

Table 1: Demographic data of the 10 participants of the study.

Number of patients	10
(male/female)	(7/3)
Mean GA	31 weeks + 5 days
Mean birth weight	1703 g (SD = 513.4)
Mean arterial PH from umbilical cord sampling	7.28 (SD = 0.06)
Mean APGAR 1	6.7 (SD = 1.19)
Mean APGAR 5	8.3 (SD = 1.1)
mean APGAR 10	9 (SD = 0.63)
Mean corrected GA, at the time of investigation	36 weeks + 3 days
Mean postgestational age at the time of investigation	33 days (SD = 22)
Mean weight at the time of investigation	2030 g (SD = 250.8)

GA: gestational age; SD: standard deviation of the mean; APGAR: Apgar-Score.

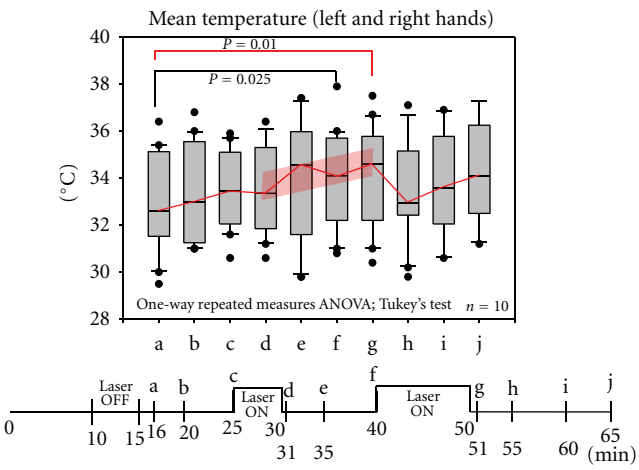

Figure 3: Results and time curve of the examination.

Table 1 gives the data of the children involved in the study.

Altogether, 20 thermographical measurings were taken of the above-mentioned measuring points. Compared to the initial temperature of 32.9°C, the skin temperature had significantly risen to 33.9°C ($P = 0.025$) after 5 min of stimulation. Equally, a significant rise in temperature was measured again after 10 min of stimulation (34.0°C) ($P = 0.01$). The maximum measured skin temperature after stimulation was found to be 37.9°C. The parameters comeasured during the examination (heart rate, oxygen saturation, end-expiratory CO_2, and breathing movements including EEG) showed no significant changes.

Figure 3 gives the results and time curve of the examination.

4. Discussion

In the context of acupuncture research, not only central but also peripheral effects, such as any changes in the blood supply of the skin due to acupuncture treatment, play a major role. Any changes in surface temperature can be made visible with the help of thermography. It was the aim of this study to obtain, for the first time, effective data on changes in surface temperature in neonates from laser acupuncture. Thermography, that is, the temperature measured by means of an infrared camera, represents a measuring procedure that has been used before in adults for acupunctural research. The wavelength range of thermal and infrared energy lies beyond the perception threshold of the human eye. Rather, the energy is found in the section of the electromagnetic range perceived by humans as warmth. In contrast to visible light, each object in this segment whose temperature lies above absolute zero radiates heat; the higher the temperature of an object, the more intense the infrared radiation it emits. Infrared cameras produce pictures of the otherwise invisible infrared or heat radiation and allow for very accurate temperature measurements.

Crucial advantages of this procedure lie in the optical and thus touch-free data collection and in the visualization of temperature distributions of a high local resolution. Moreover, the examination technique is of a passive nature, that is, no additional energy is supplied to the body. It is therefore totally harmless and fit to be applied on neonates [18].

The laser acupuncture needles (Laserneedle) [19] are glued to the skin for the acupuncture treatment, which means that pricking is not required. Also, these special needles allow for the simultaneous stimulation of individual point combinations or for the simultaneous stimulation of paired points. The stimulation of more than one acupuncture point using different laser acupunctural gadgets has so far only been possible one after the other, not simultaneously. Both its simple application and basic research up to now have well-established laser needle acupuncture in adults in acupunctural research [20–22].

The point selected for the examination under this study, LI4 (*Hegu*) can be quickly and easily found in adults and neonates alike. LI4 (*Hegu*), is considered one of the most effective acupuncture points for general pain control. Additionally, due to its analgetic effect, the point is applied in neonates suffering from infantile colic, an effect that has been confirmed by several studies obtained so far [11–13, 23].

With the present study carried out by us in 10 former premature babies, a significant rise in skin temperature both after 5 min and after 10 min of laser acupuncture treatment using laser needle 10 mW/685 nm was measured in the point LI4 (*Hegu*) compared to the initial temperature of 32.9°C.

Especially in premature babies and neonates whose skin is altogether thin and shows physiological and histological peculiarities, it is important to note that there is a potential danger of damage to the skin. On the one hand, the skin of neonates in the first few months of life contains the so-called fetal collagen (type III) and less elastin in proportion to the skin of adults. On the other hand, due to a lack of dermal papilla, there is a reduced dermoepidermal interaction of

epidermis and dermis. While the *stratum basale* immediately after birth runs almost parallel to the surface, increasingly in the course of the first few months dermal papilla is formed, a process which finally has the effect that a smooth interaction of both skin layers is achieved [24]. On top of that, the body function of thermal sweating is not fully mature in neonates, that is, the induction threshold to sweating in them is higher than in adults. In fact, the limit value of the induction threshold is dependent on the gestation age. With premature babies, we find anhidrosis in the first few days of life, with the sweating function only normalising in the course of the first 6–8 months of life [25]. It is these histological and physiological differences between premature babies and neonates on the one hand, and adults on the other hand, that explain the increased sensitivity of the skin of neonates showing, for example, in more redness of the skin and a higher likeliness for hematoma following needle acupuncture [26].

On average, a rise in the local temperature by 1 degree Celsius was found to occur after 5–10 min of laser acupuncture using laser needle 10 mW/685 nm. The highest measured skin temperature after stimulation amounted to 37.9°C. However, compared to the local temperatures reached in transcutaneous blood gas measuring [27] still used in neonatal intensive care units in the monitoring of premature babies and neonates [28, 29], the warming of the skin after 5 and 10 min, respectively, of laser needle acupuncture using laser needle (10 mW/685 nm) seems not to represent any risks.

Several studies in the field of both manual needle acupuncture and electrically stimulated acupuncture have revealed local and general warming effects as an indicator of a reduced sympathetic activity [30, 31]. However, the question as to whether or not changes in the surface temperature in connection with the application of laser acupuncture in premature babies and neonates are to be understood as changes in the sympathetic activity and therefore as an effect directly resulting from acupuncture could not be answered by this study. In the parameters additionally measured in the course of the examinations under this study (heart rate, oxygen saturation, end-expiratory CO_2, breathing movements including EEG) no significant changes were found.

5. Conclusion

By way of conclusion it can be said that a significantly raised skin temperature was measured both after 5 and after 10 min of application of local laser needle acupuncture. On average, this rise in local temperature amounted to 1 degree Celsius. Although only found once, the maximum temperature measured of 37.9°C revealed the potential danger of a local warming of the skin. Nevertheless, compared to the local temperatures reached in transcutaneous blood gas measuring [27], laser needle acupuncture applied by way of the procedure described above appears not to bear any risks.

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Conflict of Interests

The authors declare no conflict of interests.

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Research Article

The Effects of Scraping Therapy on Local Temperature and Blood Perfusion Volume in Healthy Subjects

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Objective. We aim to study the therapeutic effects of scraping by investigating the changes of temperature and local blood perfusion volume in healthy subjects after scraping stimulation, and to explore the mechanism of scraping stimulation from the points of microcirculation and energy metabolism. *Methods.* Twenty-three health subjects were included in this study. Local blood perfusion volume and body surface temperature was detected at 5 min before scraping stimulation, 0, 15, 30, 60 and 90 min after scraping using Laser Doppler imager and infrared thermograph. *Results.* Significant increase was noted in the blood perfusion volume in the scraping area within 90 minutes compared to the baseline level and non-scraping area ($P < 0.001$). Compared with non-scraping area, an increase of body temperature with an average of 1°C was observed after scraping stimulation ($P < 0.01$). *Conclusion.* Scraping can significantly improve the blood perfusion volume and increase the temperature in the scraping area, promoting the local blood circulation and energy metabolism.

1. Introduction

Scraping, called Gua Sha in Traditional Chinese medicine (TCM), is one of the unique non-medicinal external therapies of TCM under the guidance of the theory of meridians and acupoints. It involves using a smooth-edged instrument for surface frictioning to intentionally raise transitory petechiae and ecchymosis [1, 2]. To date, scraping has shown pain-relieving effects on myalgia and chronic pain [2–5], and can improve blood stasis and inflammation [6]. Although several reports indicated the effects of scraping therapy in clinical and experimental practices, the mechanism is still not clear. Recently, Tian et al. reported blood perfusion volume increased immediately after skin scraping in rabbits using laser Doppler imager [7]. In this study, we aim to determine the changes of the local blood perfusion volume and skin temperature after scraping in healthy subjects.

2. Methods and Materials

2.1. Laser Doppler Imager. PeriScan PIM II Laser Doppler Perfusion Imager (LDPI; Perimed AB, Jarfalla, Sweden) was used to measure skin perfusion volume. A low power 670 nm wavelength was applied. A medium scanning pattern was used. The image primitive was set as 0.75 mm × 0.75 mm. The image size was set at 40 mm × 40 mm. The apparatus was connected to a PC computer to obtain the blood flow images of the body surface. The laser blood flow image and visual image of the detected areas were measured via LDPI 2.5 Image Software. The blood flow of the body surface was measured by Doppler frequency shifts which is proportional to a blood flow-related variable and is expressed in arbitrary perfusion unit (PU) [8]. The blood perfusion volume and the body position at different time points were analyzed after comparing the laser blood flow images and direct images demonstrated by the Laser Doppler Perfusion Imager.

2.2. Infrared Thermograph. WP-1 type of infrared thermograph with a temperature resolution of 0.08°C was applied for thermal images in our study. Based on the infrared radiation photography, the apparatus was connected to a PC computer to convert thermo energy into temperature. The distributions and changes of body temperature were displayed as colorful images. A 3.41 version image processing software was used to analyze the data obtained from the images.

Table 1: Blood perfusion volume at different time points in scraping area and non-scraping area (PU) , ($n = 23$, $\bar{x} \pm$ SD).

PU	Scraping area (right)	Non scraping area (left)	P values
Before scraping	0.469 ± 0.103	0.453 ± 0.105	$P > 0.05$
immediately	0.966 ± 0.203	0.465 ± 0.089	$P < 0.001$
15 min	0.685 ± 0.158	0.483 ± 0.076	$P < 0.001$
30 min	0.586 ± 0.075	0.510 ± 0.080	$P < 0.001$
60 min	0.553 ± 0.064	0.504 ± 0.061	$P < 0.001$
90 min	0.558 ± 0.066	0.514 ± 0.052	$P < 0.001$

2.3. Scraping Stimulation. Scraping stimulation was performed by trained therapists using a buffalo horn scraper and a skin lubricant (Jinlongkang, Beijing Jinlong Kang Er Fu Scraping Cupping Research Institute, Beijing, China) to decrease friction. Scraping was conducted on the erector spinal muscle above the back spine (from C7-T10) along the bladder meridian of the right side. The scraping areas is 6-7 cm in width and 20 cm in length. Infrared thermal images were collected at scraping area from C7 to T7. Laser Doppler images were collected in two areas at the scanning centers of bilateral sides of the back spine (scraping area and non-scraping area at the opposite side) respectively. The areas were 4.5 cm lateral to the spinous process of the 4th thoracic vertebra.

2.4. Subjects. Twenty-three healthy subjects (12 males, 11 females) aged from 20 to 40 years old were enrolled after physical examination. Laboratory room temperature were maintained at 24°C–27°C without direct sunlight, infrared radiation, and indoor/outdoor ventilation.

2.5. Experimental Procedure. The subjects were seated in a square stool in the laboratory with their back exposed. Before collecting the infrared temperature images, the subjects were needed to stay calm for 15 min to adapt to the room temperature. Infrared temperature images were collected at a sitting position. Then laser Doppler images of both sides of the selected areas at a prone position. After scraping for 5 min, both infrared temperature images and the laser Doppler images of the above areas mentioned were collected immediately after scraping (0 min), 15 min, 30 min, 60 min, and 90 min after scraping respectively.

2.6. Data Collection. For infrared thermal images, the subject sat erectly at a distance of 1.5 m to the infrared thermograph. Then the detected area was determined and fixed with a calibration circle. The thermographic imaging system was input into a PC computer to save the infrared images and thermal images. For laser Doppler imaging, the blood perfusion volume of the selected scraping area on the right back and symmetrical non-scraping area on the left side were collected by laser Doppler imager. The images were processed by LDPI 2.5 imaging software for offline analysis.

2.7. Statistical Analysis. Data were all presented as mean ± SD. Statistical analysis was performed using SPSS 17.0 Software. A Student's t test was performed for the analysis of changes of temperature and blood perfusion volume between pre- and post-scraping, and scraping and non-scraping at different time points. $P < 0.05$ was considered as statistical significance.

3. Results

After scraping, all the 23 subjects (100%) reported obviously warm accompanied by slight pain at the scraping area. They all felt relax and comfort after scraping. It was observed that the skin became slightly red, and then subcutaneous hyperaemia and subcutaneous bloody spots were found in the local scraping area.

3.1. Changes of Blood Perfusion Volume before and after Scraping. Significant increase of blood volume was observed in the scraping area compared with the baseline level. PU values were 1.0-fold higher compared with the baseline level (0.966 ± 0.203 versus 0.469 ± 0.103, Table 1). Significant difference was noted in the blood perfusion volume within 90 minutes after scraping compared with the non-scraping area. ($P < 0.001$; Figures 1 and 2, Table 1).

3.2. Changes of the Local Skin Temperature before and after Scrapin. As is shown by infrared thermograph, the skin temperature of the scraping area increased significantly with the average temperature increased more than 1°C. Compared with the skin temperature obtained in the opposite non-scraping area and the scraping area before stimulation, significant increase of skin temperature was observed within 90 minutes after scraping, respectively ($P < 0.05$, Figures 3, and 4, Table 2).

3.3. Correlation of Changes of Temperature and Blood Perfusion Volume in the Scraping Area. With regard to the skin temperature and local blood volume obtained within 90 minutes after scraping, a close correlation was noted between skin temperature and the local blood volume in the scraping area ($r = 0.383$, $P < 0.01$, Figure 5). Both temperature and blood flow perfusion values were still higher 90 min after scraping compared with the baseline level (Tables 1, and 2).

4. Discussion

Scraping, called Gua Sha in TCM, is one of the physical stimulating therapies. Previous reports indicated that physical therapies such as acupuncture, moxibustion, massage, scraping and cupping basically shared similarities in their functions and mechanisms as they all developed from external

Table 2: infrared temperature at different time points in the scraping area and non-scraping area ($n = 23$, $\bar{x} \pm$ SD), $^{**}P < 0.01$.

(°C)	Scraping area (right)	Non scraping area (left)	P values
Before scraping	33.057 ± 1.116	32.989 ± 1.137	$P > 0.05$
Immediately	34.837 ± 0.743	33.233 ± 0.851	$P < 0.001$
15 min	34.703 ± 0.614	33.633 ± 0.673	$P < 0.001$
30 min	34.343 ± 0.855	33.640 ± 0.733	$P < 0.001$
60 min	34.123 ± 0.769	33.688 ± 0.674	$P < 0.001$
90 min	34.065 ± 0.838	33.771 ± 0.69	$P < 0.01$

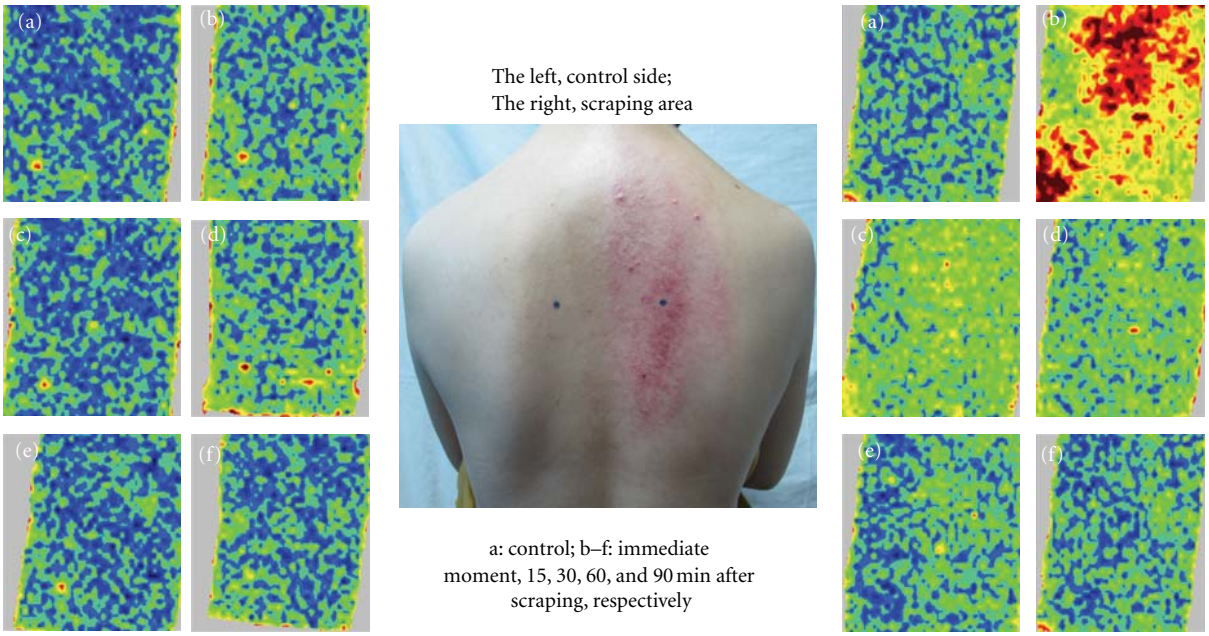

Figure 1: Visual image (middle) taken at 5 min after scraping showed that the skin of the scraping area turned apparently red. Laser Doppler images (left, non-scraping side; right, scraping side) showed the blood perfusion volume. Images (a)–(f) were taken at 5 min before scraping, 0 min, 15 min, 30 min, 60 min and 90 min after scraping stimulation, respectively.

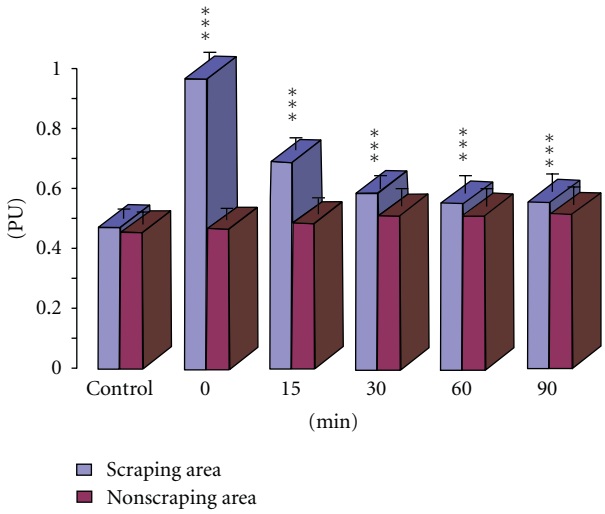

Figure 2: Changes of blood perfusion volume in scraping area and non-scraping area. $^{***}P < 0.001$, compared with non-scraping area at the same time point.

stimulating therapies [9]. In the 56th Chapter of *Plain Questions*, an ancient works in TCM, it mentioned that "the 12 meridians and collaterals distributed in their relevant cutaneous regions". Zeng (1999) reported that the scraping performed by stimulating the collaterals on the surface of the body was efficient for the treatment of certain diseases. Therefore, the author speculated that the efficiency of scraping therapy is closely related with the function of collaterals [10]. Though several studies reported the effects of scraping therapy in clinical practices [2–5], its mechanism is still not well defined. In this study, Laser Doppler imager and infrared thermograph were used to detect the effects of scraping therapy on local temperature and blood perfusion volume of human body surface. Macroscopic observations and infrared images showed apparent changes of the local skin color and temperature before and after scraping. Furthermore, quantitative analysis indicated scraping could increase the local microcirculation and metabolism of subcutaneous tissues.

Skin, covering the body surface, contains abundant capillaries functioned as the major organ for temperature regulation and body defense. Under normal conditions, the blood

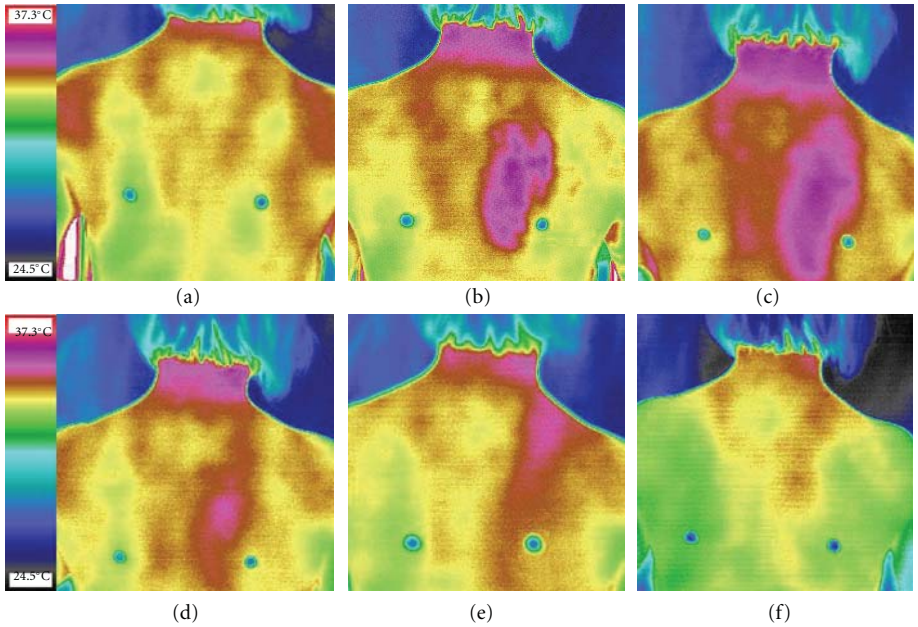

Figure 3: The infrared thermograph images showed the skin temperature of the right body side (scraping) increased significantly after stimulation. Skin temperature increased in the scraping area and extended onto the opposite side and the neck 15 min after scraping. The local temperature increase lasted about 1 hour. (a)–(f): image otabined at 5 min before scraping, 0, 15, 30, 60, and 90 min after scraping.

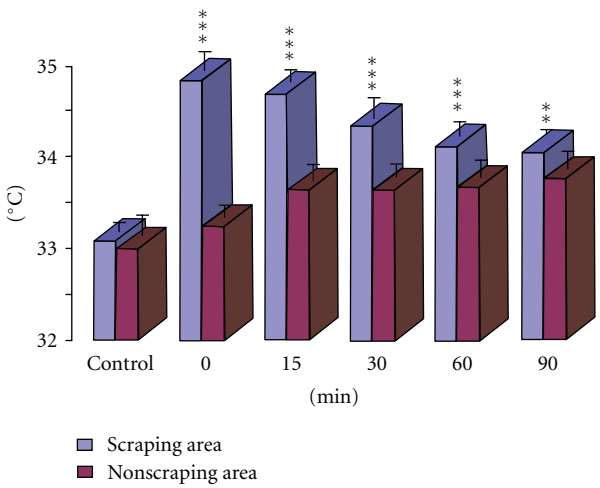

Figure 4: Changes of temperature in the scraping area and non-scraping area. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with non-scraping area at the same time point.

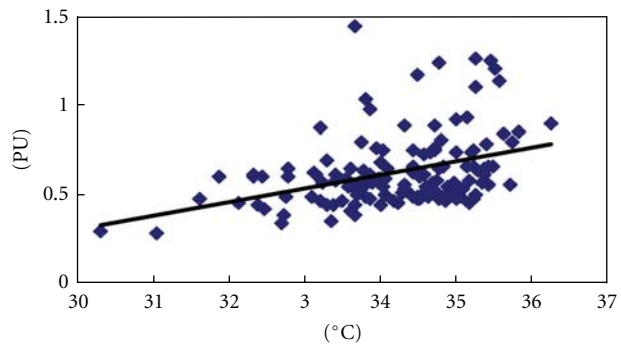

Figure 5: Correlation analysis between temperature and blood perfusion volume in the scraping area.

volume of microcirculation is in accordance with the metabolism level of the tissues and organs to keep a dynamic balance. The capacity and rate of substance exchange of external and internal capillary mainly depended on the open volume and permeability of the true capillary. The present study showed that the the blood flow volume in the scraping area significantly increased, especially immediately after scraping. The values of the blood flow increased 1.0-fold higher in the scraping area than those of the non-scraping area (Table 1). Our study is in accordance with the previous report which indicated that Gua Sha caused a 4.0-fold increase in microcirculation PUs at the scraping area for the first 7.5 minutes together with a significant increase in surface microcirculation during the entire 25 minutes of the study period following scraping stimulation ($P < 0.001$) [2]. The obvious increase of blood perfusion volume indicated that scraping stimulation could reflexively regulate the sympathetic vasodilator nerves to relax the precapillary sphincter, increase the local volume of blood flow and the amount of the opening capillaries directly, and promote local blood circulation. Scraping stimulation was possible to cause partial subcutaneous bleeding of the capillaries, resulted in hyperaemia or blood stasis [7], which can otherwise promote the metabolism of the tissues and improve local microcirculation [11–14]. According to the infrared thermograph images, a significant increase was noted in the scraping area. As is shown in Table 2, an average of 1°C was noted after scraping

stimulation. Under normal conditions, temperatures at both sides of the body back are nearly the same and symmetrical [15]. Our results also indicated that scraping could lead to a long-lasting (60 min) increase of temperature in the adjacent tissues and even further (Figure 3). It could affect these functions of the surrounding tissues. The effects of scraping to an extended area lies in that it causes more vessel dilation and increase of blood flow volume in the adjacent tissues as the cutaneous arteries trunk on the back are interconnected with each other to form a vessel network [16].

Generally, the blood circulations in human body surface were stable. Once the pressure and muscle relaxation of scraping extruded subcutaneous capillary, capillary network reconstruction and expansion was induced, which resulted in changes of cutaneous blood volume and skin temperature [12, 17]. This phenomenon indicated that scraping could change the subcutaneous micro-vascular pressure, leading to vascular dilation and increase of local temperature and the volume of blood flow of the scraping area. Previous study showed that heat could increase the temperature of the tissues, dilate the capillaries, increase local blood circulation, promote blood and oxygen supply, and strengthen the metabolism of the local tissues [13]. Based on our results, a strict correlation was detected between the blood perfusion volume and skin temperature ($r = 0.383$, $P < 0.01$, Figure 5)

Scraping is performed according to the location of acupuncture points along meridians [18]. According to the previous report, thermal conductivity along meridians and beneath tissues was more remarkable than other parts of the body [19]. In addition, a positive correlation between the therapeutic effects and microcirculatory changes of the suffered areas or relevant points was found [20]. Moreover, a remarkable increase was noted in microcirculation and blood perfusion volume after scraping stimulation in the meridian and points [21]. Our study indicated that the responsive areas of scraping extended to the bladder meridian on both sides of the back spine. embodied by mainly by capillary dilation, obvious temperature change and expanded blood perfusion volume of the scraping areas. Generally, scraping of a tolerable intensity is a positive stimulation on the skin, and can helps to increase the metabolism of the local and adjacent tissues as well as activate physiological functions of the body. The increased temperature and microcirculation could reversely remove the microcirculatory obstruction, especially for arteriole angiectasis and spasm [22]. Scraping, stain stimulation mode, could change the skin color of the local scraped area and produce warming or even slightly pain. A variety of scraping stimulation performed on body surface would help to relieve the muscular spasm and improve the local metabolism of tissues, reduce the tension of blood vessels and nerves, and eliminate or reduce the negative impact of somatic disorders on visceral functions [23]. Therefore, it is an effective way for removing the microcirculatory obstruction.

In our study, Laser Doppler and infrared thermal imaging techniques were used for the first time for the detection of the skin temperature and blood volume in healthy subjects. The effect of scraping therapy was analyzed to clarify the mechanism of scraping from microcirculation and energy metabolism. Our study provided theoretical and clinical guidances on the research of meridians and collaterals for further studies. Further studies about the effects of the different scraping techniques on pressure changes of subcutaneous microcirculatory system, and the influences of scraping stimulation on meridians and collaterals should be performed in the near future.

Acknowledgments

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Research Article

Sino-European Transcontinental Basic and Clinical High-Tech Acupuncture Studies—Part 4: "Fire of Life" Analysis of Heart Rate Variability during Acupuncture in Clinical Studies

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This fourth part of a series of Sino-European high-tech acupuncture studies describes the first clinical transcontinental teleacupuncture measurements in two patients (cervical spine syndrome and tachycardia; both 27 years old) from the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, China. The electrocardiographic data were transferred to the Stronach Research Unit for Complementary and Integrative Laser Medicine and the TCM Research Center in Graz via conventional internet connections. Data analysis was performed in Graz using a new "Fire of Life" heart rate variability analysis. Analysis was performed without any technical problems in both subjects. Heart rate decreased significantly during acupuncture in the two patients from Beijing. At the same time, total HRV increased during acupuncture. The different influences of HRV (respiratory sinus arrhythmia, blood pressure waves, etc.) could be clearly documented using the new "Fire of Life" analysis.

1. Introduction

Recently, we performed several transcontinental acupuncture studies. Parts 1–3 of this series summarize some of our animal experimental and first clinical results, performed between institutions from Graz, Austria, Beijing, China, and Harbin, China [1–3]. Computer analysis of heart rate (HR) and heart rate variability (HRV) allows the identification of specific patterns in the fluctuations of the electrocardiogram (ECG) which reflects the effects of individual mechanisms involved in cardiovascular regulation. Based on the automatic assessment of these patterns, new scientific tools for evaluating the features of cardiovascular control have been developed [4, 5].

HRV has been investigated in normal subjects of various age groups and also in different cardiovascular diseases such as acute myocardial infarction, congestive heart failure, arterial hypertension, diabetes mellitus, and different autonomic dysfunctions [6, 7]. Beside HRV power spectral analysis, the so-called "Fire of Life" analysis (Huntleigh Healthcare, Cardiff, UK) is a new method of visualization of HRV, which has been described only in few scientific publications by our research group [8–12].

The aim of this study was to demonstrate the new "Fire of Life" HRV analysis in two patients from the Capital Medical University in Beijing. In both patients, the same type of monitoring equipment was used (Figure 1).

2. Materials and Methods

2.1. HRV Monitoring. An HRV medilog AR12 (Huntleigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria) system was used for cardiac monitoring in Beijing. The system is designed for a monitoring period of more than 24 hours. The sampling rate of the recorder is 4096 Hz.

Therefore, R waves can be detected extremely accurately. All raw data are stored digitally on special memory cards. The data can be read by an appropriate card reader connected with a standard computer. The dimensions of the used HRV recorder are 70 × 100 × 22 millimeters, the weight is about 95 grams with batteries (compare Figure 1).

2.2. HRV Data Analysis. HRV is measured as the percentage changes in sequential chamber complexes (RR intervals) in the ECG. HRV can be quantified over time using registration of percentage changes in RR intervals in the time domain as well as the changes in the frequency range by analysis of electrocardiographic power spectra. Parameters are recommended by the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [13]. Calculation of ECG power spectra is thought to provide an understanding of the effects of sympathetic and parasympathetic systems on HRV [1–7, 13]. Early work pointed out a few bands in the spectrum of HRV that could be interpreted as markers of physiological relevance. Associated mechanisms are thermoregulation which can be found in the very low-frequency band, blood pressure, and respiratory effects [13].

The new "Fire of Life" software analyzes HRV and displays it in a new way to help judge the function of the autonomic nervous system. Viewing this innovative kind of analysis can help to visualize how well the human body reacts to acupuncture. For offline inspection all ECG raw data can be displayed on a screen.

2.3. Patients. The investigations were performed in two patients (both female and both 27 years old) at the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University. One of them (patient A) had a cervical spine syndrome and the other one (patient B) tachycardia. Both subjects were not taking any medication. The registration of the noninvasive parameters was in accordance with the Declaration of Helsinki of the World Medical Association.

2.4. Procedure. The identical study design was used in both patients and included the following steps: three "Skintact Premier F-55" ECG electrodes (Leonhard Lang GmbH, Innsbruck, Austria) were fixed on the chest. The measurement procedure and the 5-minute segments (altogether 40 min) are shown in Figure 2.

2.5. Acupuncture Points. The following acupuncture points were used in the two patients: patient A (diagnosis: cervical spine syndrome) received manual needle acupuncture at Fengchi (GB20), Neiguan (PC6), and Tianzhu (UB10) and patient B (diagnosis: tachycardia) at Neiguan (PC6). For manual acupuncture stimulation, sterile single-use needles (length: 30 mm; diameter: 0.3 mm, Huan Qiu, Suzhou, China) were inserted perpendicularly to the skin at the respective acupoint(s). The needles were stimulated clockwise and counterclockwise for 15 seconds each, with two rotations per second, resulting in 30 rotations per stimulation. The stimulation was performed immediately after inserting the needle, 10 minutes later, and before removing the needle (see Figure 2).

Figure 1: HRV equipment from Graz used for the clinical investigations at the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University in China.

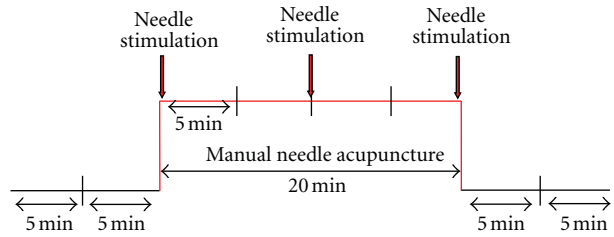


Figure 2: Recording profile. Each analysis segment consisted of 5 minutes. Altogether, a recording session of 40 minutes was performed in each patient.

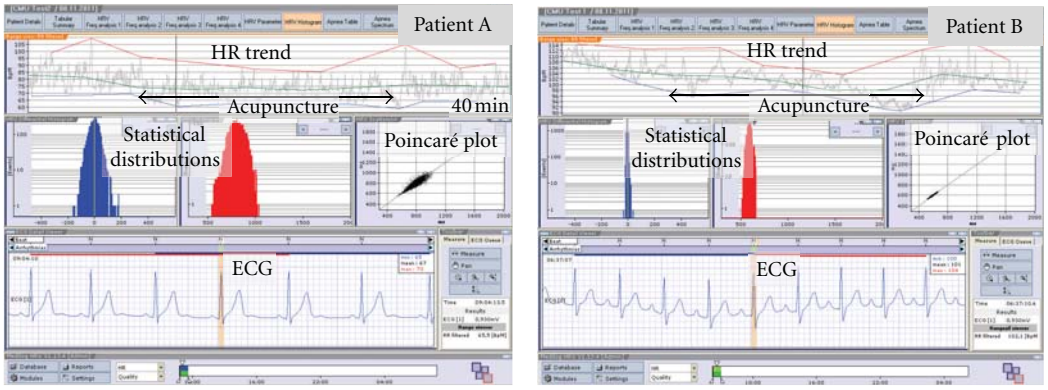
3. Results

Data acquisition and data transfer over a distance of more than 7,600 km between China and Europe were performed without any technical problems.

3.1. Standard Analysis. Figure 3 shows the HR trends (upper panel), the statistical distribution of the RR intervals (middle panel, left and middle), the Poincaré plot (middle panel, right), and the raw ECG (lower panel) which was transferred from Beijing to the TCM Research Center in Graz.

The HR data from patient A over a period of 40 minutes are shown in Figure 3(a) (upper panel). At the beginning of the recording session, the mean HR was about 80/min. There are some minor artefacts during this period caused by movement. In the following acupuncture period, the patient was lying comfortably on a bed. The mean HR during this period was 70/min in patient A (Figure 3(a)) and about 100/min in patient B (Figure 3(b)). After finishing acupuncture, HR increased again slightly in both subjects (A: 75/min, B: 105/min).

3.2. HRV Scatterplots. The "Poincaré" plot is a technique taken from nonlinear dynamics [4, 8]. Figure 4 shows two Poincaré scattergrams in which each RR interval is plotted



(a) (b)

Figure 3: Data analysis of the ECG in the two patients (A and B). Note the decrease in HR in both patients during acupuncture.

as a function of the previous RR interval. These graphical representations of cardiovascular dynamics result in elliptical types of shape (patient A). The ellipse is fitted onto the so-called "line of identity." Standard deviation of the points perpendicular to the line of identity, denoted by SD_1, describes short-term RR variability due to the respiratory component of HRV. The standard deviation along the line of identity, denoted by SD_2, describes long-term variability [14]. In Figures 4(a) and 4(b) the two patients produced two ellipses of different shape and magnitude. Patient A (Figure 4(a)) showed a higher HRV associated with a big ellipse. Patient B (Figure 4(b)) produced an extremely reduced ellipse in which the RR points gravitate around the mean RR and the line of identity.

3.3. HRV Frequency Domain ("Fire of Life" Analysis). The results of the "Fire of Life" HRV analysis of both patients are shown in Figure 5.

At the end of the acupuncture period (25–30 min), a small influence of respiratory sinus arrhythmia (frequency range 0.37–0.40 Hz) is recognizable in patient A (Figure 5(a)). This influence is much smaller in patient B (frequency range 0.28–0.30; Figure 5(b)). In addition the influence of blood pressure waves (frequency ~0.12 Hz) can be observed in patient A. The frequency range <0.05 Hz may also contain slowly changing effects from the renin angiotensin system and temperature regulation. However, the analysis and quantification of these latter parameters only make sense in long-term recordings. After acupuncture, during the last two resting periods (Figure 5(a)), the three main components of HRV analysis (respiratory sinus arrhythmia (very small), blood pressure influence, and thermoregulatory effects) are prominent in patient A. Similar effects, however extremely reduced, are shown in Figure 5(b) in patient B. Not only total variability (reduction of the "Fire of Life" in patient B) but also specific frequency component increases (e.g., influence from blood pressure waves and respiratory sinus arrhythmia) are noticeable. The neuromodulation of HRV is not as pronounced in patient B as in patient A. The "Fire of Life" burns much brighter in patient A than in patient B which can be seen in Figure 5 at the first glance.

4. Discussion

Transcontinental medical studies are rare [15–19]. Main fields of application are surgery [15], epidemiological assessments [16–18], and introducing new fields for academic health centers [19]. However, our research team was the first to perform "teleacupuncture" between Asia and Europe [10, 20, 21]. These first Sino-European transcontinental studies are at the moment mainly based on HR and HRV data acquisition and analysis using new modern methodological approaches like the "Fire of Life" analysis described in this fourth part of the series [1–3].

HRV is an index value of the neurocontrol of the heart. HRV can be quantified by simple calculation of the standard deviation of RR intervals of the cardiac cycles (total HRV) in the time domain [4, 13]. In addition, complex analyses of HRV in the frequency domain using different spectral analysis methods are possible [4, 13]. It is interpreted as a brainstem reflex with an afferent arc via the vagus and glossopharyngeal nerves and an efferent arc mainly via vagal fibres [22]. HRV has stochastic and rhythmic components. With spectral analysis variability can be classified into individual ranges which represent biological rhythms. The following influences can be distinguished for different ranges of HRV: (i) respiratory sinus arrhythmia (approximately 0.15–0.5 Hz); centrally nervous respiratory impulses and interaction with pulmonary afferents; (ii) the so-called "10-s-rhythm" (approximately 0.05–0.15 Hz); natural rhythm of cardiovascularly active neurons in the lower brainstem (circulatory center and its modulation by feedback with natural vasomotoric rhythms via baroreceptor feedback); (iii) longer wave HRV rhythms (approximately < 0.05 Hz); effects from the renin angiotensin system or temperature regulation as well as metabolic processes [4, 8, 13, 22, 23].

The scope of HRV is not yet completely clear, but it is known that there are intraindividual and interindividual variances, and that HRV depends on age, circadian variations (sleep-wake-cycle), physical condition, and mental and physical exertion. HRV can also be affected by diverse conditions such as age-related diseases (diabetic neuropathy, renal failure, essential hypertension, cardiac disorders, coronary

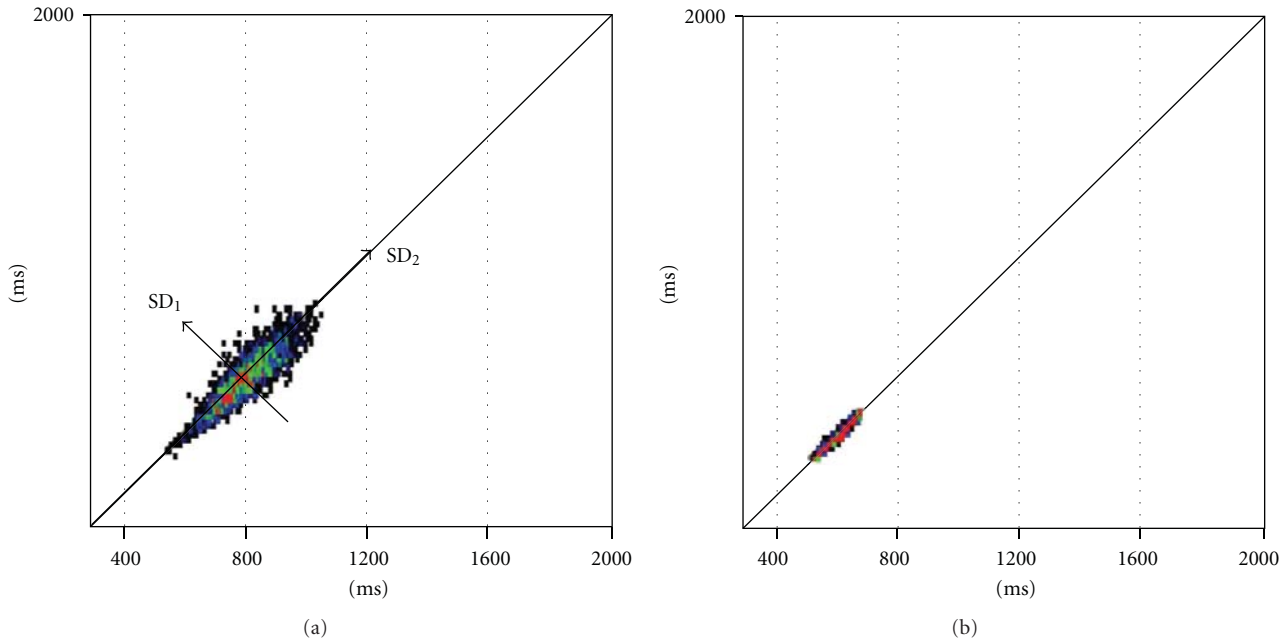


Figure 4: Quantitative beat-to-beat analysis of RR intervals (Poincaré plot). The results of patient A (a) are directly comparable (same scale) to those of patient B (b). Note the different shapes of the ellipses resulting in a different total heart rate variability.

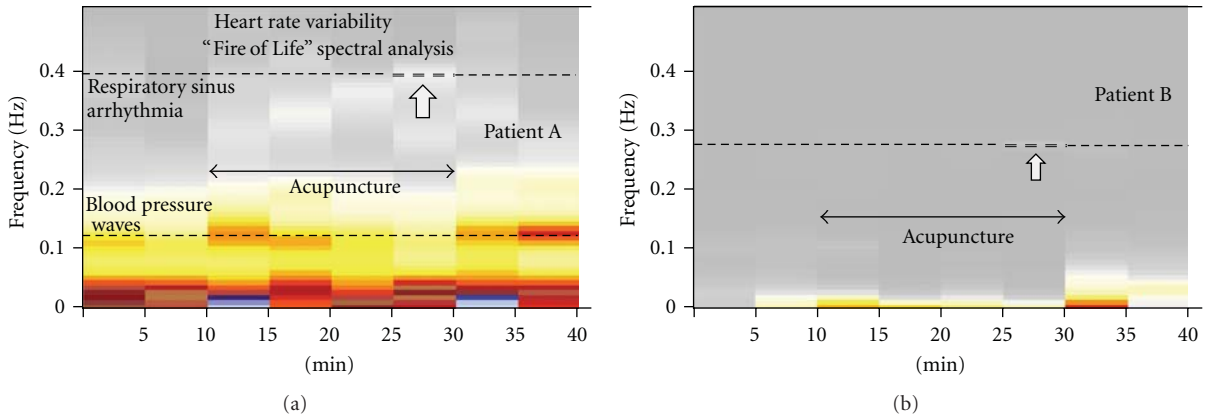


Figure 5: "Fire of Life" power frequency analyses. Heart rate variability (HRV) data of 40 minutes from patient A (a) and patient B (b) are shown.

artery disease, and intracranial lesions) and different medications [8]. The narrowness of HRV after heart transplantation [23] is similar to that seen in deep comatose patients and in brain-dead subjects [22] in whom complex reflex mechanisms are no longer generated or regulated in the brain. In contrast, heart transplantation totally interrupts peripheral autonomic afferences and efferences.

HRV can be used as reliable indicator of the state of health [4, 8, 21]. It becomes less random with the aging process and the appearance of age-related diseases. However, it has been demonstrated that in special syndromes like fatigue and stress, one can counteract this process using different preventive methods like sport [4, 8] or acupuncture [21, 24]. The latter has been demonstrated in recent investigations concerning patients with burnout syndrome as performed in a common teleacupuncture study between Beijing and Graz [21].

5. Conclusions

The following conclusions can be drawn from the results of the two patients of this preliminary study.

(i) Transcontinental data acquisition and analysis could be performed without any technical problems.

(ii) Heart rate changes significantly during acupuncture of Fengchi, Neiguan, and Tianzhu in two patients from Beijing.

(iii) Total HRV increases during acupuncture at the same acupoints as above.

(iv) The different influences of HRV (respiratory sinus arrhythmia, blood pressure waves, etc.) can be clearly documented using the new "Fire of Life" analysis.

Acknowledgments

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Research Article

NMDA Receptors of Gastric-Projecting Neurons in the Dorsal Motor Nucleus of the Vagus Mediate the Regulation of Gastric Emptying by EA at Weishu (BL21)

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A large number of studies have been conducted to explore the efficacy of electroacupuncture (EA) for the treatment of gastrointestinal motility. While several lines of evidence addressed the basic mechanism of EA on gastrointestinal motility regarding effects of limb and abdomen points, the mechanism for effects of the back points on gastric motility still remains unclear. Here we report that the NMDA receptor (NMDAR) antagonist kynurenic acid inhibited the gastric emptying increase induced by high-intensity EA at BL21 and agonist NMDA enhanced the effect of the same treatment. EA at BL21 enhanced NMDAR, but not AMPA receptor (AMPAR) component of miniature excitatory postsynaptic current (mEPSC) in gastric-projecting neurons of the dorsal motor nucleus of the vagus (DMV). In sum, our data demonstrate an important role of NMDAR-mediated synaptic transmission of gastric-projecting DMV neurons in mediating EA at BL21-induced enhancement of gastric emptying.

1. Introduction

Functional gastrointestinal disorders are frequently encountered in ordinary clinical practice. A large number of studies have been conducted to explore the efficacy of electroacupuncture (EA) for the treatment of gastrointestinal motility disorders [1–8]. However, the mechanism of acupuncture on gastrointestinal (GI) motility remains to be clarified. Weishu (BL21), as one of the most common points for gastrointestinal disorders, can accelerate gastric motility [9]. Previous reports demonstrated that EA at various frequencies (1–100 Hz) may stimulate the somatic afferent nerves innervating the skin and muscles of the body and different stimulation procedures are able to excite different somatic afferent fibers and regulate gastric motility through different mechanisms[5, 10, 11].

The inhibitory cutaneojejunal reflex is apparently propriospinal, since it existed in spinal animals, whereas the facilitatory reflex could be supraspinal [11]. The dorsal motor nucleus of the vagus (DMV) plays a critical role in regulation of gastric motility. Receptors of glutamate and GABA in the dorsal vagal complex are involved in regulation of GI motility. GABAA and NMDA receptors are colocalized within DMV neurons projecting to the stomach [12]. Microinjection of glutamate into the DMV enhanced gastric motility [13–15]. On the other hand, microinjection of GABAA antagonists, bicuculline, into the DMV induces dose-dependent increases in gastric motility and secretion [16–18]. Microinjection of GABAA agonist, muscimol, into the DMV abolished bicuculline-induced increases in gastric motility and gastric acid secretion [17, 18]. GABAB receptor antagonist, baclofen, induced outward current of the

gastric-projecting neurons at the DMV [19]. EA stimulates glutaminergic neurons in the brainstem resulting in improvement of stress-induced delay of gastric emptying [20]. In current works, we investigated the functional role of NMDA receptors (NMDARs) and GABA receptors (GABARs) in this process and found that high-intensity EA at BL21 enhanced gastric emptying through NMDARs of DMV.

2. Materials and Methods

2.1. Animals. Male Sprague Dawley (180–200 g) rats were purchased from the Institute of Laboratory Animal Sciences, CAMS & PUMC (Beijing, China), and the Shandong Laboratory animals Center. In this study, all manipulations and procedures were carried out in accordance with The Guide for Care and Use of Laboratory Animals issued by USA National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of China Academy of Chinese Medical Sciences, as well as the Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine. Rats were housed ($23 \pm 1^\circ$C) in groups and maintained under a 12 hours light/dark cycle with food and water available *ad libitum* [21, 22].

2.2. Gastric Emptying Study. As described previously [23], After 24 h of fasting, rats were given preweighed pellets (1.5 g) for 10 min. After the start of feeding (10 min), the residual pellets were withdrawn. Food intake was measured by weighing the spilled and uneaten pellets. Rats were killed by decapitation 90 min after the start of feeding under the ketamine (80 mg/kg) and xylazine (8 mg/kg) anesthesia. The stomach was surgically isolated and removed. The gastric content was recovered from the stomach, dried, and weighed. Solid gastric emptying was calculated according to the following formula

Gastric emptying (%) = (1 − (dried weight of food recovered from stomach)/weight of food intake) × 100 [23].

2.3. Microinjection in DMV. A glass micropipette (ID 0.04 mm, OD 0.12 mm, WPI, USA) with a tip diameter of ~30 μm was stereotaxically implanted at 0.1 to 0.6 mm rostral to calamus scriptorius (CS), 0.3 to 0.6 mm lateral from the midline, and 0.5 to 0.9 mm below the dorsal surface of the medulla. Microinjections of glutamate or GABA receptor (GABAR) antagonist were performed bilaterally via a Hamilton syringe connected to the micropipette, with the movement of the meniscus monitored by a dissecting microscope. Injections were given in volumes of 5 μL over a period of 45–60 seconds. EA-evoked responses were repeated 10 min after the dorsal motor nucleus of the vagus (DMV) microinjection. The locations of microinjection were confirmed by histological verification.

2.4. Histological Verification of Injection Sites. The microinjection site in the brainstem was marked by pontamine sky blue. After fixing in vivo with 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M PBS (pH 7.4), the brainstem was sectioned at 30 μm, and the sections were stained with 0.3% neutral red. The marked microinjection site was located by microscopic examination. Only those data with histological and chemical confirmation were accepted.

2.5. Electroacupuncture Stimulation. BL21 was determined by anatomical marks based on the description in textbooks and previous reports [24]. Briefly, BL21 is located on the back, below the spinous process of the 12th thoracic vertebra, 1.5 cun lateral to the posterior midline (about 8 mm in rats). 10 min after drug administration, BL21 was bilaterally stimulated with a 2-3 mA pulse of 1 ms duration at a frequency of 50 Hz for 15 minutes. The stimulation intensity of a strong nociceptive stimulation which can activate C-fiber (7.68 ± 0.53 mA) was given by a pair of needle electrodes inserted 3 mm depth into the skin. The control acupoint Zhongwan (CV12) located on the median line of the upper abdomen, 15 mm above the umbilicus [22], was also inserted to a depth of 3 mm and stimulated with the same above mentioned intensity. The electrical current for EA was generated by a stimulator (Master-8, Israel).

2.6. Retrograde Labeling. The crystals of retrograde neuronal tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ($DiIC_{18}(3)$; DiI) (Molecular Probes, Eugene, USA) was used to label gastric-projecting neurons of the DMV in 14-day-old male Sprague Dawley rats (Institute of Laboratory Animal Sciences, CAMS & PUMC, Beijing, China). As described previously [25, 26], after anesthetizing deeply with ketamine/xylazine and performing an abdominal laparotomy, DiI crystals were applied to one gastric region per rat (either the major curvature of the fundus or corpus or the antrum-pylorus). To confine the site of application, the crystals were embedded to the application site using a fast-settling epoxy resin that was allowed to harden for several minutes. After closing the laparotomy with 5/0 suture, the animal was placed in the chamber warmed under a radiant heat lamp until normal activity was restored. The animals were then returned to their home cages and allowed to recover for 10–15 days before brain slices were collected.

2.7. Brain Slice Preparation. Thin brainstem slices were prepared from retrograde-labeled rats as described previously with several modifications [25, 26]. Briefly, the rat was sacrificed after being deeply anesthetized with ketamine/xylazine. The whole brain was then removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, bubbled with 95% O_2/5% CO_2, osmolality 300–310 mOsm. After removing the cerebellum, the brainstem was transected rostrally at the level of the pons and again at a point several millimeters caudal to the CS. A vibratome (VT1200S, Leica, German) was used to cut four to five coronal slices (250 μm thickness) containing the DMV. The slices were incubated at 37°C for at least 45 minutes in oxygenated ACSF before use.

2.8. Whole-Cell Recording. A single slice was transferred to the recording chamber and kept in place with a slice anchor

(Warner Instruments, Hamden, USA). The retrograde-labeled DMV neurons were identified under a Nikon E600 microscope (Nikon, Tokyo, Japan) equipped with tetramethylrhodamine isothiocyanate epifluorescence filters. Electrophysiological recordings were made under bright-field illumination after the identity of a labeled neuron was confirmed. The slice was continuously superfused with oxygenated ACSF (2 mL/min) at room temperature. Recording solution containing (in mM): 145 K-gluconate, 7.5 KCl, 9 NaCl, 1 $MgSO_4$, 10 HEPES, 0.2 EGTA, 2 Na-ATP, 0.25 Na-GTP, adjusted to pH 7.4 with KOH, osmolality 290–300 mOsm, was used to back-fill recording electrodes (DC resistance: 5–7 MΩ). Miniature excitatory postsynaptic currents (mEPSCs) were recorded with an Axopatch 200B amplifier (Molecular Devices, USA) and filtered at 2 kHz with a lowpass filter, and data were digitized at 10 kHz and stored online using the pClamp 9 software. The perfusion solution contained 30 μM bicuculline and 1 μM TTX, and the membrane was held at −60 mV. Each data is averaged with 100 events at least. Data were analyzed with the Mini Analysis program (Synaptosoft, Leonia, USA) and WinWCP software (Strathclyde Institute of Pharmacy and Biomedical Sciences, Canada).

2.9. Data Analysis. Data was shown as mean ± SEM. For significance evaluation, data sets with normal distribution were analyzed by unpaired t-test for two groups or one-way ANOVA followed by q test or Dunnett's test for more than two groups, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. The Effect of EA at BL21 on Gastric Emptying. To determine whether EA at BL21 may affect gastric emptying in rats, we designed an experiment in which EA with a stimulation protocol mentioned above was applied at BL21 and selected an abdomen point CV12 as a control it which same stimulation protocol was performed. As shown in Figure 1, EA at BL21 increased gastric emptying capacity (control: 53.80 ± 5.23%; BL21: 68.45 ± 6.12%, $P < 0.05$). vOppositely, EA at CV12 reduced gastric emptying (40.08 ± 4.37%, $P < 0.05$).

3.2. The Role of GABA Receptors in Regulation of Gastric Motility by EA at BL21 Point. To identify the role of GABA receptors in regulation of gastric emptying by electrostimulation at BL21, we stereotaxically microinjected GABA receptor antagonists bicuculline (for GABAA, 5 μL, 12 nM) and phaclofen (for GABAB, 5 μL, 100 nM) through a glass micropipette into DMV 10 min before EA application. Same volume normal saline was microinjected into DMV in control group. After EA for 15 min, the animals were deeply anesthetized with ketamine/xylazine anesthesia, and the stomach was then isolated and removed. The gastric content was dried and weighted. As shown in Figure 2, compared to the control (52.80 ± 4.18%), EA increased gastric emptying significantly (70.08 ± 6.37%, $P < 0.05$). However,

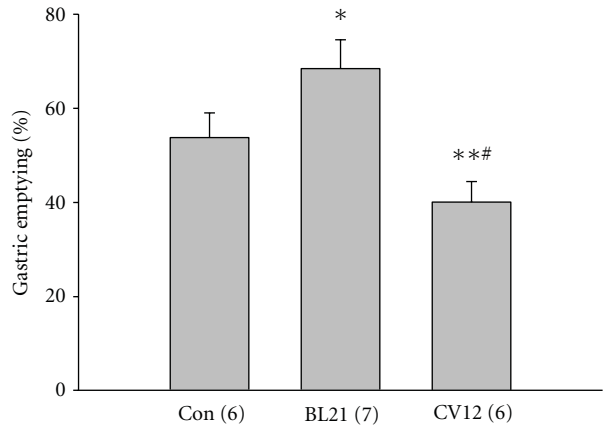

Figure 1: Effects of electroacupuncture (EA) at BL21 and CV12 on gastric emptying. EA at BL21 increases gastric emptying significantly compared to the control (*$P < 0.05$, **$P < 0.01$, one-way ANOVA followed by q test, #$P < 0.05$, compared to BL21).

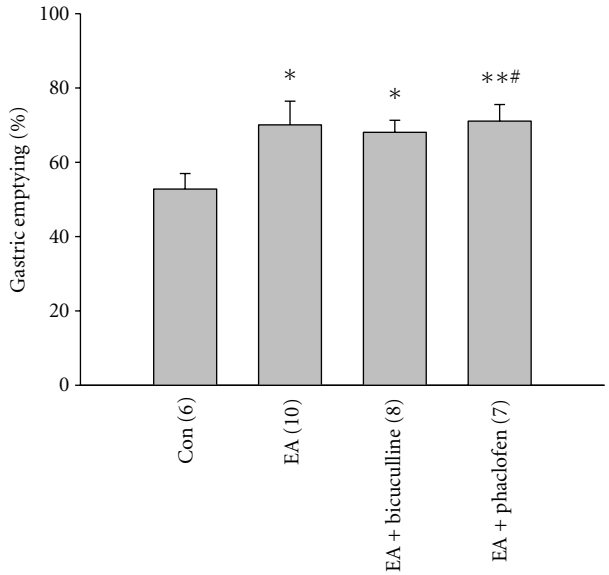

Figure 2: Effects of GABA receptor (GABAR) antagonists, bicuculline and phaclofen, on the regulation of gastric emptying by EA at BL21. EA at BL21 increased gastric emptying significantly but GABAA and GABAB antagonists, bicuculline and phaclofen, did not significantly affect the upregulation of gastric emptying induced by EA at BL21 (*$P < 0.05$, **$P < 0.01$, compared to control, #$P < 0.05$, compared to EA, one-way ANOVA followed by q test).

the emptying capacity enhanced by EA did not change significantly after injection of bicuculline or phaclofen (EA ± bicuculline: 68.07 ± 3.24%; phaclofen: 71.09 ± 4.45%, $P > 0.05$), suggesting that the emptying capacity increased by EA at BL21 might not be mediated by GABA receptors of DMV.

3.3. The Role of NMDA Receptors in Regulation of Gastric Motility by EA at BL21 Point. To identify the role of NMDARs of DMV neurons in upregulation of gastric emptying by EA at BL21, we stereotaxically microinjected NMDAR antagonists, kynurenic acid (5 μL, 0.1 mM), into DMV.

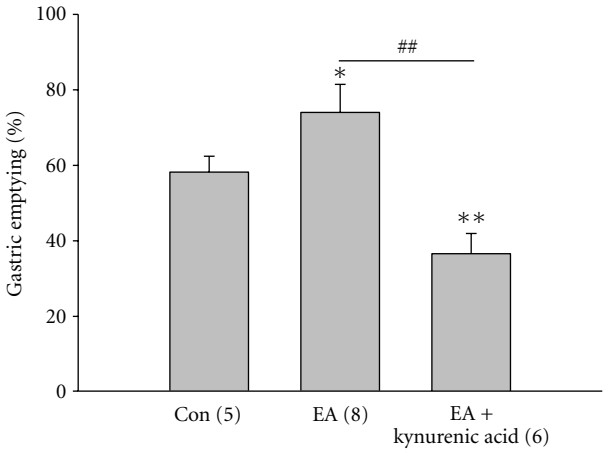

Figure 3: Effect of NMDA receptor (NMDAR) antagonist kynurenic acid on the regulation of gastric emptying by EA at BL21 EA at BL21 increased gastric emptying significantly but NMDAR antagonist kynurenic acid abolished the enhancement of gastric emptying caused by EA at BL21 ($^{*}P < 0.05$, $^{**}P < 0.01$, compared to the control; $^{\#\#}P < 0.01$, compared to EA; one-way ANOVA followed by q test).

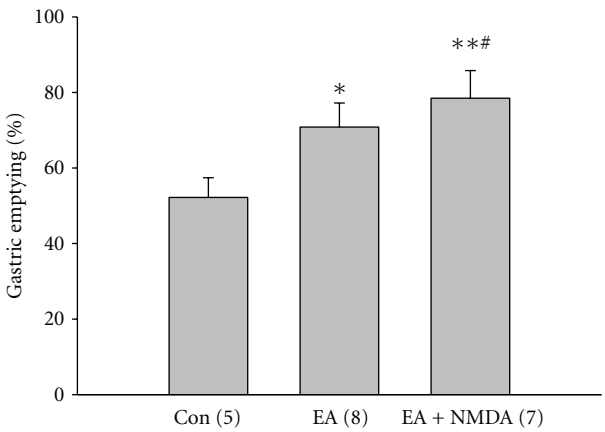

Figure 4: Effect of NMDA receptor (NMDAR) antagonist, kynurenic acid, on the regulation of gastric emptying by EA at BL21. EA at BL21 increased gastric emptying significantly. NMDAR agonist, NMDA, further increased gastric emptying caused by EA at BL21 ($^{*}P < 0.05$, $^{**}P < 0.01$, compared to the control; $^{\#}P < 0.05$, compared to EA; one-way ANOVA followed by q test).

Figure 3 showed that EA increased the gastric emptying significantly (con: 58.20 ± 4.23%; EA: 72.02 ± 7.43%, $P < 0.05$). After kynurenic acid application, the increased gastric emptying by EA decreased significantly (36.59 ± 5.37%, $P < 0.01$). To further confirm whether NMDARs play a role in the upregulation of gastric emptying by EA at BL21, NMDAR agonist, NMDA (5 μL, 10 μM), was microinjected into DMV. As shown in Figure 4, microinjection of NMDA into DMV further increases the gastric emptying induced by EA significantly (EA ± NMDA: 88.47 ± 3.31%, $P < 0.05$). These data suggested that NMDA receptors play an important role in the upregulation of gastric emptying by EA at BL21.

3.4. EA at BL21 Increases NMDA Component of mEPSC but Not AMPA Component. Having identified that the upregulation of gastric emptying by EA at BL21 is mediated by NMDARs of DMV neurons, we went on to identify the effect of EA at BL21 on synaptic transmission in DMV neurons. To address whether EA at BL21 specifically affects the NMDAR-mediated synaptic responses in gastric-projecting DMV neurons, we first used a retrograde tracing marker to label gastric-projecting DMV neurons. Most labeled neurons were localized at the medial DMV, consistent with the previous reports [25, 27, 28]. We applied EA at BL21 for 15 min in rats with retrograde labeling and performed whole-cell recording in acute brainstem slices. Firstly we recorded mEPSCs in the acute slices and separated NMDA and AMPA component using pharmacological approach (Figures 5(a) and 5(b)). In labeled neurons, EA at BL21 did not change frequency of mEPSCs significantly (data not shown). However, as shown in Figure 5(c), EA at BL21 increased NMDA component significantly (control: 116.37 ± 15.83 pA·ms; EA: 170.72 ± 17.05 pA·ms, $P < 0.01$); in contrast, it had no significant effect on AMPA component (control: 257.05 ± 35.41 pA·ms; EA: 263.33 ± 23.70 pA·ms). Figures 5(d) and (e) showed representative traces of mEPSCs recorded in the unlabeled neurons. The frequency of mEPSC did not change significantly (data not shown). Figure 5(f) showed that EA at BL21 did not cause significant changes of either NMDA or AMPA component of mEPSCs in unlabeled neurons (NMDA component: 123.45±17.37 pA·ms for control versus 128.35± 20.34 pA·ms for EA; AMPA component: 247.37 ± 27.15 pA·ms for control versus 240.42 ± 31.38 pA·ms for EA). The above results suggested that EA at BL21 enhances gastric emptying through upregulating NMDAR-mediated synaptic transmission of gastric-projecting DMV neurons.

4. Discussion

In the present study, we found that EA with a high intensity at BL21 increased gastric emptying in rats. NMDARs play a crucial role in this process. Enhancement of NMDAR-mediated synaptic transmission in gastric-projecting DMV neurons is required for this potent regulation of gastric emptying.

The emptying of liquids from the stomach is primarily a function of the pressure gradient between the stomach and the duodenum. Intragastric pressure is generated by gastric contractions, mainly from the proximal stomach [23, 29]. Therefore, gastric emptying of liquids seems to reflect mainly fundal activity. On the other hand, it has been generally accepted that solid gastric emptying is regulated by the coordination of the antrum, pylorus, and duodenum [29, 30]. The antral pump and pyloric opening are of paramount importance for emptying solids. Large solid particles are retained in the stomach by the pyloric closure and are retropelled and triturated in the antral mill [29, 31].

Somatovisceral reflexes responsible for regulation of visceral organs are strongly associated with the effects of acupuncture. Previous studies well documented that stimulating different skin area or points can produce different

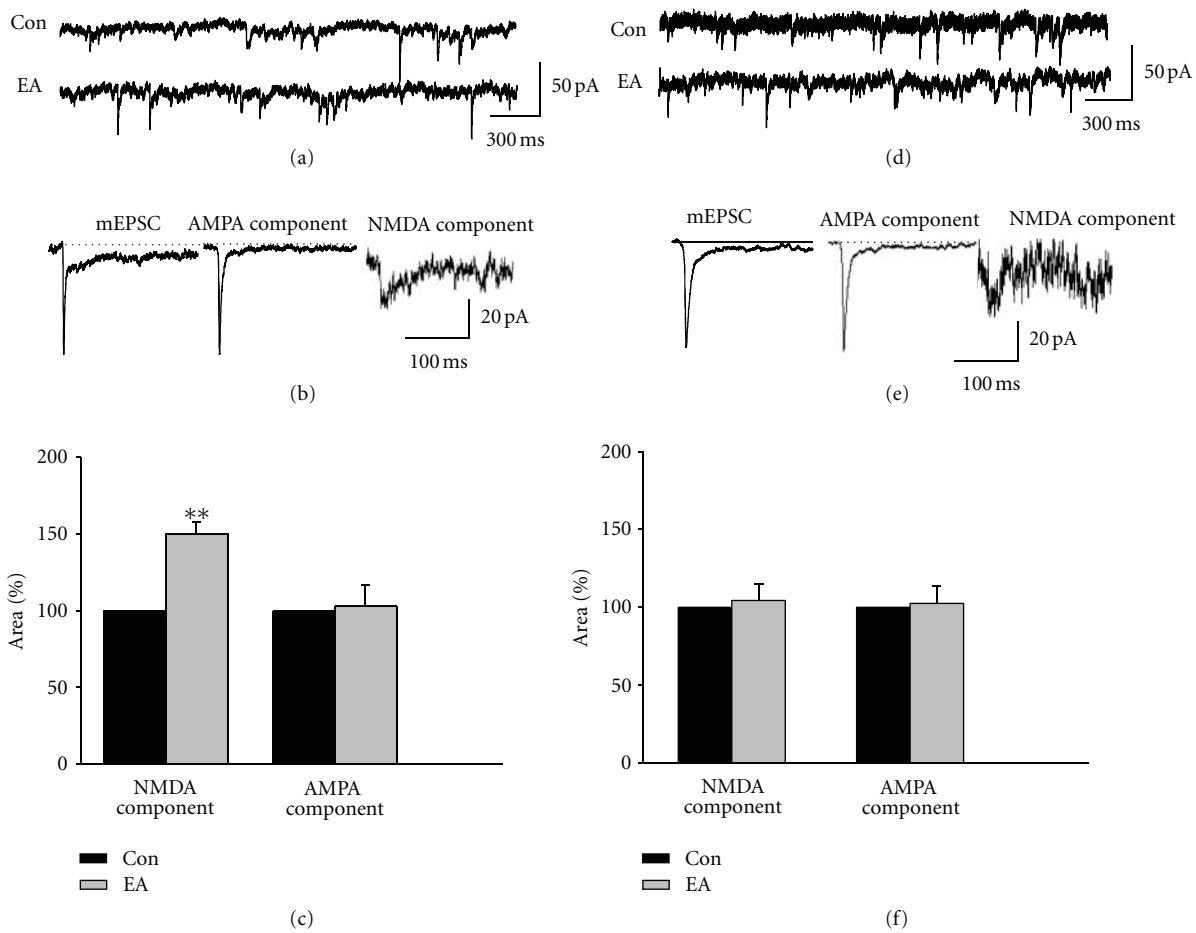

Figure 5: Effect of EA at BL21 on mEPSCs in labeled and unlabeled DMV neurons. (a) Representative traces of mEPSCs of labeled neurons in control and EA groups. (b) Representative AMPAR and NMDAR components of mEPSC (average over 100 events) in labeled neurons. From left to right: mEPSC containing AMPAR and NMDAR components, isolated AMPAR component, isolated NMDAR component. (c) With regard to labeled neurons in EA group, NMDAR component was increased significantly, but AMPAR component did not change significantly ($^{**}P < 0.01$, unpaired t test, $n = 12$ neurons for each group). (d) Representative traces of mEPSCs of unlabeled neurons in control and EA groups. (e) AMPAR and NMDAR components of mEPSC (average over 100 events) in unlabeled neurons. From left to right: mEPSC containing AMPAR and NMDAR components, isolated AMPAR component, isolated NMDAR component. (f) With Regard to unlabeled neurons in both control and EA groups, neither NMDAR nor AMPAR component changed significantly ($n = 8$ neurons for each group).

effects on gastric motility [5, 10, 11]. BL21, as one of the most common points for functional gastrointestinal disorders in the literature of traditional Chinese medicine, did not attract enough attention to its effect on these diseases. In this study, we applied EA at BL21 to observe its effect on gastric emptying and the mechanism responsible for this reaction and selected abdominal point CV12 as a control. Our results determined that EA at BL21 can mostly increase gastric emptying significantly; in contrast, EA at CV12 decreased gastric emptying significantly. Here we would like to point out that although most cases showed the upregulation of gastric emptying by BL21, in some cases EA at this point failed to accelerate or even inhibited gastric emptying, similar to the previous reports [9, 32]. What caused this phenotype will be further investigated in the future. However, regarding CV12, the results are very consistent.

Glutamate and γ-aminobutyric acid (GABA) are major excitatory and inhibitory neurotransmitters within the central nervous system (CNS) [20]. DMV, as a nucleus sending efferent projections to the gastrointestinal tract, received excitatory or inhibitory information from nucleus of the solitary tract, hypothalamus, and so forth. Endogenous glutamate and GABA are the major neurotransmitters controlling the excitability of DMV motor neurons in brainstem slice preparations [28]. It is generally accepted that their inhibitory and excitatory effects on the excitability of DMV neurons are mediated directly via activation of postsynaptic GABAA receptors and both NMDA- and non-NMDA-type glutamatergic receptors, respectively [12, 33, 34]. Our current study found that NMDAR antagonist kynurenic acid abolished the acceleration of gastric emptying by EA at BL21 and agonist NMDA increased gastric emptying, indicating that EA at BL21 accelerates gastric emptying through the glutamate pathway of DMV. Our finding that EA at BL21 increased NMDAR components of mEPSC but did not change AMPA component in gastric-projecting

DMV neurons suggests that NMDAR-mediated synaptic transmission of gastric-projecting DMV neurons plays a predominant role in this process.

5. Conclusions

In general, our works demonstrated that the upregulation of gastric emptying by EA at BL21 could be due to increasing NMDAR-mediated synaptic transmission in gastric-projecting DMV neurons.

Authors' Contribution

X. Zhang and B. Cheng contributed equally to this work.

Acknowledgments

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Research Article

Technical Parameters for Laser Acupuncture to Elicit Peripheral and Central Effects: State-of-the-Art and Short Guidelines Based on Results from the Medical University of Graz, the German Academy of Acupuncture, and the Scientific Literature

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The scientific literature in the area of laser acupuncture is rather large; however, the actual mechanisms and effects have not yet been proven in detail. Since the early days of laser acupuncture, there are still many open questions concerning technical parameters of this innovative technique. In this paper, we report about the most important technical parameters (wavelength, output power, power density, energy density, dose range, and continuous or pulsed laser) for laser acupuncture and present quantitative results for optimal laser stimulation, which allow eliciting reproducible effects in the periphery and in the brain. There are several position statements on laser acupuncture and also several review articles in scientific literature concerning clinical effectiveness of laser acupuncture. For example, the Australian Medical Acupuncture College stated recently that "the optimal energy density for laser acupuncture and biostimulation, based on current clinical experience, is 4 J/cm^2". However, our results of previous research studies and of this paper clearly show that dose must be adjusted according to the individual responses.

1. Introduction

In the Western world, it is less known that one of the medical pioneers of laser acupuncture comes from China. The surgeon Zhou used laser acupuncture in China as a type of controlled anesthetic method for dental indications since 1979 [1].

Zhou developed interesting techniques which involve irradiation of different acupuncture points. For extractions in the lower jaw, one single acupuncture point (Hegu; LI4) was irradiated for five minutes with a helium-neon laser equipment using a laser beam of 2.8–6 mW focused to a red spot on the acupuncture point [2]. The Chinese oral surgeon Zhou also works with a CO_2 laser within the laser therapy range of 0–100 mW, which he considered already at that time more effective than the other one. Zhou performed more than 10000 tooth extractions with this laser acupuncture anesthesia. Even though it was a Chinese doctor who pioneered laser acupuncture, it was a Canadian, Friedrich Plog, who pointed out the usefulness of laser acupuncture in this context in the Western world. He was already testing lasers instead of needle acupuncture in 1973 [2, 3].

The scientific literature in the area of laser acupuncture is rather large; however, the actual mechanisms and effects have not yet been proven in detail. In the scientific database PubMed (http://www.pubmed.gov/), more than 560 referenced publications can be found at the moment (February 2012). Recent studies using modern biomedical equipment comparing the effects of laser and needle acupuncture have contributed to a better understanding and have clearly shown that laser light can be successfully used for effective acupuncture treatment. However, since the early days of laser

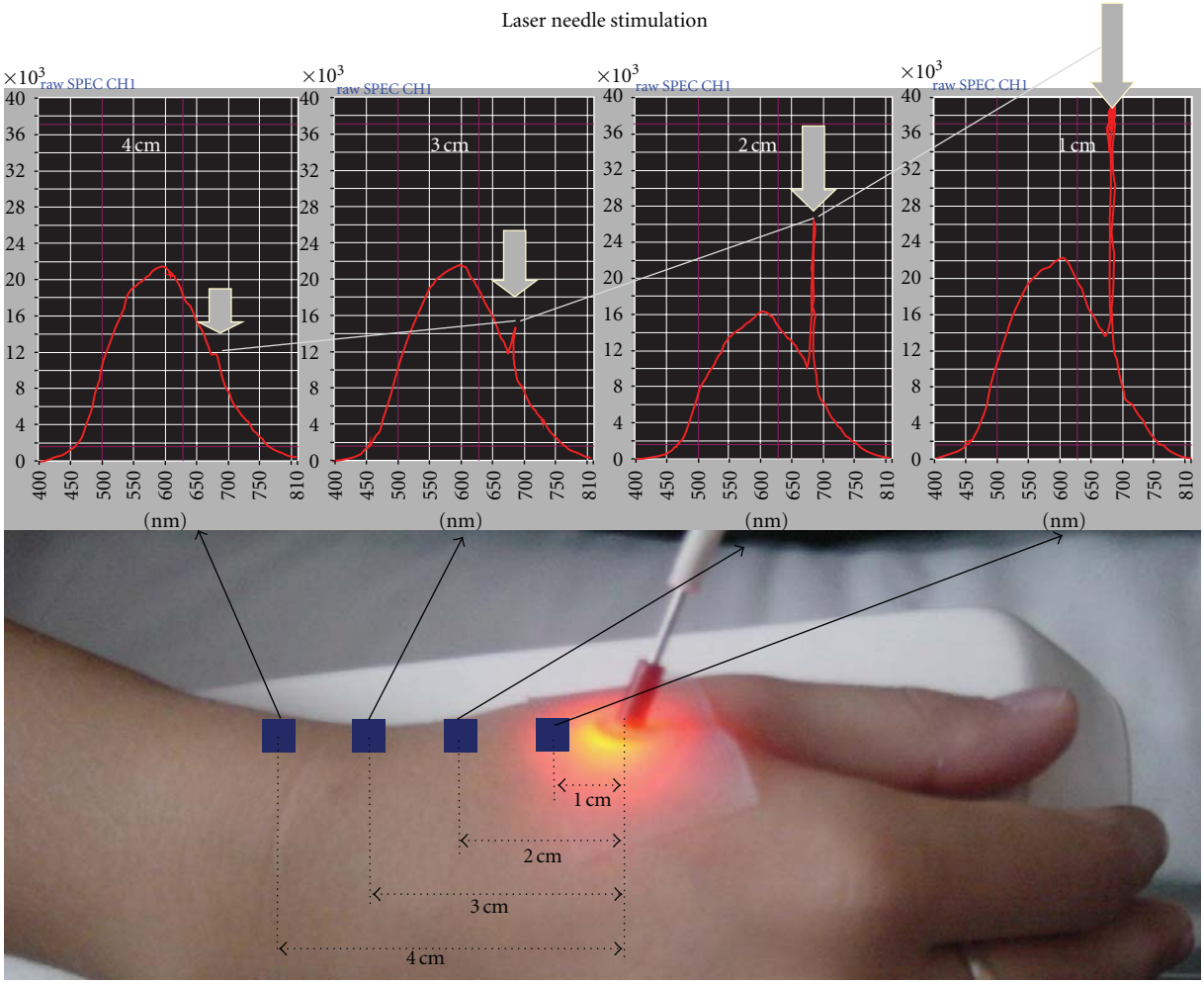

Figure 1: Light dispersion on the human skin. Note the peak in the spectrum at 685 nm (modified from [6]).

acupuncture there are still many open questions concerning technical parameters of this innovative technique.

Within this publication, only a few aspects concerning the main technical parameters should be presented. It has to be mentioned that there are several renowned associations offering recommendations for laser therapy. For example, the "World Association for Laser Therapy" (WALT) suggests dosages between 2 and 16 Joules for laser treatment [4, 5]. In the following, we will briefly describe the most important technical parameters for laser acupuncture and present for the first time quantitative results for optimal laser stimulation in acupuncture research, which allow eliciting reproducible effects in the periphery and in the brain.

2. Technical Parameters for Laser Acupuncture

The following technical parameters can significantly affect the effects of laser acupuncture treatment. In addition, there are several biological parameters which depend on the subject to be investigated. The latter influences should be discussed in another publication.

2.1. Wavelength. The question "which wavelength should be used in laser acupuncture" is sometimes related to the question "how deep does light penetrate human tissue". It is well known that red laser light has a deeper penetration depth than violet, blue, green, or yellow. Infrared light is not visible, but some authors have demonstrated that it penetrates human tissue at least as deep as visible red light. One of our own experiments using red light (685 nm) is shown in Figure 1 [6].

Light dispersion on the skin was measured using a multiparametric device (O2C Oxygen to see, LEA Medical Technology, Gießen, Germany). Figure 1 shows that even at a distance of 4 cm the red laser light from a so-called laser needle (wavelength 685 nm, output power 40 mW, and diameter 500 μm) [6] can be detected. One can conclude from this experiment that the penetration depth of red laser light with the aforementioned parameters is at least 4 cm. This is in accordance with experiments from other research groups [7]. In this example, we used a wavelength of 685 nm, as already mentioned. Other authors believe that wavelengths between 633–670 nm are the best option for laser therapy (e.g., nerve regeneration) [2]. They also describe the penetration of light

of this wavelength range to be only up to one centimeter. It should be mentioned critically that any wavelength in combination with a reasonable dose at the acupuncture point may have a biological effect. Probably other parameters like the dosage may be just as important as the wavelength. On the other hand, the dosage is sometimes not known or obtainable, for example, due to the lack of penetration [2]. In some of our "laser needle" studies [6] we have shown that good experimental and clinical results can also be obtained when two wavelengths are combined. These so-called "bichromatic" laser needles were used in several previous studies [6].

2.2. Output Power. In order to calculate the dose to be administered at the acupoint, it is important to know the output power of the laser acupuncture instrument. Higher output power results in a higher power density, and it is also important with respect to light penetration in tissue [2]. If the acupuncture laser does not only have a continuous wave mode, but also a pulsed mode, the average output power of the laser is also important. With the average output power it is also possible to calculate the dose to administer by the pulsed laser.

2.3. Power Density. When using acupuncture point treatment, one must make sure that the treatment time is not too long. The parameter power density reflects the intensity of the laser beam. Its units are watts or milliwatts per cm^2.

2.4. Energy Density. The energy density is measured in watt-seconds per cm^2 (= Joules per cm^2). Energy density is the same as dose or treatment dose. Dosage refers to the amount of energy per unit area brought to bear on tissue or cell culture [2].

2.5. Dose Range. The dose ranges used for laser acupuncture stimulation differ in the literature, from 0.001 J/cm^2 to 10 J/cm^2 and more. Tunér and Hode stated that "dose is a very complicated issue. It is a matter of wavelength, power density, type of tissue, condition of the tissue, chronic or acute problem, pigmentation, treatment technique, and so forth" [2].

2.6. Continuous or Pulsed Laser. Laser beams can be presented pulsed or continuously (see also Section 2.2). The pulsing of the laser light may interfere with other pulsing biological phenomena. This may probably have special effects, but very little is known about it today [2].

3. Results

3.1. Minimal Dose. We have shown in ultralow-level laser acupuncture stimulation in rats recently that a very low power density (about 2 mW/cm^2) of a violet laser beam (wavelength 405 nm, output power 1 mW, beam area ~0.5 cm^2, and duration 2 min) at the Baihui (GV20) acupuncture point can reproducibly modulate neurovegetative parameters (Figure 2) [8].

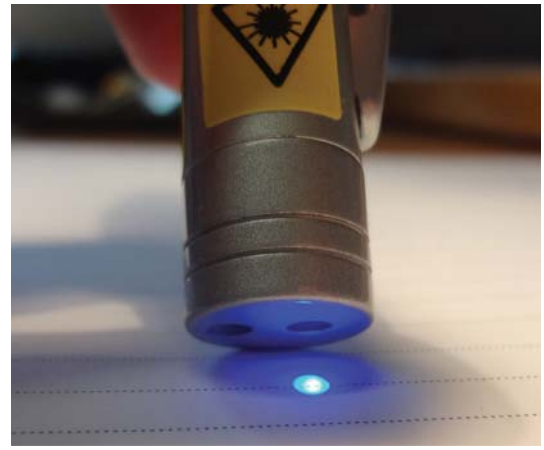

Figure 2: Violet laser stimulation with very low output power (1 mW). The beam area was about 0.5 cm^2, resulting in a power density of about 2 mW/cm^2 [8].

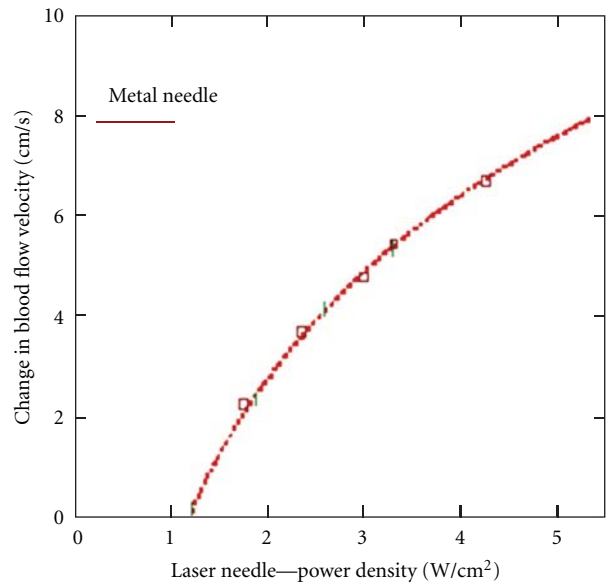

Figure 3: Change in blood flow velocity in the ophthalmic artery in dependence on the power density of laser needles during stimulation of an eye-specific acupuncture scheme. The mean changes measured in metal needle acupuncture are marked with a line (modified from [10]).

Significant biological effects using wavelengths of 633 or 670 nm at extremely low power densities (about 0.15 mW/cm^2) were recently described also by other authors [9].

3.2. Optimal Dose. Concerning this topic, own results from the Medical University of Graz can be presented [10]. Figure 3 shows the detected dependency of blood flow velocity in the human ophthalmic artery as a function of power density from laser needles.

Acupuncture of seven eye-specific acupoints leads to a significant increase in blood flow velocity in the ophthalmic

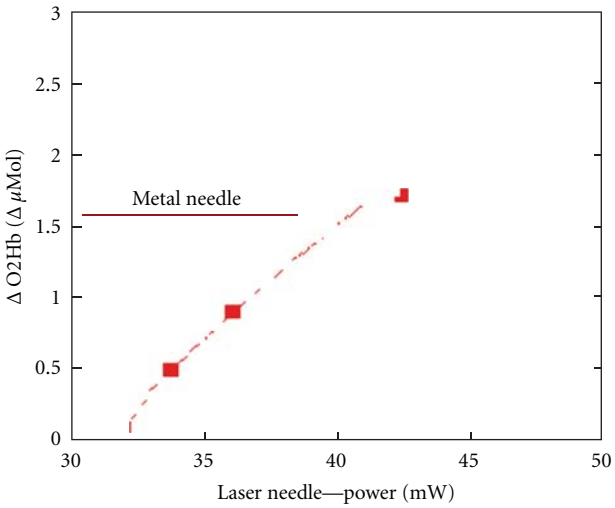

Figure 4: Changes in cerebral oxyhemoglobin concentration using a visual acupuncture scheme with metal needles and laser needles of different optical power. The curve shows the best analytical adaptation to the measurement values of laser needle stimulation (modified from [10]).

artery. Metal needles yield an increase from 10 cm/s to 18 cm/s [10].

It is obvious that changes in blood flow velocity are dependent upon the optical power densities applied when using laser needle acupuncture. The curve conveys the best analytical adaptation of measurement values. This curve satisfies the mathematical function $f(x) = c \times \ln(x + 0.5)$ [6, 10].

Measurements of changes of cerebral concentrations of oxyhemoglobin and deoxyhemoglobin were performed using near infrared spectroscopy (NIRS). Figure 4 shows one result of these measurements dependent on the optical power of the laser needle stimulation.

4. Discussion

Tunér and Hode, both very renowned researchers on laser therapy, stated recently [2]: "anyone who studies the literature carefully can become confused. Some wavelengths achieve the best effects on this and that, while others have poorer effects or none at all. Some doses lead to beneficial effects, but when the dose is increased, the effects wear off. If we treat a condition, some of the parameters we want to influence may be affected, but perhaps not all. If we administer treatment from a distance, we do not get the same effects as if we treat in contact or with pressure. Some frequencies produce effects on pain, others on oedema. What are we to believe? And what do we do to find the best dose, wavelength, and so forth?"

Studies concerning the minimal dose in laser acupuncture are rare. Yurtkuran et al. [11] investigated the effects and minimum effective dose of laser acupuncture in knee osteoarthritis. Patients received 904 nm low-level laser irradiation with 10 mW/cm^2 power density, 4 mW output power, 0.4 cm^2 spot size, 0.48 J dose per session, and 120 sec treatment time on the median side of the knee to the Yinlingquan acupuncture point SP9 (Spleen 9). Laser acupuncture, even with this small power density, was found to be effective in reducing periarticular swelling when compared with placebo laser [11]. In a recent Sino-European transcontinental animal experimental study, which was designed by our group and performed at the China Academy of Chinese Medical Sciences in Beijing, we found that ultra-low-level laser acupuncture stimulation in rats can reproducibly induce effects on neurovegetative parameters (see Section 3.1) [8].

Blood flow velocity in the ophthalmic artery in humans is an effective parameter for quantification of the effects of acupuncture treatment and is logarithmically dependent on the stimulus intensity of the laser needles. Thus, we can conclude that Weber-Fechner's law is valid for the dose-effect relationship examined here. The threshold value for optical power density (I^*) can be calculated from the registered and analytically determined effect curve, $I^* = 1.3\,\mathrm{W/cm^2}$. This indicates that the optical power density of the laser needles must be greater than 1.3 W/cm^2 in order to activate the physiological effects of acupuncture. In addition, we can see that the needle equivalence in optical power densities of the laser needles reaches $I \geq 5\,\mathrm{W/cm^2}$. We can assume that an increase in blood flow velocity in the ophthalmic artery is based on a complex cerebral reaction resulting from acupoint stimulation, preceded by multisynaptic switching of optically induced acupuncture stimulation potentials [6, 10, 12].

It is noteworthy that despite the physiological complexity, the logarithmic relationship between stimulus strength I and stimulus effect is maintained. We interpret this as obvious proof that specific effects of acupuncture underlie these logarithmic dose-effect relationships. The existence and validity of dose-effect relationships in acupuncture could be proven for the first time using the methods described [6, 10]. This statement is strictly valid only when using laser needles which trigger continuous permanent stimulation, thus allowing exact quantification of stimulus strength. To what extent low- or high- frequency modulation of laser needle light can modify proven dose-effect relationships is unclear and must be investigated in further studies. Since the postulated equivalence between metal needles and laser needles could be clearly shown in the examined context, we can conclude that classical acupuncture and its effects also should be functionally dependent on stimulus strength according to a potency rule [6, 10].

Experimental data of our research group in Figure 4 show that laser needle stimulation with an optical power of about 40 mW leads to changes in oxyhemoglobin concentration, similar to the effects when using metal needles. The equivalency between metal needle stimulation and laser needle stimulation can also be proven with these cerebral effects [13]. These experiments also yield the best analytical adaptation of the measurement results in a logarithmic function, that is, cerebral oxyhemoglobin concentration parameters also underlie a physiological dose-effect relationship.

There are several position statements on laser acupuncture [14] and also several review articles in scientific literature concerning clinical effectiveness of laser acupuncture

[15]. For example, the Australian Medical Acupuncture College [14] stated that "the optimal energy density for laser acupuncture and biostimulation, based on current clinical experience, is 4 J/cm^2". However, our results of previous research studies [6, 8] and of this publication clearly show that dose must be adjusted according to the individual responses.

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Research Article

Sino-European Transcontinental Basic and Clinical High-Tech Acupuncture Studies—Part 3: Violet Laser Stimulation in Anesthetized Rats

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The aim of this study was to determine the effect of violet laser stimulation on three acupuncture points in anesthetized rats and to test the hypothesis that violet laser light can modulate neurovegetative parameters like heart rate (HR), heart rate variability (HRV), and mean arterial blood pressure (MAP). Recordings were performed in 10 male anesthetized rats under three conditions in Beijing, and monitored with equipment from Graz, where also data analysis was performed. For stimulation a violet laser (emitted wavelength 405 nm, laser output 1 mW, continuous mode) was used. The electrocardiograms were recorded by an HRV Medilog AR12 system during laser acupuncture stimulation of the head, ear, and body (Baihui, "heart" ear acupoint, Zusanli). HR changed significantly only during ($P = 0.013$) and after ($P = 0.038$) stimulation at Baihui. Total HRV and the low frequency/high frequency ratio showed insignificant changes. There was an insignificant decrease in MAP after stimulation of Baihui acupoint. Violet laser stimulation offers a method to induce acute effects in HR and HRV in rats. Although the precise mechanism of this effect remains to be determined, alterations are significant. Violet laser stimulation on the Baihui acupoint could readily be translated to clinical studies.

1. Introduction

Basic and clinical applications of low-power laser stimulation are numerous. The field of research is characterized by a variety of different methodologies and uses of various light sources with different parameters (wavelength, output power, continuous wave or pulsed operation modes, and pulse parameters). Although in recent years longer wavelengths (650 to 900 nm, that is, red and infrared) and higher output powers (up to 150 mW) have been preferred in medical therapeutic devices, ultra-low-level laser stimulation is still a topic of animal experimental and human research [1, 2].

In the present study, we used for the first time ultra-low-level laser stimulation (405 nm; 1 mW; continuous mode) in anesthetized rats under stable conditions and analyzed the effects on physiological neurovegetative parameters. Similar to our first study in this series [3] the data were recorded for 10 rats in Beijing, China, and the data analysis was performed in Graz, Austria. A system normally used for human data analysis has been specifically adapted in Europe for these studies in rats [3].

2. Animals and Methods

2.1. Sprague-Dawley Rats and Blood Pressure Monitoring. Ten male Sprague-Dawley rats were kept in an animal house maintained at 21 ± 2°C with a 12-hour light-dark cycle and were given free access to food and water. The weight of the rats was 300–350 g. The animals were initially anesthetized with an intraperitoneal injection of 10% urethane (1.0 g/kg, Sigma-Aldrich, St. Louis, USA).

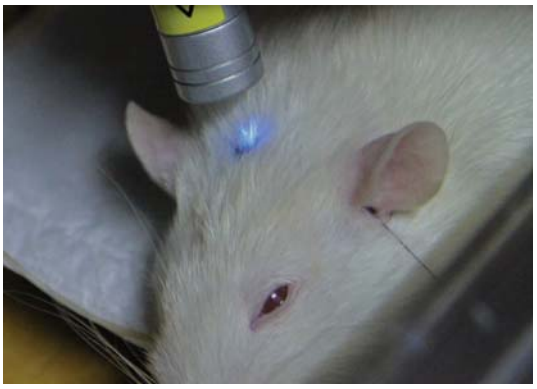

Figure 1: Violet laser stimulation in a rat at the Baihui acupuncture point.

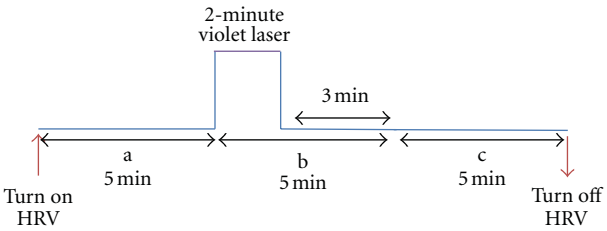

Figure 2: Experimental procedure for violet laser stimulation (405 nm) at the three acupoints.

The left common carotid artery was cannulated with a polyethylene catheter filled with physiological saline containing heparin (200 IU/mL, Sigma-Aldrich, St. Louis, USA) to record mean arterial pressure (MAP) via a blood pressure transducer (DA100, Biopac Systems, Inc., Aero Camino Goleta, USA) and amplifier (MP150, Biopac Systems, Inc., Aero Camino Goleta, USA). This signal was registered on Micro1401 and Spike2 (CED, Cambridge Electronic Design Limited, Cambridge, UK) data acquisition unit and software. The procedure was the same as in our first study of this series [3]. The depth of anesthesia was monitored by changes in MAP, and additional anesthetic (urethane 0.3 g/kg) was given if the animal showed large fluctuations in baseline AP or a withdrawal response to a pinch of the paw. After tracheal cannulation, the animals breathed spontaneously, and their core temperature was maintained at $37.0 \pm 0.5°$C by a feedback-controlled electric blanket (FHC Inc., Bowdoin, USA). The animals were sacrificed after the investigation by an overdose of anesthetics. The experiments were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* issued by the National Institutes of Health (China), and the procedures were approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences.

2.2. Electrocardiographic Monitoring in Rats. The data from electrocardiograms (ECGs) were recorded by an HRV Medilog AR12 (Huntleigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria) system. The data were analyzed using specially adapted software (Huntleigh Healthcare) [3]. The sampling rate of the recorder is 4096 Hz. All raw data from the rat experiments were stored digitally on a 32 MB compact flash memory card. After removing the card from the portable system in the lab of the Institute of Acupuncture and Moxibustion at the China Academy of Chinese Medical Sciences in Beijing, the data were read by an appropriate card reader connected to a standard computer and sent to the lab at the Stronach Research Unit for Complementary and Integrative Laser Medicine in Graz.

As described in previous publications, HRV is measured as a percent change in sequential chamber complexes called RR-intervals in the ECG. It can be quantified in the time domain and in the frequency range by analyzing the ECG power spectra [3–9]. The task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommended the HRV parameters already in 1996 [10]. The mean HR, total HRV, and LF (low frequency)/HF (high frequency) ratio of the HRV were evaluated [10].

2.3. Violet Laser Stimulation and Procedure. Three locations were selected for laser stimulation. All points can regulate cardiovascular and neurovegetative functions [11–13]. The points were identified by anatomical marks and previous reports [11–13]. Baihui (GV20) is located at the continuation of the line connecting the highest points of the ear, on the median line of the head. The "heart" ear acupoint is located at the inferior concha. Zusanli (ST36) is located on the anterolateral side of the hindlimb near the anterior crest of the tibia below the knee under the tibialis anterior muscle [11–13].

For laser stimulation, an instrument (Conrad Electronic SE, Hirschau, Germany) with a wavelength of 405 nm (violet) and an output power of 1 mW with a continuous beam was used for a duration of 2 min (Figure 1). The time scale of each stimulation is shown in Figure 2. The order of point stimulation was randomized, and the time between the investigations of the different acupoints was at least 10 minutes.

The measurement profile and measurement sessions (a–c) are shown in Figure 2. Three measurement periods were compared: one before stimulation (a); one immediately after the beginning of the 2-minute violet laser acupuncture stimulation (b); one as a second control (c). This scheme was also used, in an adapted version, in a previous investigation in rats (part 1 of this series, [3]).

2.4. Statistical Analysis. The data were analyzed using one-way repeated measures analysis of variance (ANOVA) (SigmaPlot 11.0, Systat Software Inc., Chicago, USA). Post hoc analysis was performed using Holm-Sidak test. The level of significance was defined as $P < 0.05$.

3. Results

Data analysis was performed successfully in 9 of the 10 rats. In one rat, mean HR in the control intervals was lower than 200/min and thus not included in the analysis.

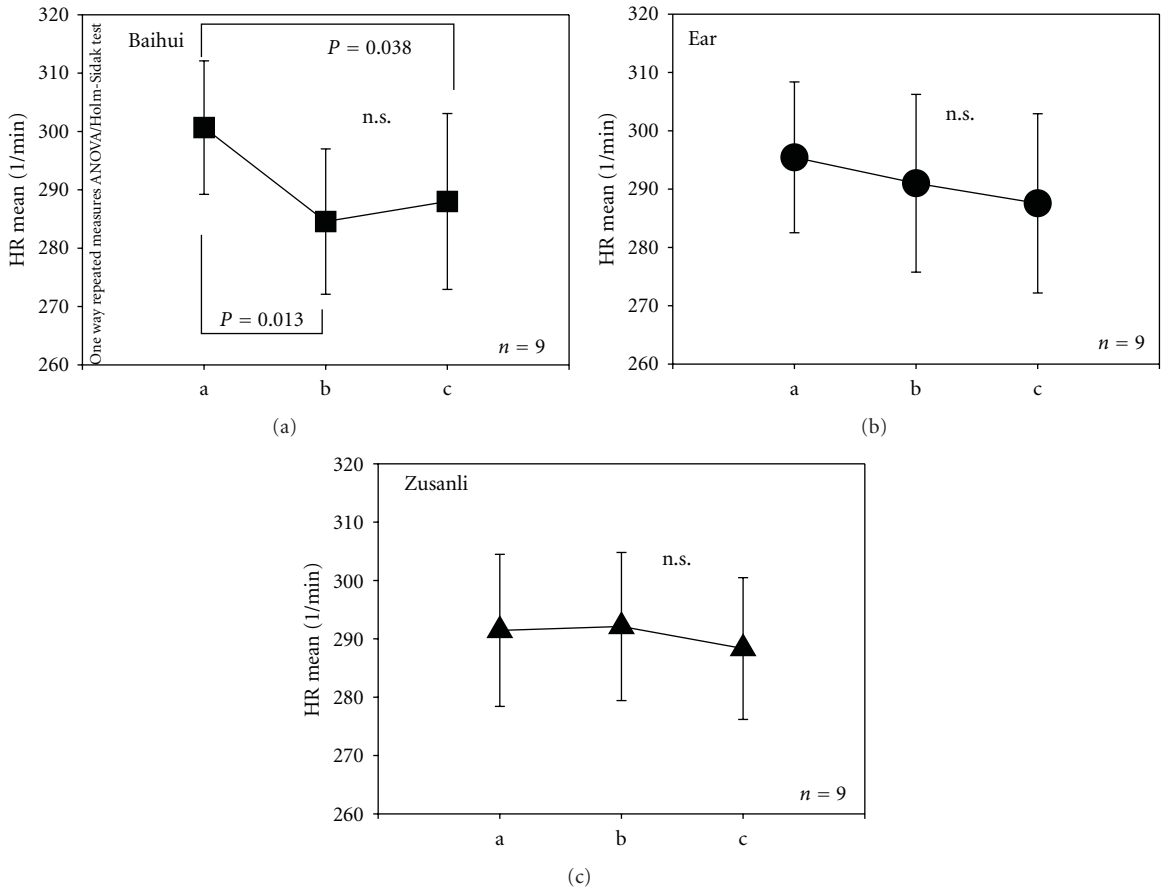

Figure 3: Diagrams displaying the mean heart rate (HR mean) and standard error of the mean (SE) of the 9 rats. There is a significant decrease of HR mean during (b) and after (c) violet laser stimulation at Baihui compared to the reference interval before stimulation (a). The different measurement phases (a–c; compare with Figure 2) and acupuncture points (Baihui; ear acupuncture: heart point; Zusanli) are indicated.

Figures 3 and 4 show the mean HR and HRV total (total heart rate variability) from the ECG recordings from 9 of the 10 rats during the three measurement phases (a, b, and c) as well as before, during, and after stimulation at the Baihui acupoint (a). The results from the stimulation of the ear and body point, respectively, are also shown ((b) ear; (c) Zusanli). There was a significant change in HR during ($P = 0.013$) and after ($P = 0.038$) the stimulation only when the Baihui acupoint was stimulated (Figure 3(a); compare also with Figure 1).

HRV total increased insignificantly (n.s.) during violet acupuncture stimulation at the acupoint Baihui and the ear acupoint (Figure 4). However, during stimulation of Zusanli, it decreased insignificantly. At the reference interval at the end of the measurement, there was an increase in HRV total only after stimulation at Baihui (Figure 4(a)).

Furthermore, continuous HR-HRV monitoring showed insignificant alterations in the LF/HF ratio after acupuncture stimulation at the three points in rats (Figure 5).

Figures 6 and 7 show computer chart records of typical experiments. Changes in blood pressure (BP), ECG, and HR after stimulation with violet laser at Baihui are demonstrated. Short-term decreases of BP and HR are shown in Figure 6 ("on effect").

In Figure 7, the continuous decrease of BP after violet laser stimulation onset is documented.

The data of the MAP of all 9 rats are summarized in Figure 8. Note the insignificant (n.s.) decrease of MAP in the control phase after stimulation with violet laser at Baihui.

4. Discussion

Laser light is a good alternative to metal needles for stimulation of acupuncture points, and it has been used successfully for several decades. However, to date there are only few studies proving the effectiveness of this kind of acupuncture stimulation. Most publications focus on red or infrared laser stimulation, and there are several relevant studies [14–20].

Violet laser acupuncture using a wavelength of 405 nm has been investigated in only a few scientific studies performed in humans by the research group in Graz [21–25]. To the best of our knowledge, it has not yet been used in animal experimental studies on acupuncture. However, some laboratory and clinical studies over the past ten years have shown that low-level laser stimulation using wavelengths of 633 or 670 nm and extremely low power densities

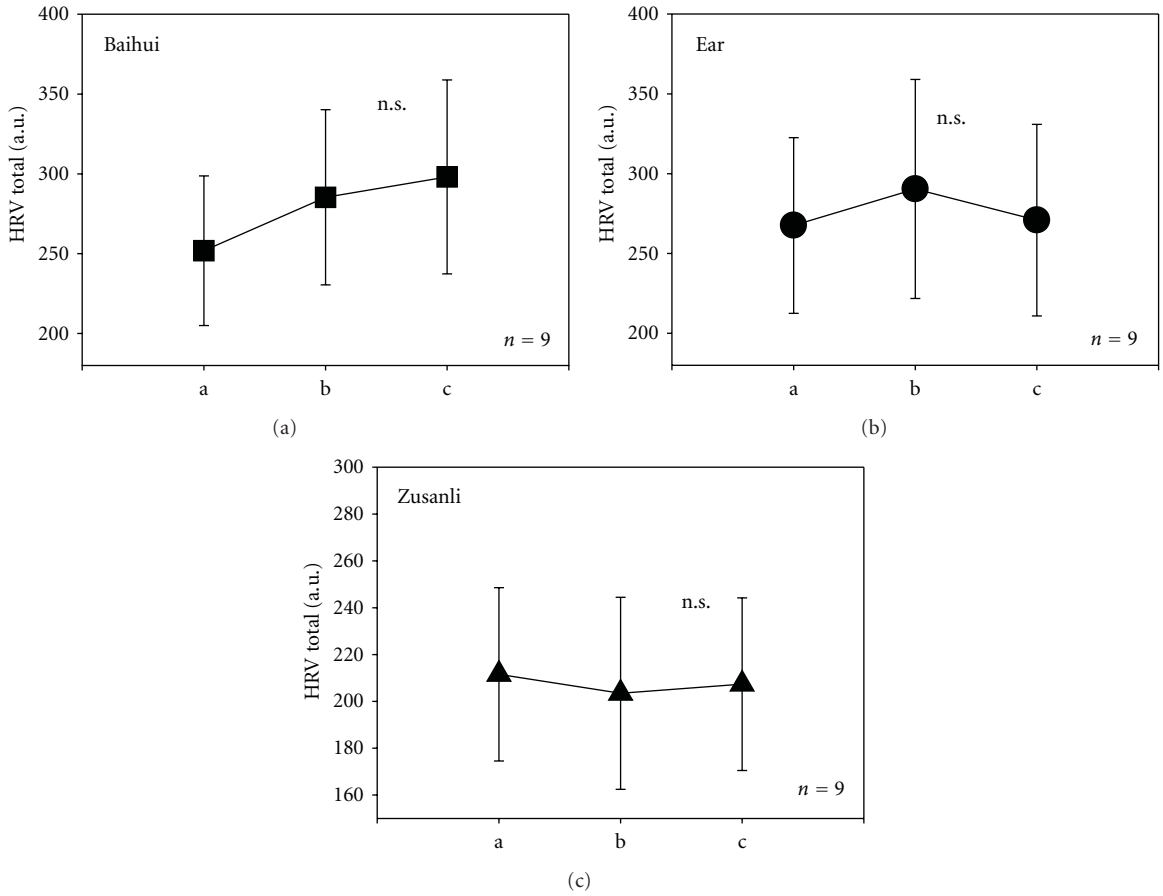

Figure 4: Graphical plots displaying total heart rate variability (HRV total) for the 9 rats. Note the marked but insignificant increase in HRV total after violet laser stimulation of the acupuncture point Baihui (a). For further explanation, compare with Figure 3.

(about 0.15 mW/cm^2) is capable of eliciting significant biological effects [2].

In the present study, we used such a low-level violet laser stimulation (405 nm; <1 mW) for acupuncture research in rats for the first time. In previous studies in rats, it could be demonstrated that photobiomodulation using light with an 810 nm and 150 mW diode laser can be used as noninvasive treatment for acute spinal cord injury, potentially acting through an immunomodulatory mechanism. This suggests that light will be a useful treatment for humans [26]. Beside application at the spinal cord, it has also been shown that pulsed infrared light alters neural activity in the rat somatosensory cortex *in vivo* [27]. In this study, infrared neural stimulation was found to evoke an intrinsic response of similar magnitude to that induced by tactile stimulation. The authors conclude that infrared light can be used safely and effectively to manipulate neural firing.

In contrast to the previous findings in humans at the Medical University in Graz, we could demonstrate in this animal experimental study that ultra-low-level violet laser stimulation ($\leq$1 mW) can modulate physiological and neurovegetative parameters after stimulating the Baihui acupuncture point. In the present study, there was also a clear on/off-effect when the laser was activated/deactivated (see typical examples in Figures 6 and 7). A similar effect with violet laser was shown in humans by Litscher et al. [21]. In that study, violet laser acupuncture (405 nm; 110 mW) at the acupoint Dazhui (GV14; on the same meridian as Baihui (GV20) in the present study) also induced an on/off-effect, but in different parameters, namely, the blood flow velocities in the basilar and middle cerebral arteries in the brain. In our present study, a more than 100 times lower output power was used in rats. Although the scalp bone of a rat is much thinner than that of a human subject, we suppose that there was no intensive direct radiation of the brain using the violet laser due to the intact skull of the rats. The on/off-effect of the violet laser stimulation could possibly be explained by the open eye of the rat; however, in human subjects, this explanation could be excluded. Of course, it would be possible to control this in future animal experimental studies.

Acupuncture stimulation at Baihui has also been investigated in other studies in rats. For example, Chuang et al. [28] stated that Baihui stimulation reduced cerebral infarct and increased dopamine levels in chronic cerebral hypoperfusion and ischemia-reperfusion injured Sprague-Dawley rats. Regular stimulation over a period of four weeks enhanced cognition and memory function of the rats.

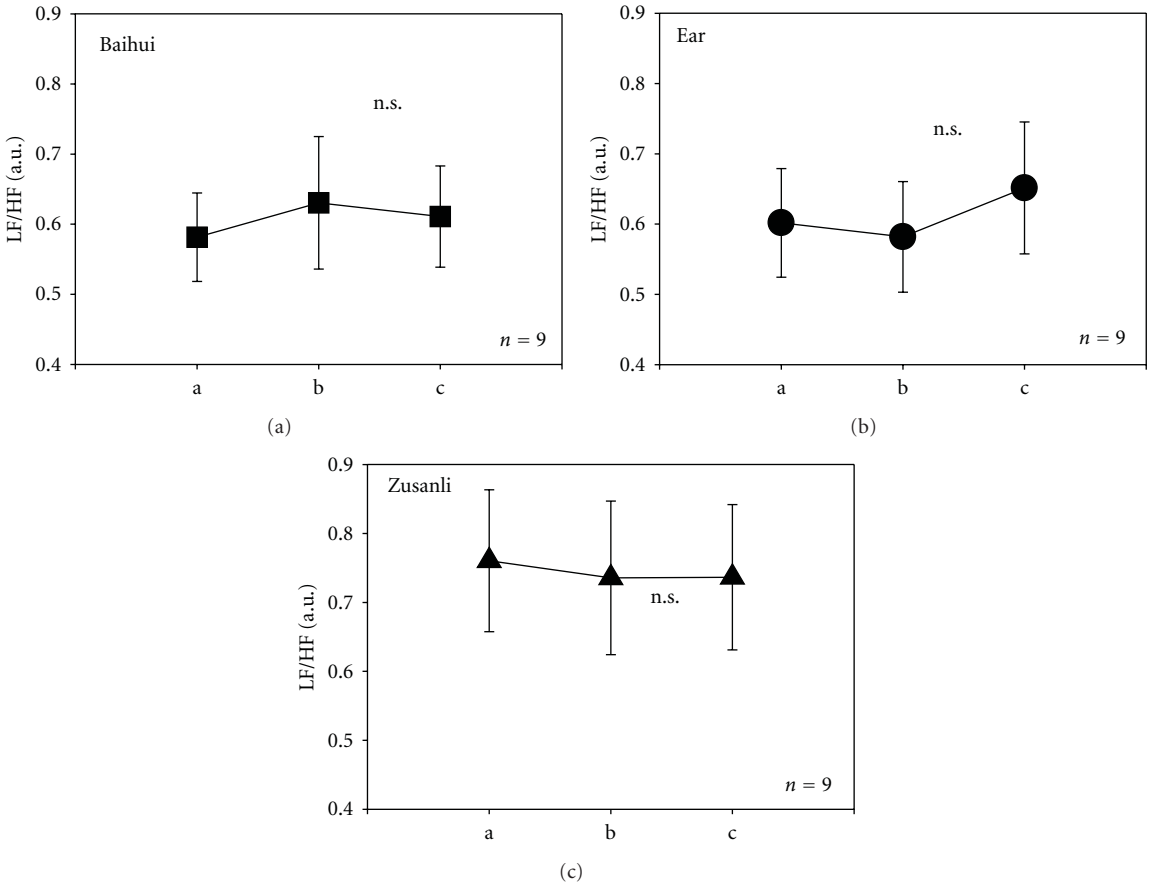

Figure 5: LF (low-frequency)/HF (high-frequency) ratio. For further explanation, see Figures 3 and 4.

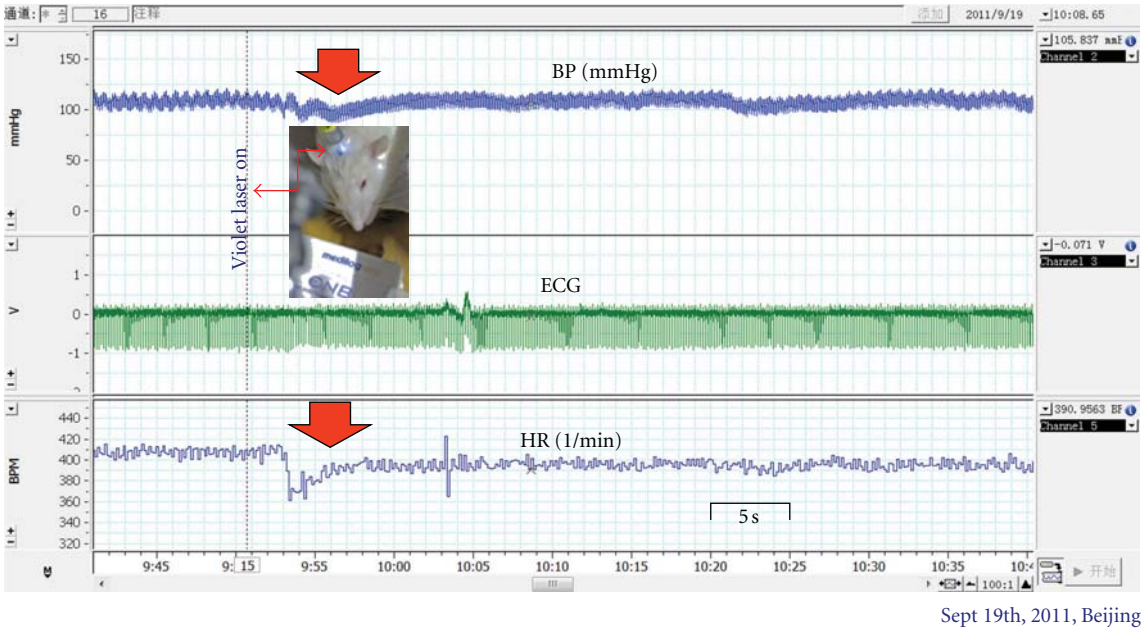

Figure 6: Original monitoring protocol of blood pressure (BP), raw electrocardiographic signal (ECG), and heart rate (HR) before and immediately after onset of violet laser stimulation. Note the decrease in BP and HR (red arrow).

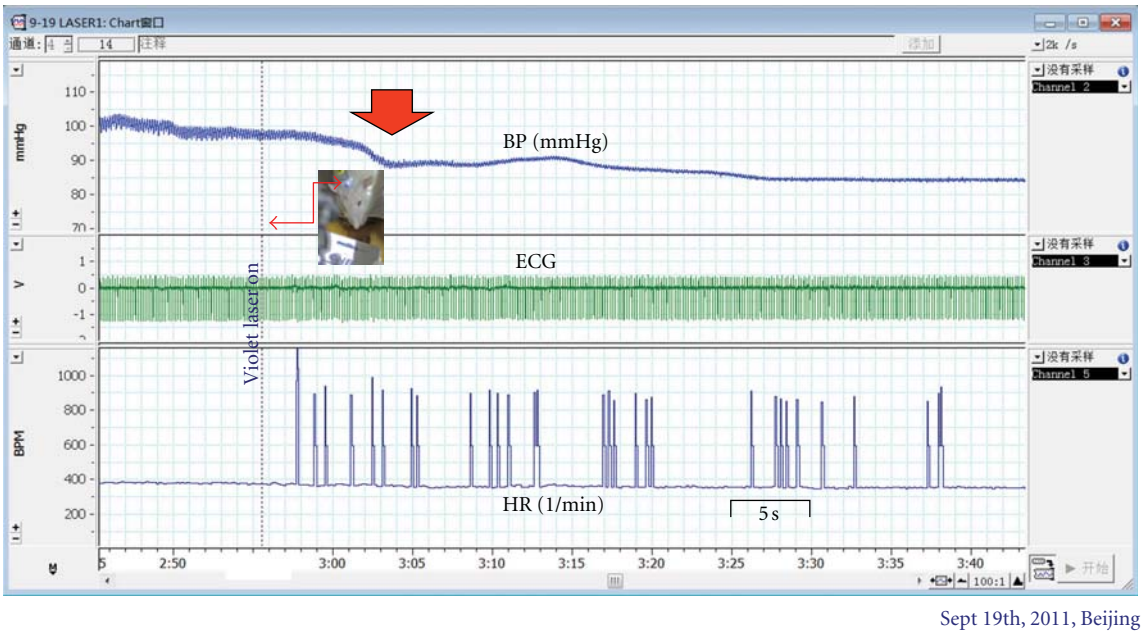

Figure 7: Original monitoring protocol of blood pressure (BP), raw electrocardiographic signal (ECG), and heart rate (HR) before and immediately after onset of violet laser stimulation.

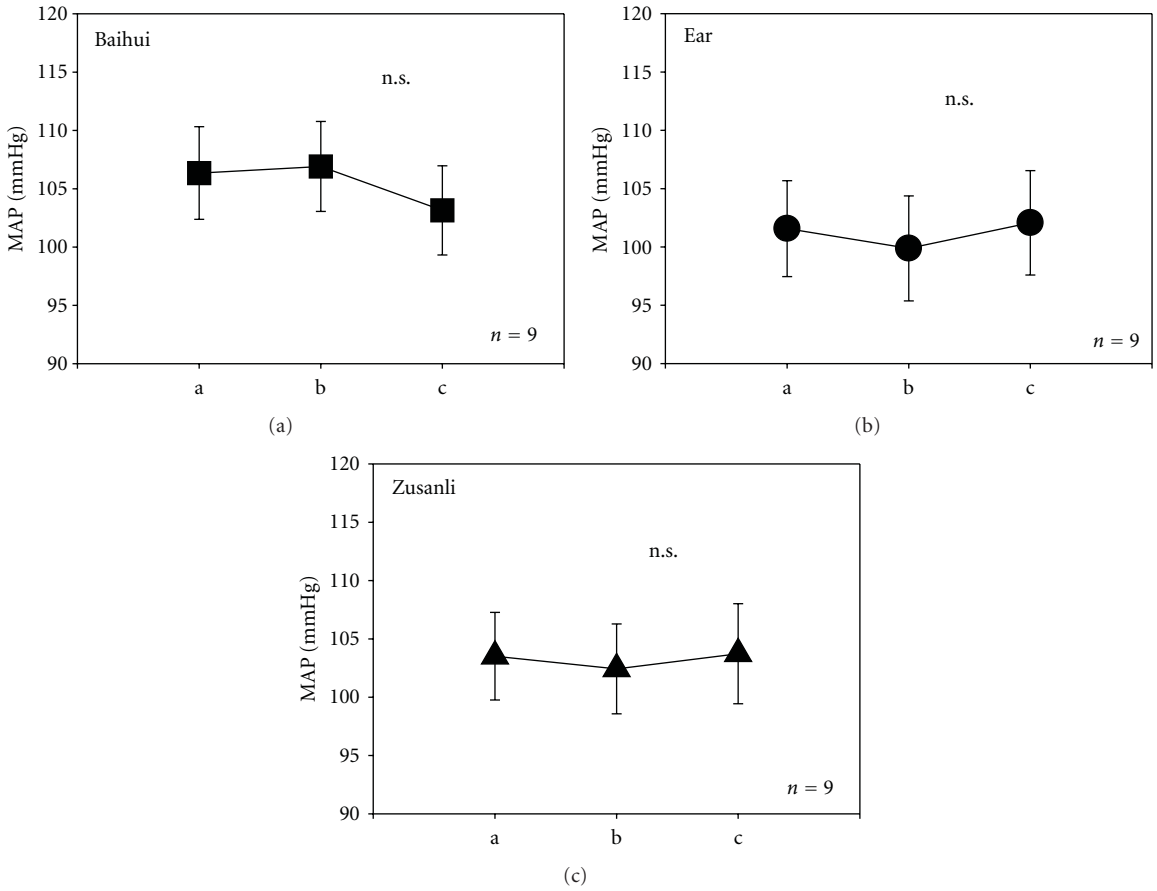

Figure 8: Diagrams showing changes in mean arterial pressure (MAP) for the three different stimulation areas using violet laser.

The use of laser light as complementary or alternative method to acupuncture needle stimulation has been promoted for some decades. However, there are only few systematic assessments of evidence to support the effectiveness of this modern technical application of acupuncture to date [29].

In several human studies, Litscher et al. [21–25] have shown that violet laser acupuncture can yield reproducible effects. These previous results were confirmed by the results of the present animal experimental study.

5. Conclusions

The following conclusions can be drawn from the results of this second animal experimental transcontinental study:

(i) heart rate changes significantly during ultra-low-level violet laser stimulation of Baihui in anesthetized rats;

(ii) total HRV changes insignificantly during violet laser application at Baihui, "heart" ear point, and Zusanli. However, there was a trend towards an increase in HRV total during and after stimulation of Baihui;

(iii) the LF/HF ratio showed no significant changes;

(iv) mean arterial pressure decreased (markedly, yet insignificantly) after violet laser stimulation of Baihui in rats.

Acknowledgments

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Research Article

Laser-Induced Evoked Potentials in the Brain after Nonperceptible Optical Stimulation at the Neiguan Acupoint: A Preliminary Report

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We report on small but reproducible human cerebral evoked potentials after bilateral nonperceptible laser needle (658 nm, 40 mW, 500 μm, 1 Hz) irradiation of the Neiguan acupoint (PC6). The results which are unique in scientific literature were obtained in a 26-year-old female healthy volunteer within a joint study between the Medical University of Graz, the Karl-Franzens University of Graz, and the Graz University of Technology. The findings of the 32-channel evoked potential analysis indicate that exposure to laser needle stimulation with a frequency of 1 Hz can modulate the ascending reticular activating system. Further studies are absolutely necessary to confirm or refute the preliminary findings.

1. Introduction

The irradiation of the skin overlying the median nerve of the wrist in humans using a helium-neon laser stimulation with a wavelength of 632.5 nm, an output power of 1 mW, and a frequency of 3.1 Hz, can produce a somatosensory evoked potential obtained at the Erb's point on the shoulder. This evidence of photosensitivity in peripheral nerves was found by Walker and Akhanjee already in 1984 [1].

The laser needle technology was invented at the University of Paderborn and first investigated scientifically at the Medical University of Graz [2]. The method does not puncture the skin; the needles are only applied at the surface of the skin.

The aim of this preliminary paper was to investigate if there are any measurable evoked potentials in the brain after "laser needle" stimulation at the Neiguan acupoint (PC6), which is also located at the wrist, near the median nerve.

2. Materials and Methods

2.1. Subject. The subject for our investigations was a 26-year-old female who was sitting in a special sound booth. The box is located at the Department of Psychology, Neurophysiology (Figure 1).

Written informed consent was obtained, and the investigations were approved by the ethics committee of the Medical University of Graz (13-048, laser needle stimulation). The subject was not taking medications and had no neurological or psychological impairments. The volunteer was informed about the nature of the investigation, as far as the study design allowed, and the measurements were performed in accordance with the Declaration of Helsinki.

2.2. Laser Needle Stimulation. Laser needle stimulation (Laserneedle GmbH, Berlin, Germany) allows the continuous stimulation of one or more acupuncture points on

Figure 1: Healthy volunteer during "laser needle" EEG (electroencephalography) experiment in Graz, Austria (with written permission of the subject).

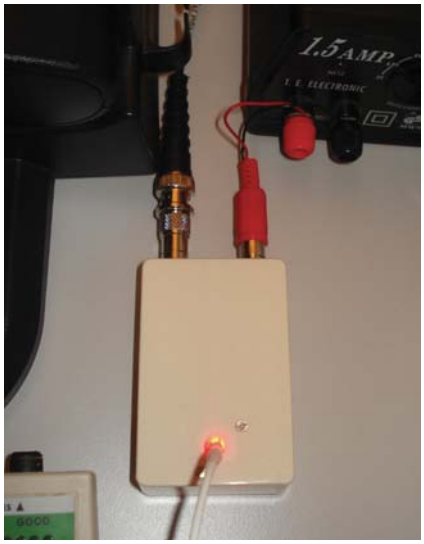

Figure 2: Trigger realization, especially for this laser needle study.

Figure 3: Bilateral stimulation of the Neiguan acupoint (PC6).

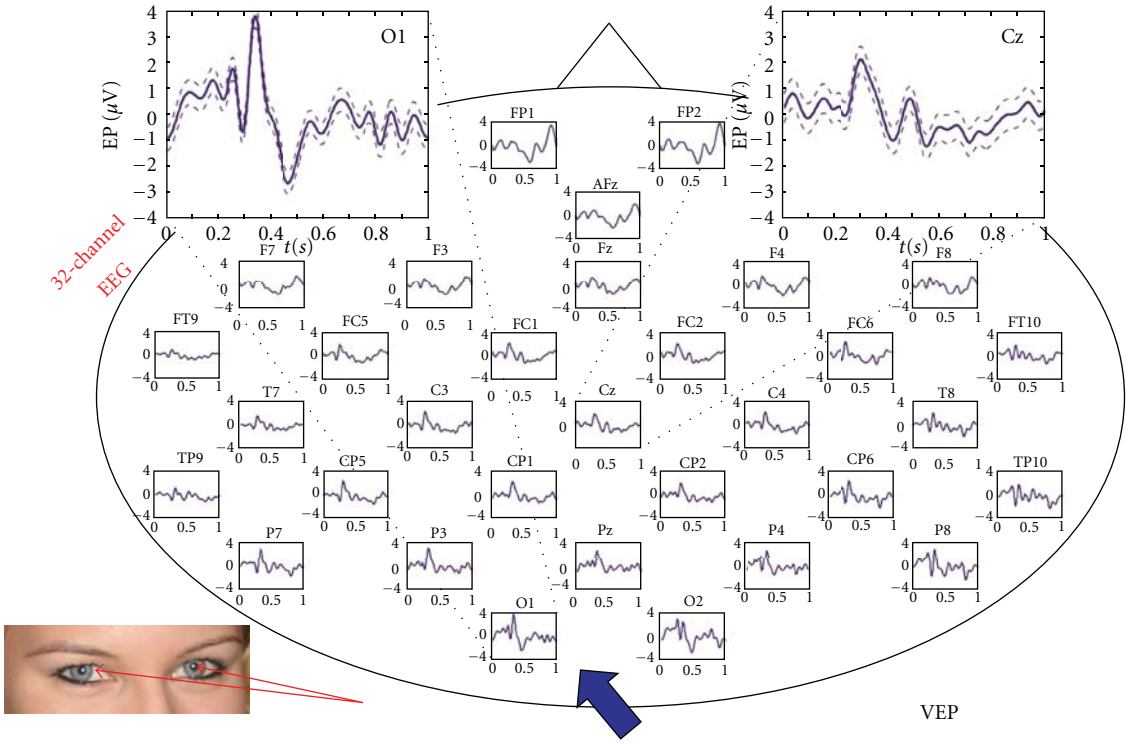

Figure 4: 32 visual evoked potentials after visual input (indirect stimulation with laser light of 658 nm). Note the VEPs are present over the entire scalp with a dominance over the occipital (blue arrow) and central regions (X-axis: s; Y-axis: μV; mean ± SE).

the body, the head, hands, or ears [2–14]. In this investigation, laser irradiation of 658 nm and 55 mW laser diodes was coupled into an optical fiber, and the laser needle was arranged at the distal end of this fiber. Due to coupling losses, the output power of the laser needles was reduced to 40 mW. The fiber core used in this study was about 500 μm in diameter. Stimulation frequency was 1 Hz. The method is described in detail in previous publications [2–14].

In order to have the laser pulses act as the sole stimulus to the average with the EEG (electroencephalography) recording system, each flash of the laser was accompanied by a 5 V pulse signal that triggered the average (Figure 2).

As in further studies [1], control experiments were performed to eliminate the possibility that the evoked potential obtained after laser irradiation was influenced by timed artifacts arising from the interface between the laser instrument and the signal averaging system.

2.3. EEG Data Acquisition and Analysis. For the EEG investigations we used two USB biosignal amplifiers (g.USBamp generation 3.0) with 16 input channels each. For all channels, the simultaneous sample rate was set to 512 Hz (24-bit resolution) with a high-pass filter at 0.1 Hz, a low-pass filter at 100 Hz, and a notch filter at 50 Hz [15].

In the present investigation, we recorded 32 EEG channels. The electrodes were positioned on the cap according to the 10-20 system; the reference electrode was placed on the nose, and the grounding electrode was placed behind the ear above the mastoid process (compare Figure 1). Electrode impedance was less than 5 kOhm in each position [15].

The software package MATLAB was used for the analysis of the evoked potentials. After a visual inspection of the raw EEG data, trials containing artifacts were marked and omitted from further analysis. Afterwards the raw EEG was band pass filtered between 0.8 and 10 Hz. Finally, altogether about 600 trials were averaged from the processed EEG.

2.4. Control Measurement. In the control measurement, the optical stimulation of the laserneedle was visible for the healthy volunteer. To avoid a direct stimulation of the eye, the subject wore eye protection glasses, and the light source was located behind a screen.

2.5. Optical Acupuncture and Placebo Stimulation. The laser needles were placed on the skin at the Neiguan (PC6) acupuncture point bilaterally (Figure 3). PC6 is situated between the tendons of the palmaris longus and flexor carpi radialis muscles, 2 cun proximal to the transverse crease of the wrist [16, 17]. The stimulation with red light (658 nm) was not felt by the subject. The volunteer has open eyes; however, the stimulation area was covered, and therefore the subject could not see whether the stimulation was on or off (compare Figure 1).

Figure 5: Reproducible evoked potentials after bilateral nonperceptible optical laser stimulation at the Neiguan acupuncture point. The evoked potentials are dominant over the central areas (red arrow) (X-axis: s; Y-axis: μV; mean ± SE).

3. Results

The result of the 32-channel visual evoked potential analysis is displayed in Figure 4.

Figure 5 shows the same procedure; however the laser light stimulation at Neiguan acupuncture point was not visible and not perceptible for the subject (compare Figure 1). Only very small but reproducible evoked potentials are detectable, mainly over the central and frontal region.

To facilitate comparison between the results of the visual evoked potentials and the laser-induced evoked potentials, the two measurements are plotted on top of each other in Figure 6.

4. Discussion

An irradiation, as from a laser, is not a stimulus found in nature. Lasers (light amplification by stimulated emission of radiation) allow brief pulses (μs to ms) with very fast rise time [18].

The laser needles used for acupuncture point stimulation in this study do not produce high temperatures; however, the penetration depth of the focused red laser light (658 nm) is about 3-4 cm [2]. Other experiments have indicated that brief high heating rate diode laser pulses can selectively activate myelinated Aδ fiber nociceptors in rats and produce pricking pain in humans, whereas for low heating rate, longer pulses can preferentially activate unmyelinated C fibers in rats and produce burning pain in humans [18–22]. There is no evidence whether these different pulse parameters will differentially activate fibers in humans.

In our present study, the laser needle stimulation (658 nm) was not felt by the subject. It is very interesting that this nonperceptible optical stimulation can lead to cortical responses. This finding appears to be unique in literature. To the best of our knowledge, there are no studies in scientific literature describing this phenomenon. Some reports concerning the effects on human brain EEG caused by manual needle stimulation at the PC6 acupuncture site are available [17]. These authors found that the frequency

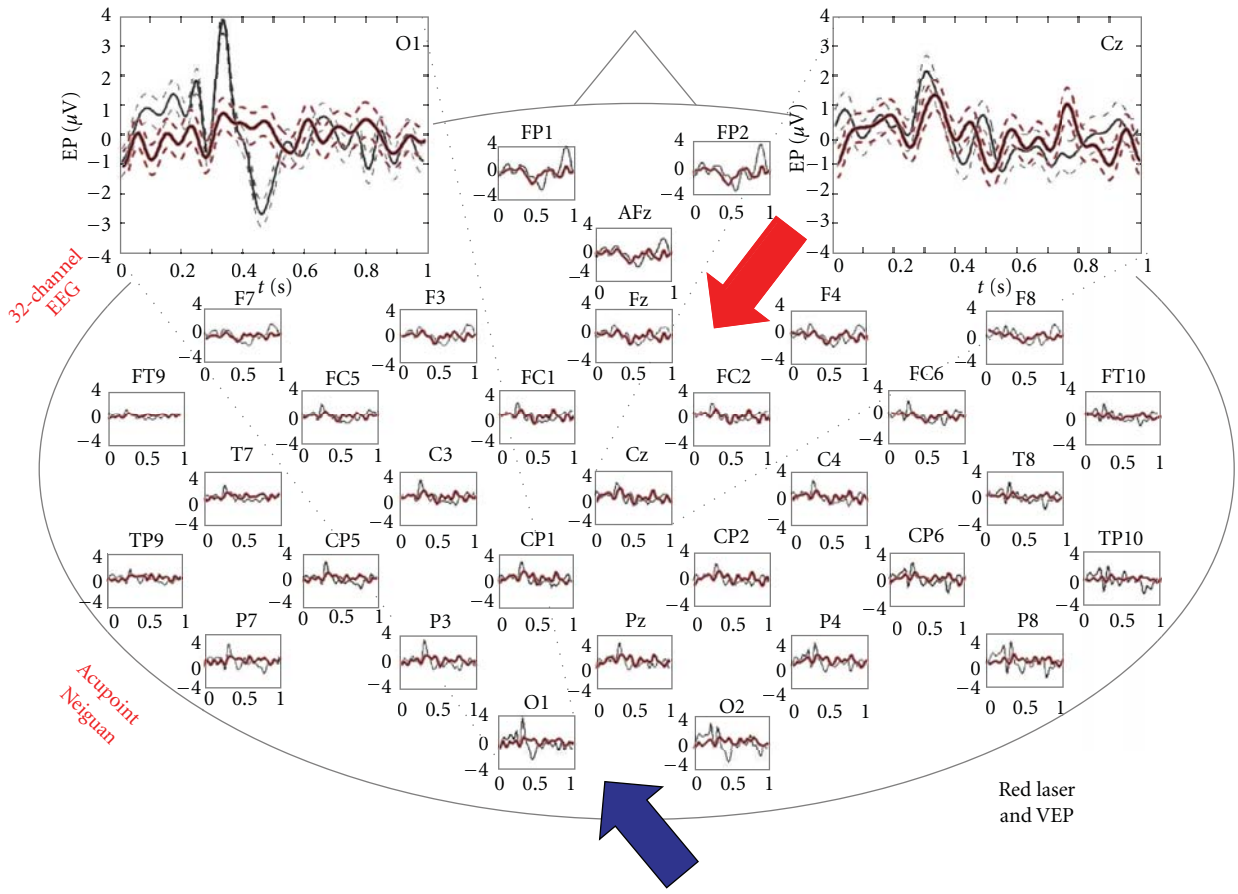

Figure 6: Comparison of visual and laser-induced evoked potentials plotted with the same scale (X-axis: s; Y-axis: μV; mean ± SE).

peaks in alpha band of 12 channels were more synchronized during and after acupuncture [17].

Conscious perception of stimuli requires two intact systems. The first one is, the specific input system which results in an evoked potential. The second one is, the unspecific system called ARAS, the ascending reticular activating system which was first investigated by Moruzzi and Magoun [23].

There is an example which is similar to the mechanism probably at work in our experiment; for example, during sleep, the ear is also in an activated state; auditory evoked potentials are possible although one does not consciously perceive the stimuli. Therefore it could be possible that laser stimulation modulates functional structures in the ascending reticular activating system.

Further studies with EEG and other neuromonitoring techniques like near infrared spectroscopy [24] and different stimulation methods (optical-cave VEP, electrical, mechanical) are in progress and absolutely necessary to confirm or refute the preliminary findings.

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Review Article

Integrative Laser Medicine and High-Tech Acupuncture at the Medical University of Graz, Austria, Europe

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At the moment, modernization of acupuncture has a high priority. On the traditional side, acupuncture has only recently been awarded the status of Intangible Cultural Heritage by the UNESCO. On the innovative side, high-tech acupuncture is a registered trademark in Austria. Acupuncture has been used for medical treatment for thousands of years. A large number of empirical data are available but the technical quantification of effects was not possible up to now. Using electroacupuncture, needle, or laser stimulation and modern biomedical techniques, it was possible for the first time to quantify changes in biological activities caused by acupuncture. This paper which serves as introduction for the special issue "High-Tech Acupuncture and Integrative Laser Medicine" of the present journal, focuses on the latest innovative aspects that underline the further enhancement and development of acupuncture. Special emphasis is given to new methodological and technical investigations, for example, results obtained from all kinds of acupuncture innovations (e.g., teleacupuncture) and integrative laser medicine.

1. Introduction

The scientific and technological progress has truly revolutionized eastern and western experimental and clinical medicine recently. The last century was certainly the most innovative phase in medical history. At the Medical University of Graz, our TCM (Traditional Chinese Medicine) Research Center has made various efforts within the last 15 years to modernize acupuncture [1–11], one of the most spectacular Eastern medical procedures. This paper article focuses on the latest aspects that underline the further enhancement and development of modern acupuncture. High-tech acupuncture comprises many different forms of stimulation and recording techniques (G. Litscher, Austrian Patent Office, no. AM 7066/2001, 202 560, valid until 2022) [12]. At the high-tech acupuncture laboratory (see Figure 1) of the Medical University of Graz, a broad spectrum of future-oriented bioengineering methods is used in joint projects with different leading acupuncture institutions in China. One can find an exemplary listing of important noninvasive procedures in Figure 2.

For the application of stimuli at the acupuncture points we use manual needle acupuncture (Figure 3(a)), laser needle acupuncture (Figure 3(b)), and electrical methods (Figure 3(c)).

For current acupuncture research, the usage of advanced exploratory tools multidirectional transcranial Doppler ultrasound sonography, cerebral near infrared spectroscopy, functional magnetic resonance imaging, different bioelectrical methods, and other highly sophisticated biomedical equipments, provides revealing insights. The obtained results are absolutely necessary for the acceptance of acupuncture by the western medical community.

2. Modern High-Tech Acupuncture Stimulation Methods

Since ancient times, metal needles are the most important tools for stimulating different acupuncture points [13]. New optical and also electrical stimulation methods were

Figure 1: High-tech acupuncture laboratory (2011) at the Medical University of Graz, Austria.

scientifically investigated by our research group within the last years. These procedures are described in the following.

2.1. Laser Acupuncture (405 nm). Up to now, violet lasers are used only in special areas in medicine [14, 15], because it is a new and still expensive invention. In acupuncture research, violet laser was applied only in a few scientific investigations until now, which were published by our research group [12, 16–20] (Figures 4(a) and 4(b)).

Violet laser needle acupuncture is a new optical method for stimulating different acupuncture points continuously and simultaneously. A wavelength of 405 nm, an output power of 110 mW, and a diameter of 500 μm were used for our experimental investigations. The system consists of 10 semi-conductor injection laser diodes (Figure 5).

Each single needle can emit a different wavelength. We used a continuous wave mode (CW). Due to coupling losses, the output at the tip of the needle is about 100 mW. Irradiation usually lasts 10 min (600 sec), and, therefore, optical power density was very high (range: kJ/cm^2) [19]. The violet laser needles are placed vertically at the skin and trigger painless but perceptible stimulation at the acupuncture point.

Violet laser acupuncture was made possible only due to latest inventions. Nakamura et al. [21] developed small, convenient blue and violet lasers which had not been available before. The acupuncture laser equipment used in our studies operates, as already mentioned before, at a wavelength of 405 nanometers. It is worth noticing that this wavelength is not in fact blue but appears to the eye as violet, a colour for which the human eye has a very limited sensitivity (Figure 6).

The violet laser does not have similar penetration depth in human skin as for example the red or infrared laser described in the next subsection (violet: approximately 2 mm versus red/infrared: 2-3 cm [12, 22, 23]); however, there is an evoked deQi-sensation, which is a prerequisite for effective acupuncture stimulation. DeQi is described by patients and volunteers as heaviness or like an electrical current running along the treated meridians. If red (685 nm) or infrared (785 nm) lasers are used, the patients normally do not notice when the laser is started. So in the beginning of the treatment they also do not feel any deQi sensation. Several minutes later (5–10 min) many patients report a pleasant warm and sometimes vibrating feeling in some treated areas [24].

In an experimental pilot study we found that violet laser stimulation increases temperature (mean ~1.5°C) and microcirculation (mean ~20%) at the acupoint Hegu (LI.4) significantly and immediately (1 min) after stimulation onset (Figure 7) [12]. The main interesting finding of our second publication concerning violet laser acupuncture was that heart rate decreases significantly within an interval of 5 min after violet laser stimulation onset at the acupoint Neiguan (Pe.6) [16]. Four interesting studies performed recently [17–20] will be presented in the respective subsections in the recording methods section (temperature distribution, microcirculation monitoring, cerebral blood flow velocity, and arterial stiffness and wave reflection).

2.2. Laser Acupuncture (685 nm and 785 nm). The first bichromatic laser needles (685 nm and 785 nm) were developed at the University of Paderborn, Germany (Dr. Detlef Schikora), and the first clinical investigations were performed in Lauenförde, Germany (Dr. Michael Weber). The first scientific experiments and publications on this field of research started in 2002 at our Medical University of Graz, Austria [22, 25–29]. A new laser needle acupuncture system based on red and infrared laser light is shown in Figure 8.

Multichannel laser needle acupuncture allows the simultaneous stimulation of individual point combinations [22, 26]. Variations and combinations of acupuncture points according to TCM are possible on the body, or at the ear and hand using Korean or Chinese hand acupuncture. The bichromatic laser needle method is based on systems with 8–12 separate semiconductor laser diodes and emission wavelengths of 685 nm and 785 nm. The system consists of flexible optical light fibers, which conduct the laser light with minimal loss to the laser needle. Thus, a high optical density can be achieved at the distal end of the laser needle. The intensity of the laser needles is optimized in such a way so that the volunteer or patient does not immediately feel the activation of the needle (30–40 mW per needle; diameter 500 μm; duration 10 min; power density ~20 J/cm^2 per acupuncture point). More details regarding this method are described in previous studies and books [22, 28, 29].

2.3. Electroacupuncture. Ear acupuncture can be performed using ultrathin permanent needles (P-Stim, Biegler GmbH, Mauerbach, Austria). A generator located behind the ear produces electrical stimulation impulses that are transferred to the acupuncture points on the ear via the needles (Figure 9).

After selection of the ear acupuncture points, a position tape previously prepared with the P-Stim application pointer is applied. This procedure is repeated until all acupuncture points are marked. Then, the needles can be taken up by the application pointer and applied. The wires are connected to the needles by snapping conductive plastic rings over the needles. Electrical stimulation is performed using a constant AC (alternating) current of 1 mA; impulse duration is 1 ms, stimulus frequency is 1 Hz. This method can be

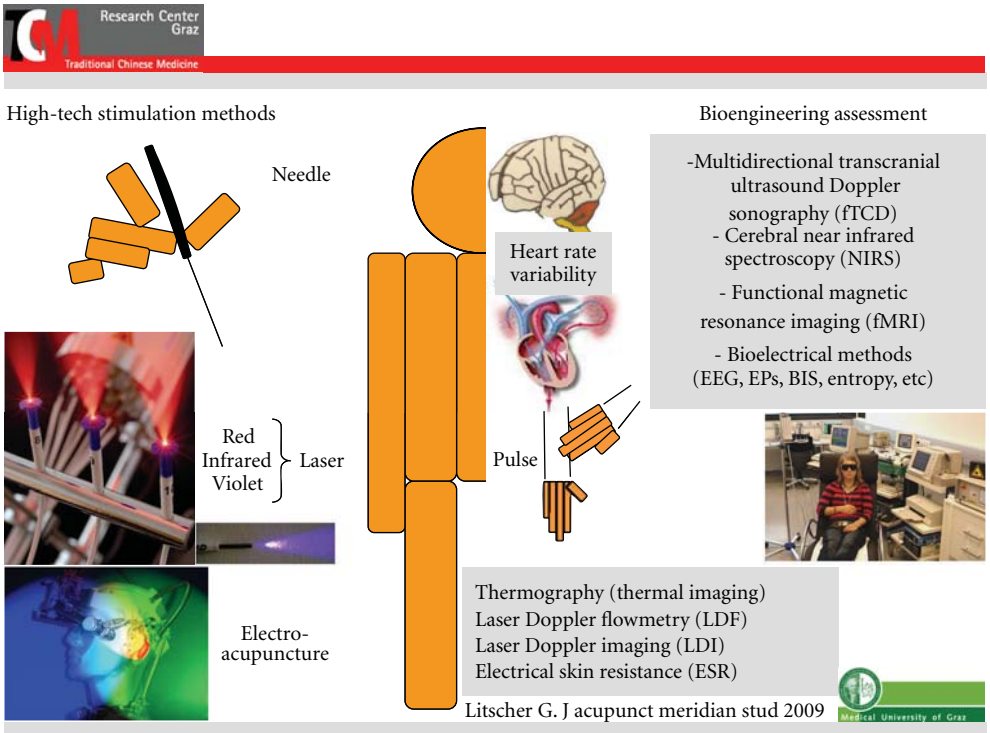


Figure 2: High-tech biomedical methods for manual and computer-based stimulation and quantification of peripheral and cerebral effects of acupuncture. All these procedures are used at the Medical University of Graz, Austria (modified from [12]).

used for pain treatment and was developed by the Medical University of Vienna (P-STIM; Biegler, Austria). This kind of electroacupuncture has also been investigated at our TCM Research Center [30, 31]. In the past, traditional acupuncture and electroacupuncture were characterized by the fact that these methods were confined to the clinic and/or the physicians practice. P-Stim allows continuous, intermitting stimulation up to several days combined with absolute mobility of the patient [32].

Manual needle acupuncture, laser needle stimulation, and electroacupuncture are based on totally different physical principles, and the stimulus intensities are not directly comparable. However, all three methods can induce similar effects in the brain and in the periphery. This has been shown in studies comparing metal needle acupuncture and laser needle stimulation [27] as well as in investigations comparing optical and electrical stimuli [33].

3. Modern High-Tech Acupuncture Recording Methods

We have been using many different methods of bioengineering assessment for our investigations over the last years. In this paper, we describe methods for biosignal analysis in the periphery and also for the central nervous system. Heart rate variability measurements and analyses complete the methodological spectrum (cf. Figure 2).

3.1. Peripheral Effects

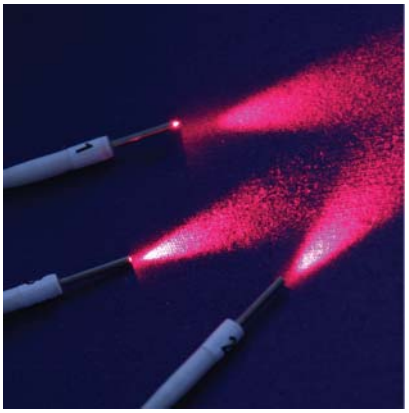
3.1.1. Thermography. Thermal imaging involves measurements of the surface temperature using an array of infrared sensors installed in an infrared camera. This imaging allows for the simultaneous measurement of temperatures of multiple points on the skin and is also a reference for the surrounding temperature. For a study performed in our lab recently [19], we used a FLIR i5 infrared camera (Flir Systems Inc., Portland, USA) with a wavelength range of 7.5–13 μm (Figure 10). Temperature distribution measurements were possible in the range between 0°C and +250°C. The data were transferred to a notebook computer using ThermaCAM Researchers Pro 2.8 software (Flir Systems Inc., Portland, USA).

We investigated ten healthy volunteers (mean age ± SD: 24.9 ± 3.3 years; 5 f, 5 m) before, during, and after stimulation using a noninvasive violet (405 nm) laser needle at the Dazhui (GV.14) acupoint. The results showed significant ($P < 0.001$) increases in temperature at the region of interest around the acupuncture point (Figure 11).

In two persons, it was demonstrated that needle acupuncture and placebo (deactivated laser) did not have the same temperature effects [19].

Quantitative thermal imaging is becoming an important method in acupuncture research. Infrared thermography enables the measurement of patients' or healthy volunteers' skin surface temperature profiles without influences caused

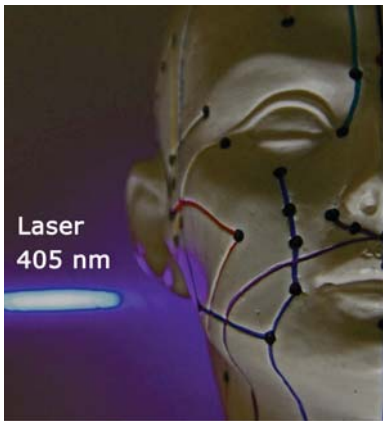
(a)



(b)

(c)

Figure 3: Acupuncture stimulus application: manual needle acupuncture (a), laser needle acupuncture (b), and electrical acupuncture stimulation (c).



(a)

(b)

Figure 4: Innovative laser stimulation with a violet laser beam.

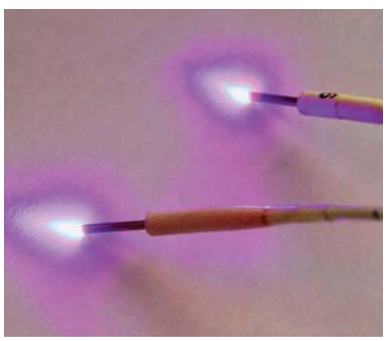
by direct contact of probes to the skin. Therefore, thermography is also a useful method for evaluating peripheral effects of acupuncture.

There are still methodological limitations of thermal imaging used in acupuncture research. The validity of the method for proving meridian structures according to the view of TCM must be considered critically and analyzed scientifically [34, 35].

3.1.2. Laser Doppler Flowmetry (LDF). Using noninvasive laser Doppler flowmetry it is possible to quantify peripheral changes in microcirculation during different methods of acupuncture stimulation [2]. An important parameter is Flux (product of mean blood flow velocity and concentration of red blood cells). The measurements described in Figure 12 were performed with the LDF-monitor DRT4 (Moore Instruments Ltd., Millwey, Axminster, UK). Edge frequencies were 20 Hz and 22.5 kHz. The temperature unit (5–50°C) had a resolution of 0.2°C and an accuracy of 0.5°C. Two standard probes (DPIT, diameter 8 mm, length 7 mm) were used. Flux was recorded at a distance of 3 cm distal from the acupoint Dazhui (Flux 1) and at a control point proximal to the upper arm (Flux 2). The exact position of this control point was 2 cun above LI.14 and 1 cun medially from the line connecting LI.14 and LI.15. The control point was chosen because no application of pressure occurred in this location, and the region of interest for the thermographic measurements is not influenced by the sensor temperature (cf. Figures 10 and 11).

Three measurements of microcirculation parameters in a 29-year-old female volunteer during placebo, violet laser, and metal needle acupuncture are shown in Figure 12. Compared to violet laser acupuncture, stimulation using

(a)



(b)

(c)

Figure 5: Multichannel system (a) with violet laser needles (b) for acupuncture stimulation (c) used at the Medical University of Graz.

needle acupuncture enhanced the effect, whereas the placebo procedure (deactivated laser) did not lead to any significant change [18].

LDF is a technique used for investigating changes in microcirculation. It allows objectifying circulation within the microcapillary range without strongly influencing tissue structures. The principle is based on the Doppler shifting of light when light hits moving particles (erythrocytes).

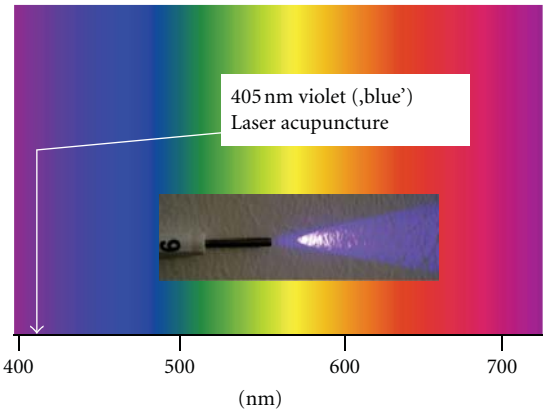


Figure 6: Portion of the electromagnetic spectrum that is visible to (can be detected by) the human eye. Electromagnetic radiation in this range of wavelengths is called visible light. A typical human eye will respond to wavelengths from about 390 to 750 nm. Our violet laser for acupuncture works at a wavelength of 405 nm (modified from [17]).

3.1.3. Laser Doppler Imaging (LDI). Like LDF, laser Doppler imaging (LDI) is also based on the laser Doppler principle whereby data can be obtained directly from the reflected light and is displayed as a color-coded picture showing the distribution of tissue perfusion. LDI is a further advancement of punctual LDF. Here, laser light with low output is directed to the surface of the skin. A servomotor directs the laser light to a maximum of 4096 different positions from one measurement point to the next [36].

The "PIM II" laser Doppler perfusion measurement system from Lisca AB (Linköping, Sweden) includes a scanning head, an optoisolator unit, an AD converter, and a computer. The scanning head comprises a laser unit, an optical system for gradual scanning, and an optode detector unit. The optode isolator unit includes circuits for signal evaluation, stepper motor drive, and circuits to guarantee galvanic isolation between the scanning head and the computer.

A solid laser (670 nm) scans the tissue surface step by step. The reflected light is checked for Doppler effects at 4096 positions of the tissue surface. Monochromatic laser light penetrates each tissue area to a depth of several hundred micrometers. When a moving particle such as a red blood cell is hit, one part of the photons experiences a frequency shift according to the Dopplers principle; the remaining photons in stationary tissues do not underlie these changes. A small part of the reflected light comprises changed and unchanged light and thus influences the photodetector in the scanning head. The combination of light on the photo detector surface results in a change in photo current determined by the Doppler-shifted particles of light. Major Doppler frequency shifts caused by the high velocity of moving blood cells usually increase the frequency of photocurrent, whereas the peak of fluctuation reflects the number of moving blood cells. If this fluctuation element of photo current is guided through a filter and the immediate energy is calculated, a signal for tissue circulation can be derived (i.e., mean velocity

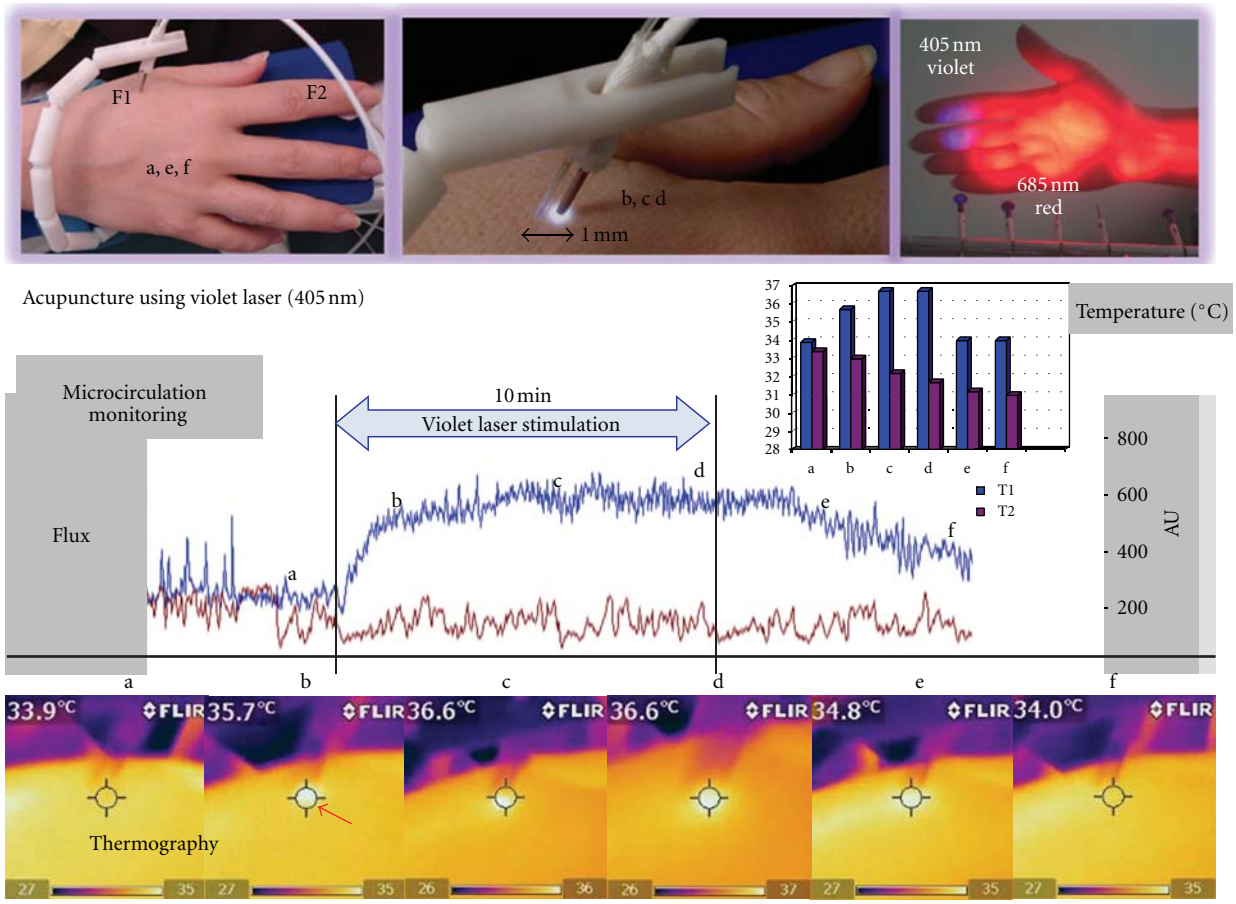


Figure 7: The first violet laser acupuncture using a wavelength of 405 nm. Note the significant increase in microcirculation (Flux1, F1; blue curve) 1 mm beside the violet laser needle at the acupoint Hegu (LI.4). Temperature at this location (T1, same as F1) was measured using thermal infrared imaging (Flir i5). Note also the increase of temperature from 33.9°C to 36.6°C in the region of interest in this volunteer. Flux2 (F2) and temperature2 (T2, location same as F2) were recorded at the index finger (modified from [12]).

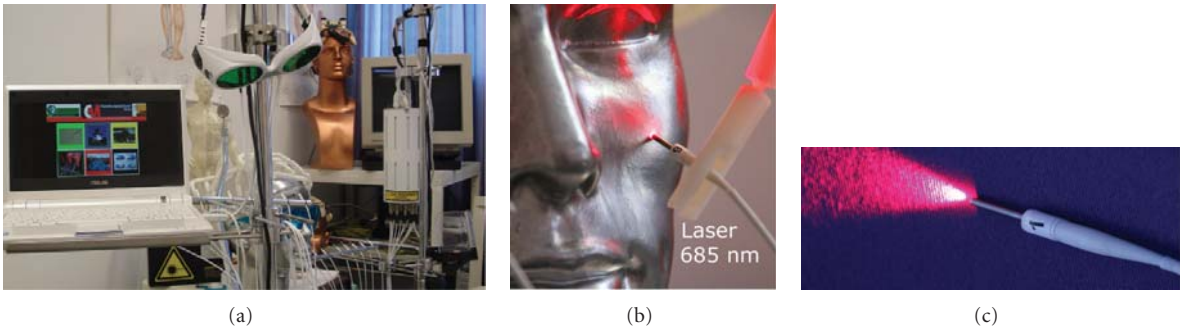


Figure 8: Multichannel laser acupuncture (a) using bichromatic laser needles with red (685 nm) and infrared (785 nm) light (b) and (c).

multiplied by the concentration of blood cells within tissue distribution).

Any relative movement between laser light and tissue must be avoided during Doppler signal registration in order to prevent artifacts. The system guarantees this with its patented step-by-step scanning method. The laser light remains fixed in relationship to tissue, while the reflected light is analyzed. For registration of light reflection, laser light remains at each single measurement point for about 50 ms.

Figure 13 shows a continuous microcirculation monitoring of the right foot in a 62-year-old patient with cryoglobulinaemia before, during, and after manual needle

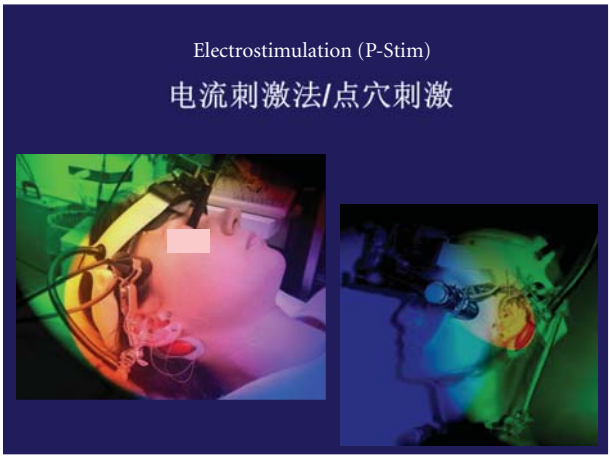


Figure 9: Ear electroacupuncture using a system developed in Austria.

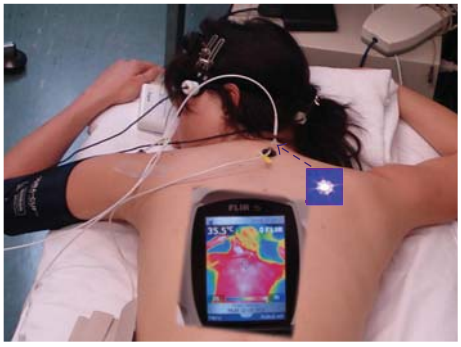


Figure 10: Thermographic monitoring during violet laser acupuncture at the Medical University of Graz. The position of the violet laser needle at the acupoint Dazhui (GV.14) is marked with a violet arrow. Modified from [19].

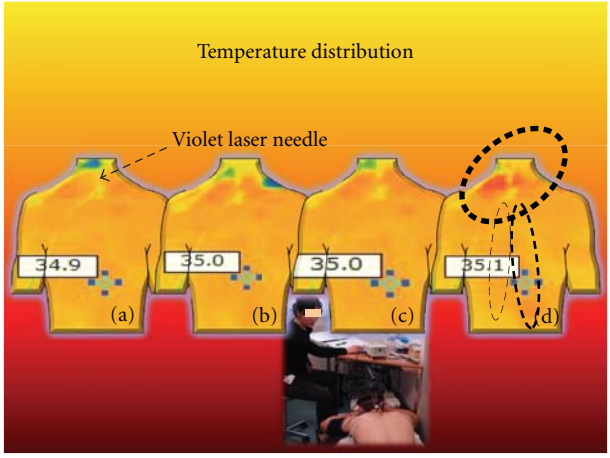


Figure 11: Four thermal images from a 23-year-old healthy male volunteer before (a), during (b) and (c), and after (d) violet laser stimulation at the acupoint Dazhui (GV.14). Note the significant ($P < 0.05$) increase at the shoulder and also the increase in skin temperature at the "far field" location around the Zhiyang (GV.9) area of the same meridian. Modified from [19].

acupuncture at Jie Xi (St.41) acupoint [37]. In addition to visual classification, calculation of alterations of mean perfusion was performed.

These results suggest that the new biomedical method of laser Doppler imaging may be useful to monitor peripheral effects of acupuncture on microcirculation. LDI studies on acupuncture show that needle acupuncture and also laser needle acupuncture can lead to quantifiable changes in microcirculation parameters in addition to the changes in temperature of skin surface.

3.1.4. Electrical Skin Resistance. A scientific literature research (http://www.pubmed.gov/) shows more than 600 articles (January 2012) referring to electrical skin resistance measurements. Most of them are case reports. Only eighteen studies meet the criteria for further evaluation with regard to evidence-based medicine [38, 39]. These studies can be divided into acupuncture studies ($n = 9$) and the so-called "meridian studies" ($n = 9$). Five out of nine acupuncture point studies showed positive association between acupuncture point and lower electrical resistance and impedance. Four studies on this topic showed contrary results. The present limitations of the method are based on the one hand on the small tip of a pen-shaped device, which is pressed manually against the skin by the examiner and on the other hand on the repeated pressing of the electrode tip into the skin or scratching over the skin surface. Therefore an electrical skin resistance mapping consisting of many electrodes is desirable and has already been performed recently by our group and others [39–41].

The newly developed multichannel skin resistance measuring system from our group [40, 41] is used to characterize the variability in electrical resistance measurements in and around an acupoint and a nonacupoint. The system measures the skin resistance at 48 points, both absolutely and continuously. With software developed along with the hardware, both a high-resolution measurement and a graphical presentation of possible changes in electrical resistance in the region of interest are possible [41].

Figure 14 shows the results of a study performed in 10 male volunteers, ages 20 to 30 years (mean age ± SD: 24.6 ± 2.5 years) [42]. The aim of this study was to measure the skin resistance of an acupuncture point compared to a placebo point using the new system. The point Kǒngzuì (Lu.6) and a placebo point on the same level of the acupoint but located on the ulnar side of the heart meridian were used. These points were determined by an experienced acupuncture practitioner. Two measurements were carried out per person. The results of the electrical characterization (skin resistance) of the areas surrounding the acupuncture point and the placebo point were then compared. The measurements of skin resistance at the acupuncture point showed lower impedance values than those taken from the placebo point on the same arm. A significant ($P < 0.01$; ANOVA on ranks) difference of the values was found. Measured values on the acupuncture point were significantly lower (by 106 kOhm; mean values placebo point: 1218 kOhm, mean values acupuncture point: 1112 kOhm) [42].

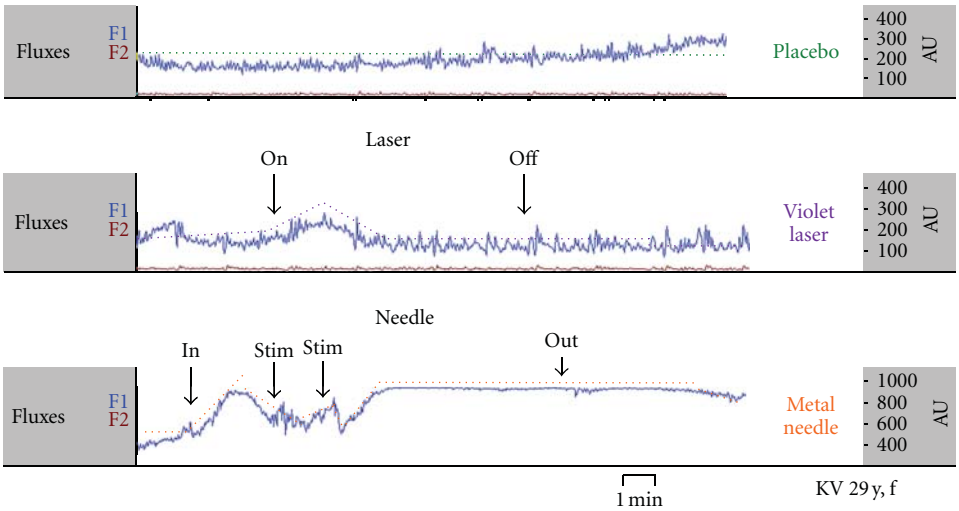


Figure 12: Increase of Flux1 (Dazhui acupoint area) during different kinds of acupuncture stimulation (violet laser and metal needle acupuncture) and during placebo (deactivated laser). Modified from [18].

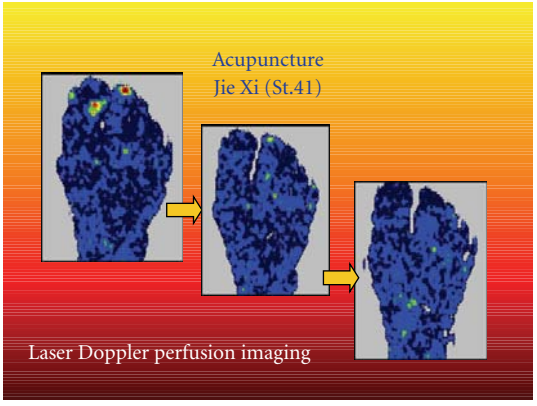


Figure 13: Laser Doppler perfusion imaging of the foot of a 62-year-old patient: red/yellow/green/blue: highest relative perfusion; black: lowest perfusion. Note the increase in mean perfusion (change from black to blue) in the entire region of interest. Modified from [37].

The electrodermal mapping is an innovative method for highly precise skin resistance measurements [40–42].

3.2. Cerebral Effects

3.2.1. Multidirectional Transcranial Ultrasound Doppler Sonography (TCD). Ultrasound waves are mechanic, matter-bound density waves with frequencies of >20 KHz. These are produced by electric alternating voltage being applied to piezoelectric crystals ("transducer"). The waves propagate in biologic tissue (with the exception of bone) at a nearly constant speed (~1550 m/s). The waves are totally or partially reflected and weakened by scattering and absorption at biologic-acoustic border regions. Ultrasound waves of low intensity (<10 mW/cm^2; diagnostics) are considered to be harmless [43, 44].

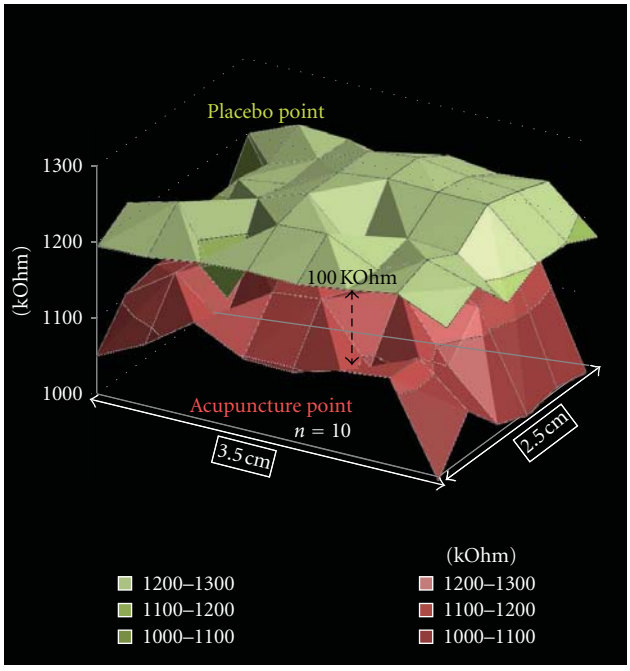


Figure 14: Graphical analysis of 48 channels of electrodermal skin impedance (average values of $n = 10$ persons) at an acupuncture point (below) and a nonacupuncture point (placebo point; above). Note the mean difference between the two surrounding areas is about 100 kOhm. Modified from [42].

Investigations of the neuronal correlates of acupuncture in the human brain were limited by the lack of noninvasive continuous measurement methods such as multidirectional transcranial Doppler sonography in the past. In different studies using these new techniques we have revealed the existence of specific acupoint-brain correlations. Some of these correlations are summarized in Figure 15. For example,

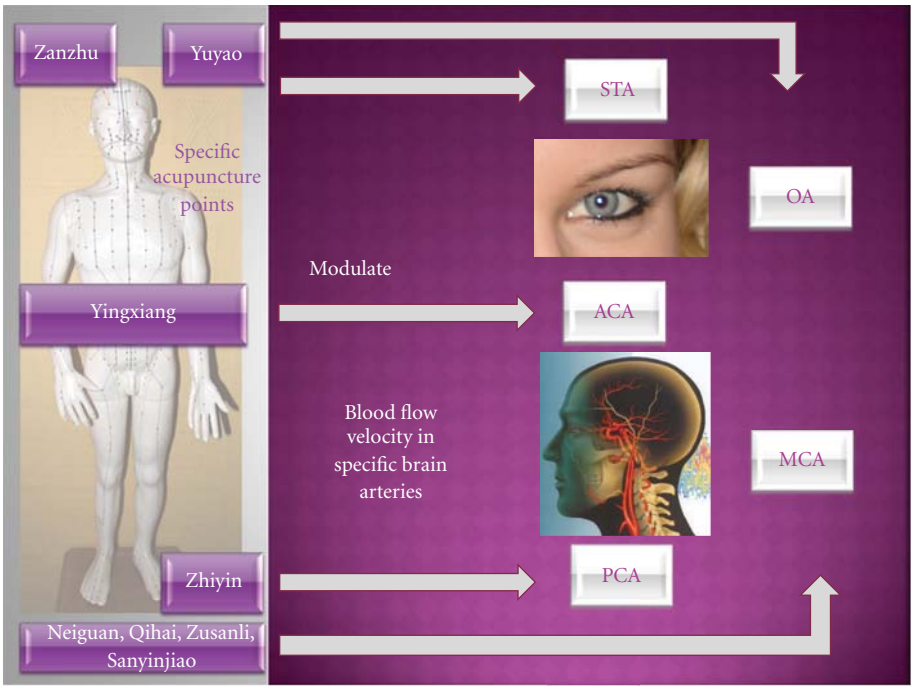
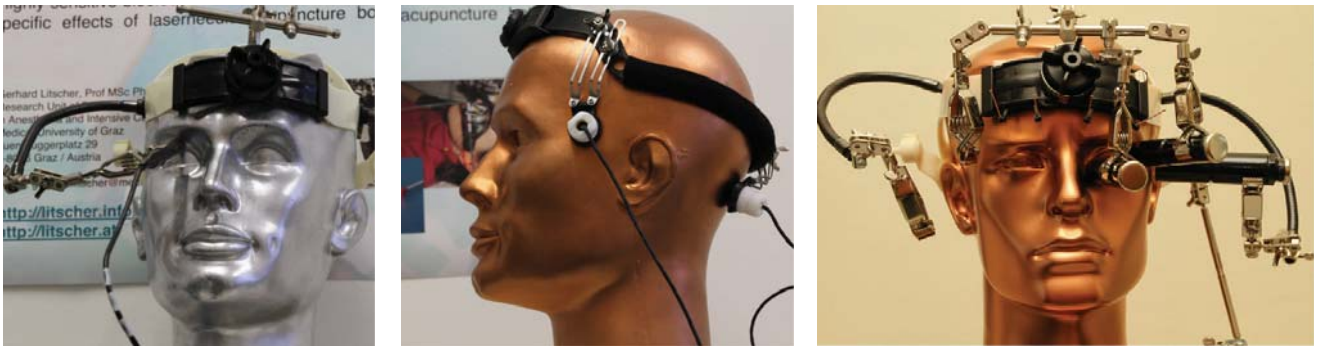


Figure 15: Evidence from multidirectional transcranial ultrasound Doppler sonography: specific acupuncture points (left) modulate blood flow velocity in specific brain arteries (STA supratrochlear artery, OA ophthalmic artery, ACA anterior cerebral artery, MCA middle cerebral artery, and PCA posterior cerebral artery).



(a) (b) (c)

Figure 16: "Windows to the brain" for transcranial ultrasound investigations: transorbital (a) and (c), transtemporal ((b) left, (c) right) and transnuchal ((b) right). The probe holder constructions were developed at the TCM Research Center in Graz, Austria.

acupuncture points traditionally implicated for visual functions were shown to modulate the blood flow velocity of the corresponding cerebral arteries [45–50].

The middle cerebral artery (MCA) with its different branches can usually be investigated at a depth of 3-4 cm, the media main trunk usually lies at a depth of 5.5–6.5 cm. Blood flow velocity in the internal carotid artery (distal part at a depth of 60–65 mm) as well as in the MCA can be determined by transtemporal entry. With increasing depth of measurement volumina a part belonging to the anterior cerebral artery (ACA) can also be determined. If the ultrasound probe is turned slightly in the dorsal and caudal direction, the origin of the posterior cerebral artery (PCA) can be reached at a depth of 65–75 mm.

A restriction of intracranial TCD results could be ultrasound transmission through the skull. According to the literature, particularly older women could only be examined sufficiently in 50 percent of the cases. However, optimal transtemporal registration was not possible in 5–10% of men and younger women either [51].

Figure 17: Helmet construction from our research group for the first proof of changes in blood flow velocity in the brain caused by acupuncture. Modified from [45].

In addition to the three transtemporal acoustic windows (front, middle, and rear), transorbital and transnuchal entry for TCD-monitoring is also possible (Figure 16).

Measurement of blood flow profiles in the ophthalmic artery (OA) was performed with a probe, applied lateral to the cornea on the closed bulb of the eye (cf. Figure 16(c)). Reduction in transmission energy should be achieved as far as possible in order to avoid direct exposure of the lens to sonic waves. A signal from the OA is registered at a depth of 40–50 mm by a probe turned slightly in the central direction [44].

The foramen magnum offers a further path of entry for ultrasound measurements (cf. Figure 16(b) right). Dependent upon anatomical variations, the basilar artery (BA) lies at a depth between 70 and 110 mm.

For the ultrasound measurements in acupuncture research we have also developed innovative helmet constructions already in the late 1990s (Figure 17) [45–47].

In the year 1997, our interdisciplinary research team was able to scientifically prove that acupuncture needles can increase blood flow velocity in the brain [45]. The computer- and robotic-controlled biosensors and probes integrated in a special measurement helmet, coupled with light, ultrasound and highly sensitive bioelectrical monitoring methods yield reproducible results indicating that the blood flow velocity in the MCA is higher and the oxygen supply in the brain is increased by acupuncture in healthy volunteers [44, 46].

In tests performed with healthy volunteers in 1999, we could prove that acupuncture does not only lead to *general* changes in blood flow velocity in the brain but to *specific* changes (i.e., different regional localization; cf. Figure 15). Thus, stimulation of acupuncture points on the hand or outer side of the foot (e.g., Zhiyin), which according to TCM are often connected to the optical system, leads to an increase in mean blood flow velocity in the PCA, which supplies the occipital center of the brain. At the same time, the blood flow velocity in other cerebral arteries remains nearly unchanged. These effects can only be registered up to now when light stimulation is performed. Comparative investigation of points at the inner edge of the foot did not show changes in blood flow velocity in this particular cerebral artery [48].

Studies with a crossover design using different acupuncture schemes in the same subjects were performed to exclude the placebo effect during acupuncture as much as possible. Each person was treated with an optic acupuncture scheme A, which according to TCM should improve vision, and with another scheme B to improve perfusion in the middle cerebral artery. When using the optic scheme, a significant increase in mean blood flow velocity in the STA and OA occurred, whereas the flow velocity in the MCA remained nearly unchanged. In reverse, scheme B led to a significant increase in blood flow velocity in the MCA with nearly unchanged flow profile patterns in the optic arteries. Several crossover studies were performed and confirm the initial results of selective changes in cerebral perfusion after acupuncture [3, 48–50].

In a recent study of our TCM Research Center Graz in Austria, which was performed 2010 in [17] in our acupuncture lab, violet laser acupuncture has been investigated in context with blood flow velocity of the BA and MCA (see Figure 18). The aim of that study was to provide selective evidence of specific effects of violet laser acupuncture on mean cerebral blood flow. Again a transcranial Doppler sonography construction was used to monitor blood flow profiles in the BA and MCA simultaneously and continuously. In that controlled study the acupuncture point Dazhui on the upper back was tested with ten healthy volunteers. In addition to an on/off-effect, violet laser stimulation increased the blood flow velocity in the BA significantly compared with the reference interval before laser acupuncture. In the MCA, only minimal, insignificant changes in blood flow velocity were seen. Metal needle acupuncture at the same point intensified the effects; however, blood flow profiles did not change significantly during and after stimulation with deactivated violet laser (placebo) [17].

All our investigations performed with transcranial Doppler sonography demonstrate that acupuncture produces specific and reproducible effects on brain blood flow velocity. However the study designs and also our technology cannot explain the underlying mechanism.

3.2.2. Cerebral Near Infrared Spectroscopy (NIRS). Near-infrared spectroscopy (NIRS) is a noninvasive optical technique for assessment of functional activity in the human brain [47]. The sophisticated technique uses an optical window in the near infrared (NIR) light spectrum. The first in vivo results in humans were reported by Jöbsis in 1977 [52]. Within a spectral range of approximately 630 to 1300 nm, light can penetrate the cranium and reach sufficient depth to allow investigation of the metabolism

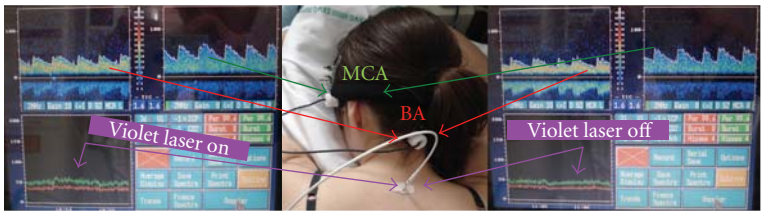


Figure 18: Example of the on/off effect on mean blood flow velocity at the beginning and end of violet laser acupuncture in a healthy female volunteer. Modified from [17].

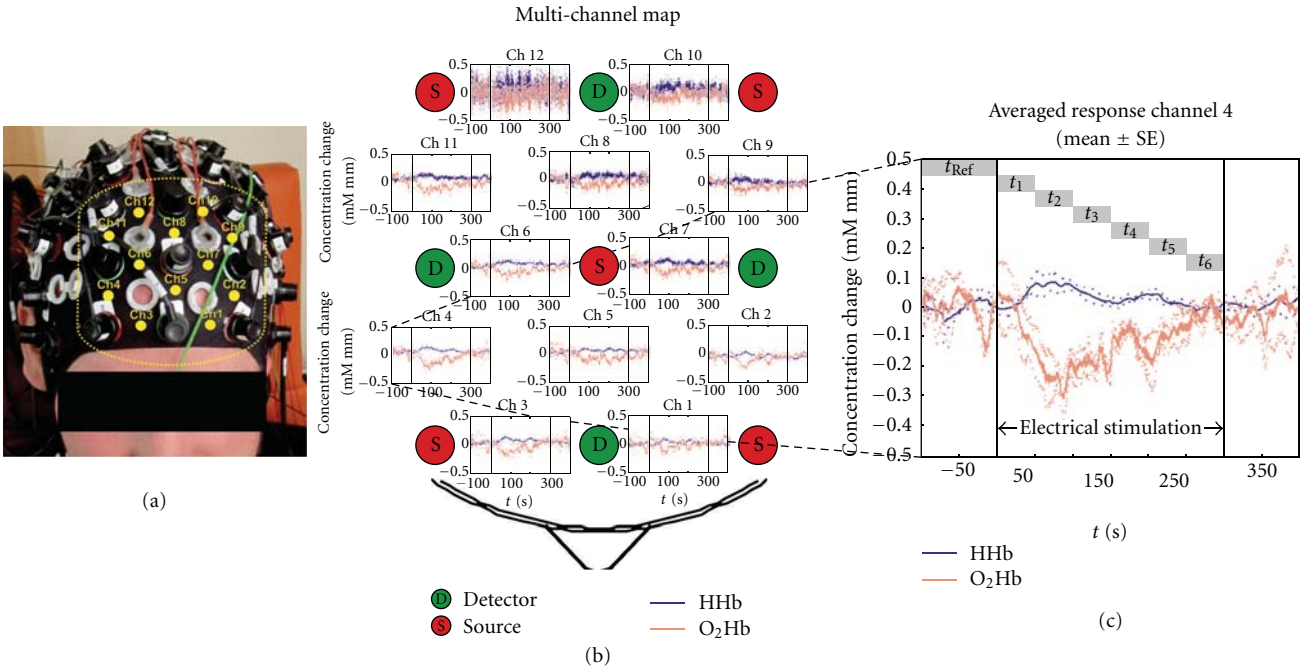


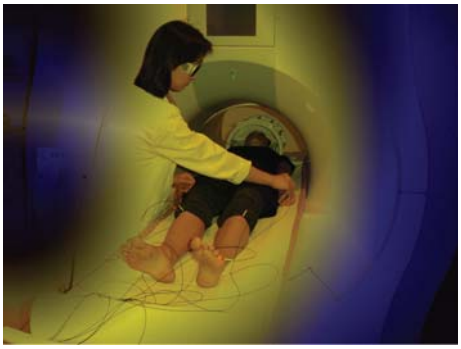
Figure 19: First 50-channel NIRS measurement at the Graz University of Technology (modified from [54]). (a) Electrode cap with detectors and emitters for recording NIRS parameters. The 12 channels presented in the multichannel map in (b) are numbered (Ch1-Ch12). (b) Concentration changes of oxy- (red lines) and deoxyhemoglobin (blue lines) in a map. (c) Averaged responses (mean ± SE (standard error)) of channel 4 before, during, and after electrical stimulation of Battlefield ear acupuncture points.

in the cerebral cortex. Existing reports on the use of NIRS during acupuncture are mainly focused on one channel recordings. For a review concerning NIRS and acupuncture see Litscher, 2006 [5].

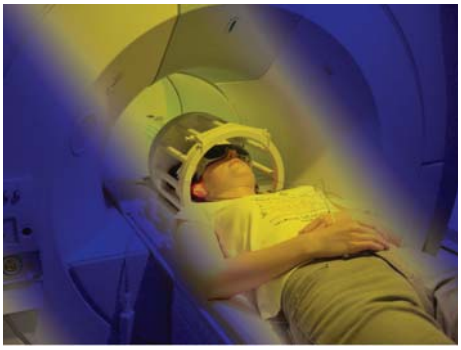
Different instrumental components are used in our NIRS acupuncture research. One system is the NIRO 300 (Hamamatsu Photonics, Hamamatsu, Japan) and another the INVOS 5100 Oximeter (Somanetics Corp., Troy, USA). Tissue oxygenation index (TOI) and regional cerebral oxygen saturation (rSO_2) were calculated in percent from the proportion of oxygenated and deoxygenated hemoglobin. Normal values lie between 60 and 80 percent [47]. The most important results of our NIRS studies can be summarized as follows [44, 53].

Needling and laser stimulation of a placebo point did not lead to marked changes in cerebral NIRS parameters during or 5 minutes after acupuncture. The combination of Korean hand acupuncture (E2) and Chinese hand acupuncture (Yan Dian) as well as TCM body acupuncture (Zanzhu (UB.2) and Yuyao (Ex.3)) and combined body, ear, and hand acupuncture lead to a marked increase in O_2Hb and a simultaneous decrease in HHb. This effect was still present 5 minutes after removing the needles or deactivating laser needle stimulation. One case of minimal contrary behavior in O_2Hb and HHb is present when needling or performing laser stimulation at both ear points (eye and liver). Standard monitoring parameters such as blood pressure did not show any significant changes during all types of acupuncture or combined acupuncture methods.

In a recent study we performed multichannel measurements using an optical neuroimaging system (NIRScout 1624, NIRx Medical Technology, Berlin, Germany) [54]. The system is intuitively operated through a graphical user interface (GUI). The GUI displays measured data for all channels in real time. This system uses wavelengths of 760 and 850 nm and the power is 10 mW per wavelength. The multi-channel system measures the change of O_2Hb and HHb in the unit of mM mm (m(mol/L)∗mm) and consists



(a)



(b)

Figure 20: First functional magnetic resonance imaging during laser needle acupuncture at the body (a) and ear (b). These measurements were performed at the Medical University of Graz.

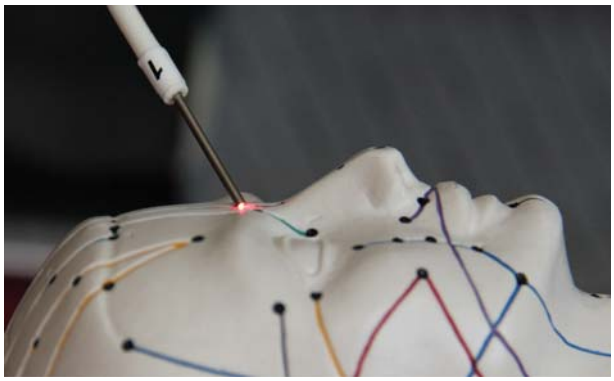


Figure 21: Optical stimulation of the acupuncture point Yintang using a painless laser needle.

(a)

(b)

Figure 22: Electrical stimulation at the Yintang acupoint area (light blue point in (a)) at the TCM Research Center Graz. Measurement position (b). With permission of the healthy volunteer and modified from [44, 67].

of 16 light emitters and 24 photodetectors. The distance between source and detector was 3 cm, and the sampling rate was set to 3 Hz. A special designed electrode cap was used to apply the noninvasive optodes (optical electrodes) above the frontal and motor areas to the intact skull (Figure 19), resulting in a total of 50 measurement channels.

Battlefield acupuncture was developed in the course of researching a more efficient auriculotherapy system for rapid relief of pain [31]. The objective of that study was to investigate possible changes of NIRS parameters in the frontal area of the brain during electrical stimulation of Battlefield acupuncture points. For the first time a 50-channel NIRS recording has been performed to get new insights into the possible cerebral effects of ear acupuncture. Electrical ear stimulation (constant current 1 mA; duration 1 ms, frequency 1 Hz) was used to stimulate the ear points. The main outcome measures were concentration of O_2Hb and HHb in the brain tissue. Regional decreases of O_2Hb in the frontal area were found in the 50-channel recordings, reaching their maximum within 100 seconds of stimulation onset [54].

3.2.3. Functional Magnetic Resonance Imaging (fMRI). Functional magnetic resonance experiments have also been successfully used to investigate changes in cerebral activity during acupuncture [4, 44, 55] (see Figure 20). The method is based on the indirect representation of neuronal activity and the resulting metabolic and circulatory changes, particularly the relative changes in concentration of oxygenated and deoxygenated haemoglobin [4, 44].

Since the late 1990s, fMRI has been used to investigate the underlying mechanism of the Chinese medical treatment of acupuncture. The research group of Professor Zang Hee

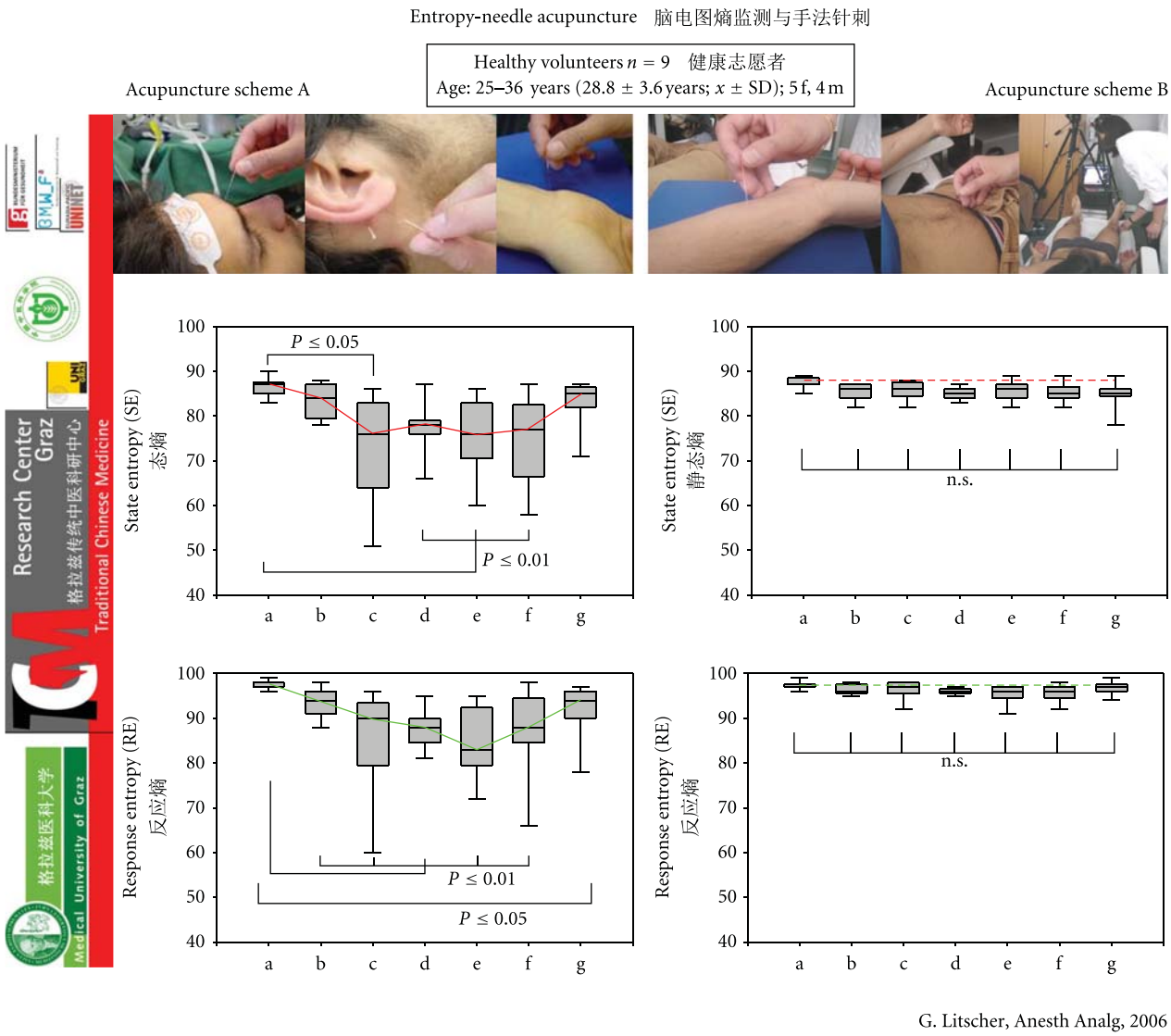


Figure 23: EEG entropy during needle acupuncture. Box-plot illustration of alteration in state (SE) and response (RE) entropy values in nine healthy volunteers 2 min before (a), during ((b) immediately after applying the needles; (c) 2 min after (b); (d) 5 min after (b); (e) 10 min after (b); and (f) 15 min after applying needles), and 2 min after (g) needle acupuncture using two different acupuncture schemes (A and B). Note the significant decrease in entropy when using scheme A (sedation points). The horizontal line in the box indicates the position of the median. The ends of the bars define the 25th and 75th percentile and the error bars mark the 10th and 90th percentile. Modified from [27].

Cho from Korea was one of the first to investigate effects of acupuncture using this method [49, 56]. However, some of the authors retracted the paper from Cho et al., 1998, on June 21, 2006. In this retraction they stated that there is no point specificity, at least for pain and analgetic effects.

In the meantime, there are more than 250 publications (http://www.pubmed.gov/; January 2012) on the topic "acupuncture and fMRI." The point specificity is still discussed controversially. The first review article of fMRI papers was published in Critical Reviews in Biomedical Engineering about five years ago by the author of this contribution [4].

The review by Beissner and Henke published in 2009 [57] should be mentioned especially as it shows clearly the limitations of fMRI investigations in acupuncture research. In a further publication from the same first author [58] the following recommendations are made: standard needles should not be used in MRI; nonferromagnetic metal needles seem to be the best choice for acupuncture points outside of the transmitter coil; only plastic needles are suited for points inside the coil. Laser acupuncture may be a safe alternative, too [4, 22, 58].

Recent studies using fMRI techniques have revealed the existence of specific acupuncture point and brain correlations, for example, acupuncture points traditionally implicated for auditory, visual, sensory, motor, and cognitive functions were shown to modulate the activity of the corresponding cerebral sites [59–63] and also to induce long-lasting effects [64].

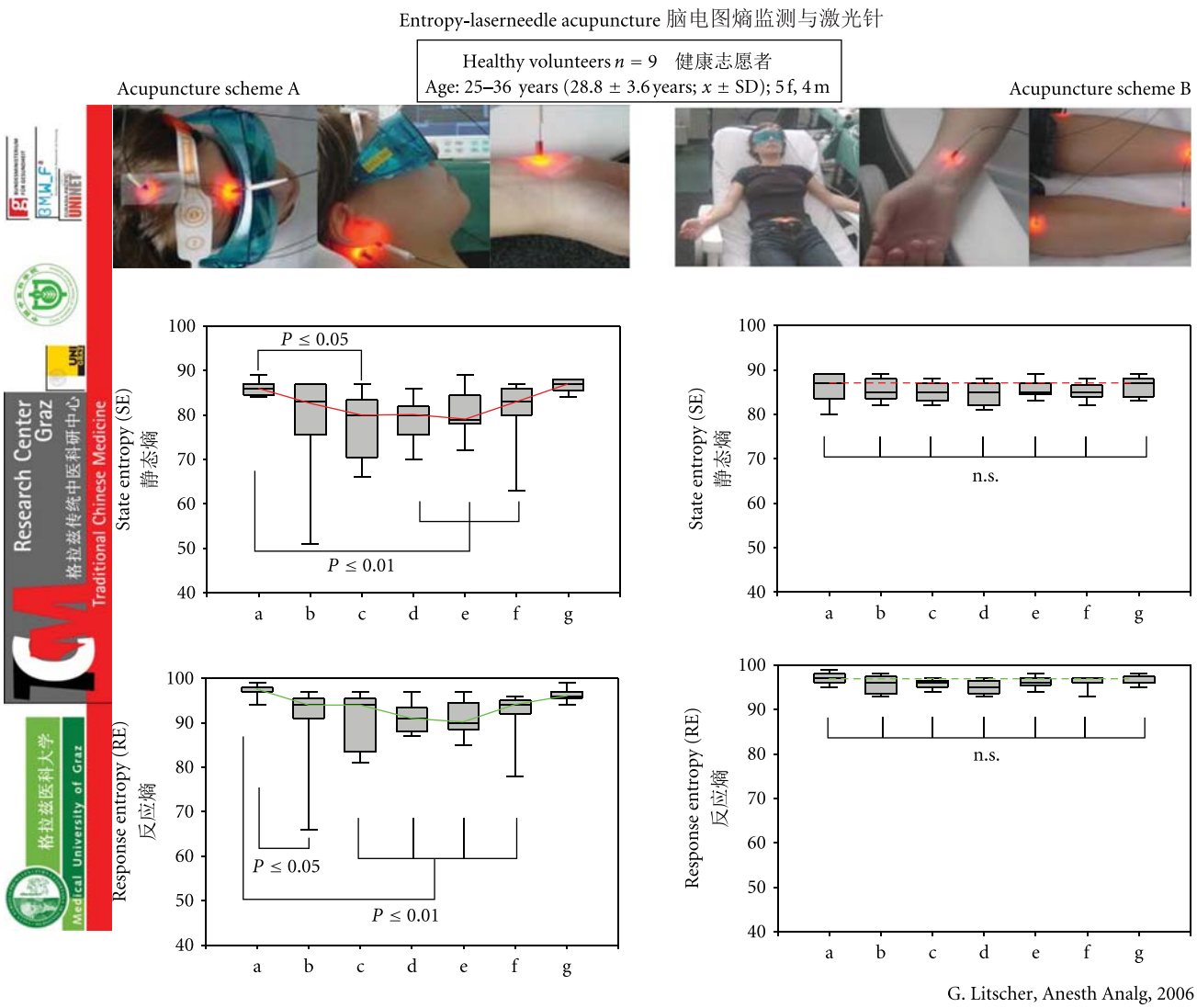


Figure 24: EEG entropy during laser needle acupuncture. Box-plot illustration of changes in state (SE) and response (RE) entropy values in nine healthy volunteers (a) before, (b)–(f) during, and (g) after laser needle acupuncture using two different acupuncture schemes (A and B). For further explanations see Figure 23. Modified from [27].

3.2.4. Bioelectrical Methods. Neurophysiologic monitoring is gaining more and more attention in acupuncture research [6]. Bioelectrical brain activity can be monitored using bispectral index (BIS) or entropy. Both are numerical descriptors of the electroencephalogram (EEG) and are mainly used for assessing depth of anesthesia.

In previous studies we have investigated the effects of acupuncture and acupressure at the acupuncture point Yintang in healthy awake volunteers. Acupressure stimulation results in statistically significant and clinically relevant reductions in BIS while needle acupuncture, laser needle acupuncture (Figure 21), and acupressure at a control point resulted in statistically significant but clinically unimportant reductions [65, 66].

Another method for stimulating the Yintang area is the application of electrical stimulation (Cefaly, STX-Med, Liege, Belgium) on the forehead (constant current: max. 30 mA; duration of rectangular electrical impulses: 100–500 μs; max. frequency: 150 Hz; stimulus duration: 20 min). This kind of stimulation can induce a state of deep relaxation and sedation as shown in our preliminary studies [67, 68] (Figure 22). The results highlight the electroencephalographic similarities of nonpharmacologically induced sedation and anesthesia.

EEG entropy is a parameter that is mainly used for quantification of depth of anesthesia and sedation. We applied this method of EEG entropy in an acupuncture study. Evidence regarding an acupuncture scheme consisting of "sedative acupuncture points" compared to an acupuncture scheme that is considered to "activate energy" was validated [27]. The investigation was performed in a group of healthy volunteers to determine whether manual needle acupuncture and laser acupuncture stimulations applied to two groups of

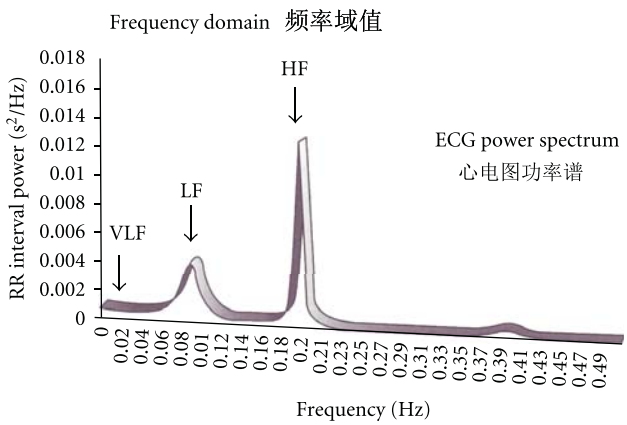


Figure 25: RR interval power spectral density (very low frequency (VLF) band, low frequency (LF) band, and high frequency (HF) band). Modified from [44].

acupuncture points (sedative versus enhancement of energy) have effects on either state entropy (SE) or response entropy (RE) or both.

A cross-over design was used. This means that two different acupuncture schemes were tested in the same volunteer on different days. The first acupuncture scheme (A) contained the so-called sedative points and included the following acupuncture points: Shenting (Du.24), Yintang (Ex.1), Sedative point 1 (Ex.8, Anmian I), Sedative point 2 (Ex.9, Anmian II), and Shenmen (He.7). Points Shenting and Yintang and their cerebral effects are well documented in the scientific literature [66]. Sedative point 1 is located at the median point of the connecting line between SJ.17 (Yi Feng) and Yi Ming (Ex.7), and sedative point 2 lies at the median point of the connection line between GB.20 (Feng Chi) and Yi Ming (Ex.7). The main indication for the latter two points is sleeplessness. Acupuncture scheme two (B) consists of points that according to TCM should support the "general availability of Qi-energy": Neiguan (Pe.6), Qihai (Ren 6), Zusanli (St.36) and Sanyinjiao (Sp.6). This scheme has been investigated by our study group and it also leads to an increase in blood flow velocity of the middle cerebral artery [3].

The study shows that two different acupuncture schemes can influence different parameters in the brain. The so-called "sedation point scheme" (A) showed a significant decrease in two entropy parameters (state entropy and response entropy) which can be interpreted as a sedation effect in the EEG (Figure 23).

In the same volunteer, a different acupuncture scheme (B), which according to TCM leads to a general increase in Qi-energy, did not yield this effect. Moreover, several preliminary tests showed that the latter scheme leads to a significant increase in blood flow velocity in the middle cerebral artery [6]. In addition, this study [27] proves that laser needle acupuncture is needle equivalent regarding the values of entropy described (Figure 24).

Electrophysiologic monitoring has been utilized to minimize neurological morbidity from operative manipulations and for monitoring during anesthesia and in the intensive care unit. One goal of such monitoring is to identify changes in brain, spinal cord, and peripheral nerve function prior to irreversible damage. However, neurophysiologic monitoring has also been effective in investigating effects of acupuncture, which may help to discover the complex mechanism.

3.3. Autonomic Nervous System Effects. In the last years, several animal [69] and human [7] experimental studies showed that acupuncture can influence the autonomic nervous system (ANS).

3.3.1. Heart Rate Variability (HRV). Already in the third century the Chinese medical doctor and scientist Wang-Shu Ho realized that variable heart beats are a sign of good health. He stated: "If the pattern of the heart beat becomes regular as the tapping of woodpecker or the dripping of rain from the roof, the patient will be dead in four days."

Today innovative research including the latest recording technology and also artificial intelligence techniques are used for data acquisition and data analysis of heart rate variability (HRV) in acupuncture research.

HRV is an index value of the neurocontrol of the heart and is measured as the percentage change in sequential chamber complexes, the so-called RR intervals, in the electrocardiogram (ECG). The registration of HRV is performed using three electrodes on the chest. It is important to simultaneously record respiration and if possible continuous blood pressure. The RR intervals in the ECG are controlled by the blood pressure control system, influenced by the hypothalamus and in particular controlled by the vagal cardiovascular center in the lower brainstem. HRV can be quantified over time using registration of percentage changes in RR intervals in the time domain as well as the changes in the frequency range by analysis of electrocardiographic power spectra [7, 44].

HRV parameters are recommended by the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology and are standard since 1996 [70].

ECG power spectral analysis is thought to provide an understanding of the effects of sympathetic and parasympathetic systems on HRV. Early work pointed out a few bands in the spectrum of HRV that could be interpreted as markers of physiological relevance. Associated mechanisms are thermoregulation which can be found in the very low frequency band (the so-called VLF band), blood pressure (LF band) and respiratory effects (HF-band) (Figure 25). In addition, total power of the heart rate spectrum is calculated for total HRV, and as an important parameter the ratio of the low frequency and high frequency band has become accepted [7, 44, 70].

The so-called "Fire of Life" analysis is a totally new method of visualization of HRV. A low frequency component at around 0.1 Hz is represented by the LF band between 0.05 and 0.15 Hz. The power output in the low frequency band is partly dependent on the sympathetic tone because

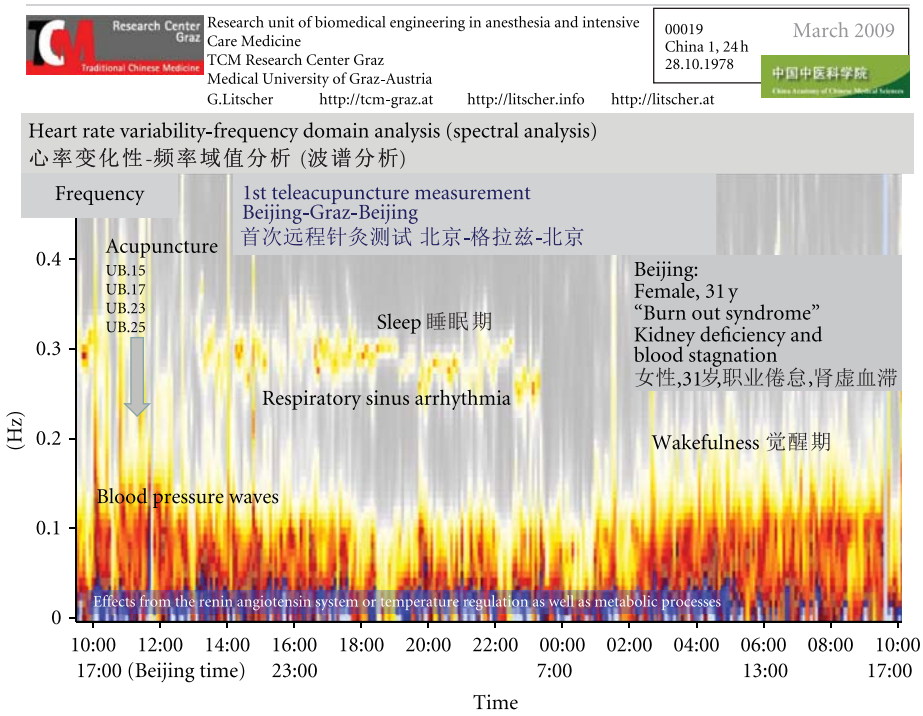


Figure 26: The first teleacupuncture measurement between Europe (Graz) and China (Beijing). The picture shows sympathetic and vagal activity and this can be used to indicate the patient's health and quality of sleep. Acupuncture points: Xinshu (UB.15), Geshu (UB.17), Shenshu (UB.23), and Dachangshu (UB.25). Modified from [71, 72].

of baroreceptor activity. Blood pressure waves of third order prove the connection of this so-called 10-second rhythm.

A high frequency component, which is represented by the HF-band, generally between 0.15 and 0.5 Hz, is associated with the breathing frequency and considered an indicator of vagal activity.

In Figure 26 the first results from a teleacupuncture pilot study together with the China Academy of Chinese Medical Sciences in Beijing are presented. The success of the manual needle acupuncture therapy can be quantified using modern technology. Up to now (January 2012), more than 150 teleacupuncture experiments have been successfully performed by our group between China and Austria [71–75].

A 24-hour ECG was recorded using a system partly developed in Austria. The raw data were transferred using internet to the TCM Research Center Graz in Austria from the patients' bedside computer in Beijing to the control computer in Graz over a distance of 7,650 km. Data analysis of different parameters of heart rate and HRV was performed immediately for control of possible therapeutic effects of acupuncture. The acupuncturists in China were informed about the findings immediately and the success of the therapy could be demonstrated objectively. This could probably be useful under special circumstances, for example, for cooperations between experts from different continents as demonstrated in our Sino-Austrian collaboration [44, 76–81].

Follow-up measurements demonstrate the success of acupuncture therapy. In this Chinese female patient an obvious sleep-wake-cycle appears during 10 acupuncture treatments within a time period of about nine weeks. The initially reduced "Fire of Life" starts to burn more brightly already after the fourth acupuncture treatment (Figure 27).

Heart rate variation depends on age (Figure 28), and there are intraindividual and interindividual variances. Apart from age, circadian variations (sleep-wake-cycle), physical condition and mental and physical exertion are important influencing factors. HRV can also be affected by diverse conditions such as age-related diseases like diabetic neuropathy, renal failure, essential hypertension, cardiac disorders, coronary artery disease, or intracranial lesions [7, 44, 70]. In all cases, different medications have to be taken into account.

HRV can be used as a reliable indicator of the state of health. However, it could be demonstrated that in special syndromes like stress one can counteract this process using different preventive methods like acupuncture. This has been shown in recent investigations concerning patients with burn-out syndrome as performed in common teleacupuncture studies between China and Austria [44, 71–75].

3.3.2. Blood Pressure, Pulse Wave Velocity, and Augmentation Index. In a recent study [20] we investigated the effects of violet laser acupuncture (see stimulation methods) on arterial stiffness and other important parameters of the functional state of heart such as the augmentation index (AIx). Pulse wave velocity (PWV) is a direct marker of arterial stiffness and the AIx one of wave reflection. Both

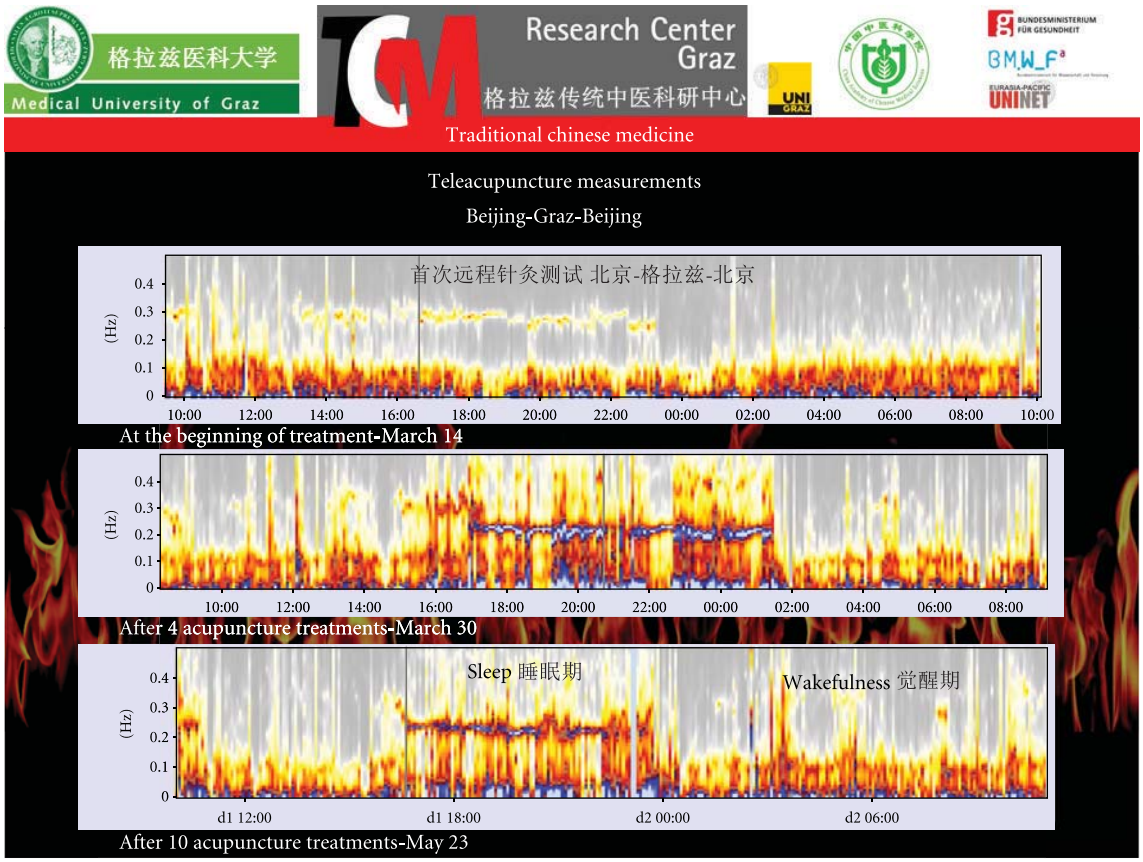


Figure 27: Follow-up measurements during a total of ten acupuncture sessions in China. Note the appearance of an obvious sleep-wake-cycle already after the fourth acupuncture treatment (modified from [72]).

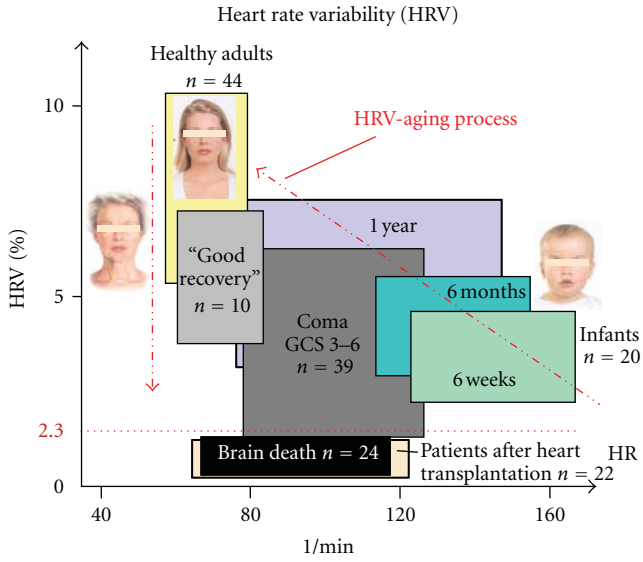


Figure 28: Heart rate (HR) and heart rate variability (HRV) and the aging process.

parameters can be registered with a cuff applied to the brachial artery (Figure 29).

The first results from a study in 10 healthy volunteers showed a marked but not statistically significant decrease in PWVaortic and an increase in AIx brachial during and after laser acupuncture at the acupoint Baihui [20]. Further studies including control measurements are necessary.

The goal of a further study [80] at our TCM Research Center Graz was to develop a new system for ear acupressure (vibration stimulation) and to perform pilot investigations on the possible acute effects of vibration and manual ear acupressure on HRV, PWVaortic, and the AIx using new noninvasive recording methods (Figure 30).

Investigations were performed in 14 healthy volunteers before, during and after acupressure vibration and manual acupressure stimulation at the "heart" auricular acupuncture point. The results showed a significant decrease in heart rate and a significant increase in total HRV after ear acupressure. The PWV decreased markedly (yet insignificantly), whereas the AIx increased immediately after both methods of stimulation. The increase in the low-frequency band of HRV was mainly based on the intensification of the related mechanism of blood pressure regulation (10-s-rhythm). Further studies in Beijing using animal models and investigations in Graz using human subjects are already in progress.

4. Concluding Remarks

Traditional Chinese Medicine (TCM), especially acupuncture, has made many important contributions to the

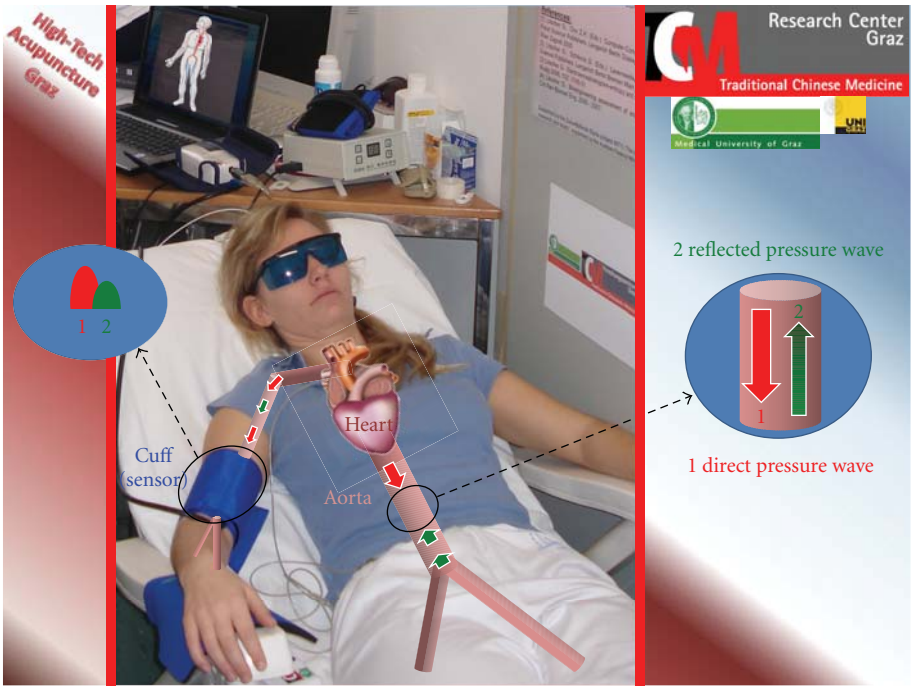


Figure 29: Measurement of the pulse wave velocity and the augmentation index using a cuff at the brachial artery in the lab of the TCM Research Center Graz at the Medical University of Graz. With permission of the volunteer and modified from [20].

Figure 30: Investigation of ear acupuncture using a new system developed at the TCM Research Center in Graz at the Medical University of Graz (with permission of all medical doctors and volunteers). Modified from [80].

medicine of the world. Using needle, laser needle, electrical stimulation, and modern biomedical recording techniques, changes in the brain and periphery can be quantified.

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Research Article

Biomedical Teleacupuncture between China and Austria Using Heart Rate Variability—Part 2: Patients with Depression

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It has been shown in previous studies that the autonomic nervous system can be affected by acupuncture. Within this study, teleacupuncture between China and Austria is used for quantifying the effects of heart rate (HR) and heart rate variability (HRV) in 33 Chinese patients (27 females, 6 males; mean age ± SD 49.5 ± 13.1 years; range 22–72 years) suffering from depression. Electrocardiographic signals before, during, and after acupuncture at the acupoint Baihui (GV20) were recorded in Harbin and analyzed in Graz using teleacupuncture. HRV data were analyzed in the time and frequency domain. Mean HR decreased significantly ($P < 0.05$) during and after acupuncture, whereas total HRV increased significantly after the third acupuncture stimulation period ($P < 0.05$) and also 5–10 minutes after ($P < 0.05$) acupuncture. The study shows that HRV could be a useful parameter for quantifying clinical effects of acupuncture on the autonomic nervous system.

1. Introduction

A recent Cochrane review identified 30 randomized controlled trials (RCTs) that evaluated manual acupuncture, electroacupuncture, or laser acupuncture in 2812 patients with major depressive disorder [1]. In this study, no consistent benefit was noted with any form of acupuncture [1]. However, our research group found acute stimulation effects on neurovegetative parameters like heart rate (HR) and heart rate variability (HRV) in patients with depression [2] and insomnia [3] and poststroke patients [4].

An innovative concept of the current teleacupuncture technology has been implemented at the Traditional Chinese Medicine (TCM) Research Center Graz in Austria (http://litscher.info/ and http://tcm-graz.at/) in 2010 in cooperation with different institutions in China over a distance of several thousands of kilometres [5–7].

This paper describes the second results from teleacupuncture measurements in patients with depression using computer-based HRV recordings before, during, and after acupuncture under standardized clinical conditions in China. The first study in patients with depression was performed using the acupuncture point Jianshi (PC5) [2] and the present study the acupoint Baihui (GV20). All analyses were performed in Graz, Austria [5].

2. Subjects and Methods

2.1. Patients. Thirty-three patients (27 females, 6 males; mean age ± SD 49.5 ± 13.1 years; range 22–72 years)

suffering from depression (Chinese diagnosis "Yu Zheng") and therefore receiving acupuncture treatment were investigated at the Heilongjiang University in Harbin. Similar to our first study the clinical evaluation of the patients was performed immediately before HRV data recording using three main scales: the Hamilton rating scale for depression (HRSD) [8], the Hamilton anxiety rating scale (HAM-A) [9], and the Athens insomnia scale (AIS) [10]. No patient was under the influence of centrally active medication. The study was approved by the ethic committee of the Heilongjiang University of Chinese Medicine (no. 2010HZYLL-030) and carried out in compliance with the Declaration of Helsinki. All patients gave oral informed consent.

2.2. Biosignal Recording in Asia and Data Analysis in Europe. The duration of RR intervals is measured during a special time period (5 min), and on spectral analysis basis HRV is determined. Electrocardiographic (ECG) registration is performed using three adhesive electrodes (Skintact Premier F-55; Leonhard Lang GmbH, Innsbruck, Austria), which are applied to the chest.

The researchers in China used a medilog AR12 HRV (Huntleigh Healthcare, Cardiff, UK) system from the TCM Research Center at the Medical University in Graz for the joint investigations. This system has a sampling rate of 4096 Hz and can therefore detect R waves extremely accurately [11]. The raw data are stored digitally on a CompactFlash (CF) 32 MB memory card. After removing the card from the portable system, the data were read by a card reader connected with a standard computer in China and then transferred to the TCM Research Center Graz via internet. With a new software [5–7] the biosignals were analyzed and HRV was displayed in a way to help to judge the function of the autonomic nervous system. Viewing this innovative kind of analysis helps to show how well the human body reacts to sport, stress, recovery, and also acupuncture [2–7, 12].

Similar to a previous studies [2–4], mean HR, total HRV, and the LF (low frequency)/HF (high frequency) ratio of HRV were chosen as 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 [13].

2.3. Clinical Acupuncture and Procedure. All 33 patients received manual needle acupuncture at the acupoint Baihui (GV 20) on the head (Figure 1). Baihui is located 5 cun directly above the midpoint of the anterior hairline, at the midpoint of the line connecting the apexes of both ears. Its use is indicated, for example, in neurological diseases like depression, headache, dizziness, epilepsy, and mania [14]. Sterile single-use needles (0.30 × 25 mm; Huan Qiu, Suzhou, China) were used. Needling was performed horizontally (angle 15°, depth about 1 cun), and the needle was stimulated clockwise and counterclockwise for 15 seconds each, with six rotations per second, resulting in 90 rotations per stimulation. Stimulation was done immediately after inserting the needle, 10 minutes later and before removing the needle (cf. Figure 2 and [2]).

Figure 1: Acupuncture at the acupoint Baihui (GV20).

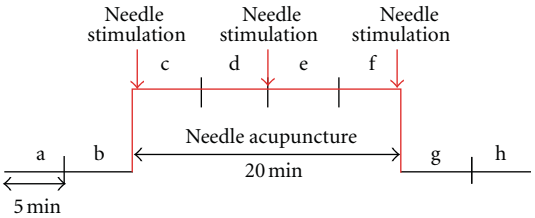

Figure 2: Measurement procedure. The data before (measurement phases a, b), during (c–f), and after (g, h) manual needle acupuncture stimulation at the acupoint Baihui (comp. Figure 1) were measured and statistically analyzed.

2.4. Statistical Analysis. Data of the 33 patients from the 2nd Neurological Department from the Heilongjiang University of Chinese Medicine in Harbin were analyzed using SigmaPlot 11.0 software (Systat Software Inc., Chicago, USA). Graphical presentation of results uses box plot illustrations. Testing was performed with Friedman repeated measures ANOVA on ranks and Tukey test. The criterion for significance was $P < 0.05$.

3. Results

Figure 3 shows the results of mean HR from the ECG recordings before, during, and after acupuncture of the 33 patients with depression. There was a significant decrease in HR during the second half of the acupuncture phase and after acupuncture ($P < 0.05$).

In contrast to this decrease in HR, total HRV increased significantly ($P < 0.05$) only after finishing the third and last needle stimulation (Figure 4, phase g). This increase was still present at the end of the measurement procedure in comparison to the second phase (Figure 4, phase h; $P < 0.05$). It is interesting that between the stimulation phases total HRV was lowered again, with the median continually increasing with respect to the previous nonstimulation phase.

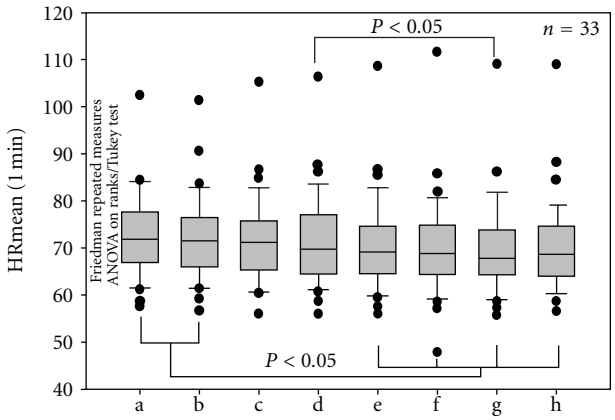

Figure 3: Mean heart rate. Box plot illustration in 33 patients with depression before (a, b), during (c–f), and after (g, h) needle acupuncture. Significant changes ($P < 0.05$) were found in the phases e–h, each compared to baseline values a, b, respectively. The horizontal line in the box gives the position of the median. The end of the box defines the 25th and 75th percentile; the error bars mark the 10th and 90th percentile.

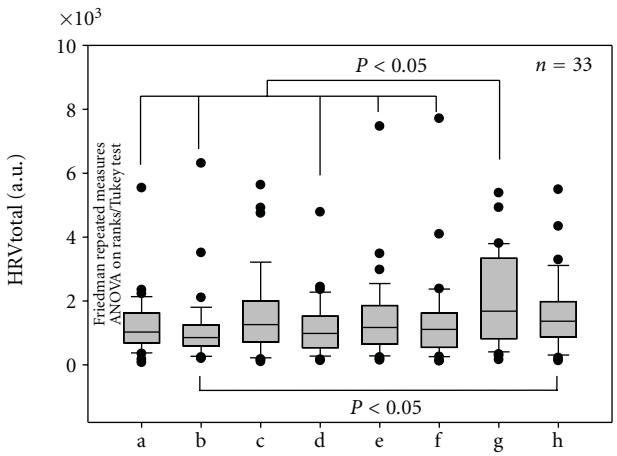

Figure 4: Total heart rate variability. Graphical box plot presentation of changes immediately after (g) and 5–10 min after (h) acupuncture. Note the increase in total HRV after each manual needle stimulation (c, e, g). For further explanations compare Figures 2 and 3.

Insignificant changes were found in the LF/HF ratio during acupuncture and can be seen in Figure 5.

The results of the different scales as described in Section 2 showed the following mean ± SD values: HRSD 20.3 ± 4.1; HAM-A 19.4 ± 4.4; AIS 12.7 ± 4.8.

4. Discussion

Depression is one of the most prevalent and fastest-growing diseases in both western and eastern worlds. New-generation antidepressants appear more effective than older drugs; however, many drugs have side effects that can affect compliance and morbidity [1]. From the point of view of TCM the main syndromes of depression are qi stagnation, and blood

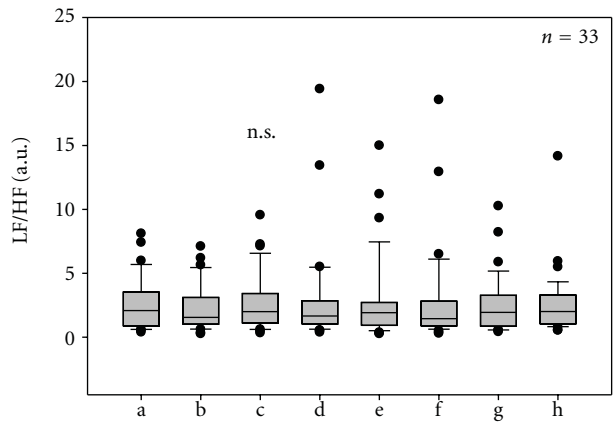

Figure 5: LF (low frequency)/HF (high frequency) ratio during acupuncture treatment in the 33 patients. For further explanations see Figure 3.

stasis, liver qi depression and transformation of fire due to qi stagnation [15]. In China, there are several preclinical and clinical studies using Chinese herbal medicine, which are the basis for design of new therapeutic programmes for treatment of depression [15]. In addition, acupuncture is also used in several evidence-based studies concerning this topic of research.

Although there are a great number of referenced publications (see Section 1 and http://www.pubmed.gov), there are only seven articles (including our first study on the topic [2]) concerning depression, acupuncture, and HRV at the moment (January 2012). These publications should be discussed in context with the results of this study in the following [2]: in 2001, Callahan [16] stated that HRV has been shown to be a strong predictor of mortality and is adversely affected by problems such as anxiety and depression. Pignotti and Steinberg [17] demonstrated that a lowering of subjective units of distress was in most cases also related to an improvement in HRV. In the third paper in 2001, Sakai et al. [18] included HRV in a general concept of behavioural health services, and the authors reported HRV as a useful parameter. In 2003, Agelink et al. [19] also undertook a study to evaluate the effects of needle acupuncture on cardiac autonomic nervous system function in patients with minor depression or anxiety disorders. In contrast to our 33 patients, the 36 patients from that group were randomly distributed into a verum acupuncture group and a placebo group. Similar to our investigations, 5-minute intervals of ECG were analyzed and the acupuncture group also showed a significant decrease of the mean resting heart rate, 5 and 15 minutes after needle application (cf. Figure 3). In the study by Agelink et al. [19], this effect was only significant in verum acupuncture in patients with minor depression or anxiety. Therefore, a relative increase of cardiovagal modulation of heart rate and physiological regulatory effects due to acupuncture stimulation could be detected in the present study, which confirms the results of other authors [19], although the acupuncture schemes were

different (He.7 Shenmen and PC7 Neiguan [19] versus PC5 Jianshi [2] and GV20 Baihui (this study)).

In a further publication, Yun et al. [20] described in 2005 the dynamic range of biologic functions. They stated that reduced variation of physical exertion, environmental stressors, and thermal gradients that characterize modern life styles may reduce the autonomic dynamic range resulting in lowered HRV and a myriad of systemic dysfunctions. Acupuncture may operate through increasing autonomic variability.

As already mentioned in the previous part (part 1) of this study [4], a systematic clinical review on acupuncture and HRV was published by Lee et al. in 2010 [21], which searched the literature using 14 data bases. Twelve RCTs met all inclusion criteria. Five RCTs found significant differences in HRV between patients treated with acupuncture and those treated with sham acupuncture (controls). The majority of the other RCTs showed inconsistent results [21]. The authors stated that more rigorous research appears to be warranted. The number, size, and quality of the RCTs that are available are too low to draw firm conclusions [21]. Another review article concerning the topic of HRV and acupuncture was published by our research group already in 2007 [12]. In this paper, it could be demonstrated that in special syndromes like fatigue and stress one can counteract the aging process using different preventive methods like acupuncture [12]. This was demonstrated in recent investigations concerning patients with burn-out syndrome as performed in a further teleacupuncture study between Beijing and Graz [5, 22].

The following conclusions can be drawn from the present clinical teleacupuncture study in patients with depression.

(1) The stimulation at the acupoint Baihui (GV20) significantly decreased HR in depression patients.

(2) Total HRV increased significantly during and after acupuncture stimulation at Baihui.

(3) We have shown that teleacupuncture at the acupoint Baihui in patients with depression shows similar effects in neurovegetative parameters like acupoint stimulation of Jianshi (PC5). In both studies ([2] and present study) the same technique in different patients but with the same disease was used.

Authors' Contribution

G. Litscher, G. Cheng and L. Wang contributed equally to this study.

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Research Article

Manual and Electroacupuncture for Labour Pain: Study Design of a Longitudinal Randomized Controlled Trial

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Introduction. Results from previous studies on acupuncture for labour pain are contradictory and lack important information on methodology. However, studies indicate that acupuncture has a positive effect on women's experiences of labour pain. The aim of the present study was to evaluate the efficacy of two different acupuncture stimulations, manual or electrical stimulation, compared with standard care in the relief of labour pain as the primary outcome. This paper will present in-depth information on the design of the study, following the CONSORT and STRICTA recommendations. *Methods*. The study was designed as a randomized controlled trial based on western medical theories. Nulliparous women with normal pregnancies admitted to the delivery ward after a spontaneous onset of labour were randomly allocated into one of three groups: manual acupuncture, electroacupuncture, or standard care. Sample size calculation gave 101 women in each group, including a total of 303 women. A Visual Analogue Scale was used for assessing pain every 30 minutes for five hours and thereafter every hour until birth. Questionnaires were distributed before treatment, directly after the birth, and at one day and two months postpartum. Blood samples were collected before and after the first treatment. This trial is registered at ClinicalTrials.gov: NCT01197950.

1. Introduction

Methodology problems in acupuncture research have previously been highlighted [1], including the area of labour pain [2]. A recent systematic review [2] concludes that further high-quality studies are needed as the findings from existing studies [3–9] on the effect of acupuncture on labour pain are contradictory and difficult to interpret for a number of methodology reasons.

One such reason is that the studies have different primary outcomes. In total, seven randomized controlled trials (RCTs) were published in this area of research between 2002 and 2009. Of them, six trials report the women's subjective assessment of labour pain intensity as a primary outcome measure [4–9]. Two studies found that acupuncture was more effective than low-intensity "minimal acupuncture" at nonacupuncture points [6, 8]. On the other hand, three studies found no difference in pain intensity between women receiving acupuncture and women receiving standard care only [4, 7, 9] or with the addition of transcutaneous electrical nerve stimulation (TENS) [4] or administering acupuncture at nonacupuncture points [7]. A further study showed that sterile water injections reduced the intensity of labour pain more effectively than acupuncture [5] and that this effect is most likely mediated via similar mechanisms to high-intensity acupuncture (where needles are more frequently stimulated).

In two studies, the primary outcome was the use of pharmacological pain relief, where women who received acupuncture treatment used epidural analgesia and/or pethidine to a lesser extent than women who received standard care [3, 4] or with the addition of TENS [4]. However, this outcome measurement may be problematic as other factors influence the decision to use an epidural, such as the local culture at the delivery ward, the availability of an anaesthesiologist; or the woman's background [10]. In addition, the quality of midwife support influences the need of pharmacological pain relief [11]. As interventions such as acupuncture involve a high degree of midwifery presence, the possibility to draw proper conclusions from studies using such outcomes may be problematic. Other outcomes associated with conditions that may influence the experience of pain have also been evaluated but with contradictory results. In some studies, acupuncture increased the degree of relaxation [4, 9] while in others it did not [5, 7]. In some it shortened the time spent in labour [6, 8], and in others it did not [4, 5, 7, 9].

Another important methodological aspect is the variation in intensity of treatment between the studies. Acupuncture with frequent manual stimulation seems to have a better effect on labour pain than treatments with less stimulation, such as minimal acupuncture [6, 8]. Also, high-intensity treatments, such as sterile water injections, seem to relieve pain more effectively than low-intensity acupuncture [5]. Previous studies therefore raise important questions that need to be explored further regarding acupuncture treatment such as type of stimulation (electrical versus manual), number of needles used, stimulation intensity, and treatment duration.

In addition to this, most previously published studies include small sample sizes, and few include power calculations assuring that the study is large enough to detect relevant differences in outcomes. The treatment protocol (i.e., a precise description of the treatment including number of acupuncture points used, stimulation type, frequency of sessions, and length of treatment period) is often incomplete or absent, and does not follow the CONSORT [12] recommendations for reporting RCT nor the STRICTA document [13], which is a complement for reporting acupuncture studies specifically. Differences in content of care in the control group and the inclusion and exclusion criteria all make the results difficult to evaluate. Some studies give limited or no information on important factors such as possible maternal and neonatal side effects, timing of the treatment, intensity of the treatment, and training and skills of the personnel administering the treatment [3, 6, 7]. Women receiving treatment from a small group of midwives who were experienced in administering acupuncture seemed to benefit more from treatment [8] than women who were treated by a large number of midwives with varying training and experience [5]. Information on biological markers of pain and stress may further increase the understanding of possible effects of acupuncture in the relief of labour pain.

Acupuncture involves puncturing the skin with thin sterile needles at well-defined acupuncture points which may be excitable muscle/skin-nerve complexes with a high density of nerve endings [14]. The needles are primarily stimulated manually or electrically. In manual acupuncture (MA) the needles are twisted back and forth until a feeling of DeQi is reached. DeQi is described as a sensation of numbness, soreness, or heaviness reflecting the activation of afferent nerve fibres. The efficacy of the treatment depends on its intensity, which is related to the number of times DeQi is reached [15, 16]. In electroacupuncture (EA), needles are stimulated electrically [17]. Frequency and intensity are parameters known to influence the effect of EA.

The physiological mechanisms of acupuncture are not fully understood, but the pain-relieving effects may be explained by western medical theories [16]. Acupuncture needles activate receptors and afferent nerve fibres, in particular Aβ/δ-fibres and C-fibres. According to Melzack and Wall, the stimulation of Aβ-fibres activates the gate control mechanism that inhibits pain transmission at spinal level [18]. To activate these pain inhibiting systems, needles should be placed and stimulated in the same spinal segment as the source of the pain [19]. There are also extra segmental, central effects. Acupuncture triggers three main groups of opioid peptides, β-endorphin, enkephalin, and dynorphin [20]. Another effect of acupuncture is that it may activate another endorphin system—the diffuse noxious inhibitory Control (DNIC) system [21].

The nociceptive stimuli of the ripening and dilatation in the cervix in the initial stage of labour, known as the latent phase, are transmitted to the posterior root ganglia of thorasic spine Th10 to lumbar spine L1-L2. As the labour proceeds, more slowly in primiparous women than in multiparous, the nociceptive stimuli originate from the spinal segments S2–S4 [22, 23]. The nervous system, the immune system, and the endocrine system are all involved in the regulation of pain and the body's response to stress [24], and pain enhancement is mediated by glial activation and the release of proinflammatory cytokines [25]. Stressful experiences increase the circulation levels of proinflammatory cytokines [26], and possible associations between labour pain management, stress, and cytokines are underinvestigated.

Control interventions are a problem in acupuncture research [27] and the use of placebo has been discussed intensively [28, 29]. Placebo interventions include minimal or superficial acupuncture, needles on nonacupuncture points (sham acupuncture), or "placebo needles", that is, needles with a handle that moves down over the needle, giving the false impression that they are inserted into the skin. A recent review demonstrates that placebo acupuncture differs from other physical and pharmacological placebo procedures in that it is associated with larger effects [30], possibly having a similar neurochemical basis with the activation of the endogenous opioid systems [19, 31], and sham acupuncture could be seen as a low-intensity form of therapeutic needling [27]. Consequently, placebo treatment does not seem to be a credible control intervention in acupuncture trials [19].

A review of previous studies indicates that acupuncture may have an effect on women's experiences of labour pain, but in order to enable a more definite conclusion further

studies with better reporting to enable valid interpretations and replicability are needed.

2. Aim and Outcome Measurements

The aim of the present study was to evaluate the efficacy of two different acupuncture stimulations compared with standard care in the relief of labour pain. Our hypothesis is that acupuncture with manual or electrical stimulation is more effective than standard care in the relief of labour pain and that acupuncture with electrical stimulation is the most effective. This paper presents in-depth information on the design of the study and the ways in which we attempt to follow CONSORT [12] and STRICTA [13] recommendations.

2.1. Primary Outcome. Experience of labour pain.

2.2. Secondary Outcomes

Use of epidural analgesia.

Experience of relaxation.

Labour outcomes: mode of delivery, pain relief, augmentation of labour, duration of labour, and perineal trauma.

Negative side effects.

Experience of midwife support.

Proinflammatory cytokines, for example, interleukin (IL)-1, IL-6, highly sensitive C-reactive protein (hs-CRP), and tumor necrosis (TNF)-alpha.

Memory of labour pain and overall childbirth experience.

Infant outcomes: Apgar score, pH, BE, and neonatal transfer.

3. Materials and Methods

3.1. Design. The study was designed as a three-armed, randomized, and controlled trial in two delivery wards in two different hospitals in Sweden. A description of the study outline is presented in Figure 1. All eligible women who gave their written consent to participate in the study were randomly allocated to one of three groups: manual acupuncture (MA), electroacupuncture (EA), or standard care (SC). The rationale of acupuncture was based on Western medical theories, and the study protocol follows CONSORT [12] and STRICTA [13] recommendations. The study is registered at ClinicalTrials.gov: NCT01197950. Process evaluation was conducted by intermittent checkups in order to assure that the intervention procedures were performed correctly and that they followed study protocol.

3.2. Participants. All nulliparous women who were in gestational week 34–36 and attended regular checkups with a midwife at the antenatal clinics connected to the two hospitals received written and oral information about the study and an address to an informative study website (http://www.akupunkturstudien.se). Women were then asked to give consent to participate in the study when admitted to the labour ward.

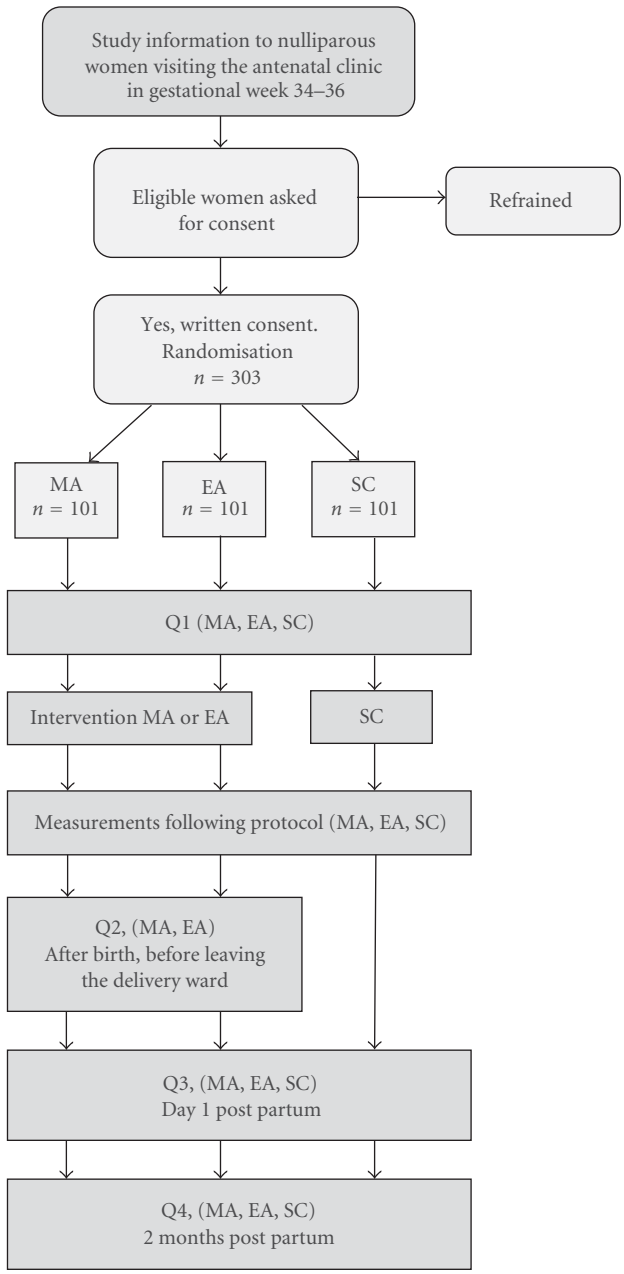

Figure 1: Study design including recruitment, randomization, interventions, and data collection. MA: Manual acupuncture, EA: Electroacupuncture, SC: Standard care, Q: Questionnaire.

3.2.1. Inclusion Criteria

Spontaneous onset of labour.

Admission to the labour ward in latent or active phase of labour.

Nulliparity.

Singleton pregnancy, cephalic presentation.

Gestation: 37 + 0 to 41 + 6 (weeks + days).

Expressed need for labour pain relief.

Knowledge of the Swedish language good enough to understand written and oral instructions.

3.2.2. Exclusion Criteria.

Intake of pharmacological pain relief medication within 24 hours prior to inclusion into the study, with the exception of paracetamol.

Preeclampsia.

Treatment with oxytocin at the time point of allocation.

Treatment with anticoagulant.

Pacemaker.

After assessment of eligibility to the study, the women were randomized into one of the three groups. The randomization was conducted in blocks of 9, 12, and 15, which were varied randomly. A computerised random number generator generated a list of codes from 1 to 303, with each code linked to one of the three groups. Sequentially numbered, opaque, sealed envelopes were prepared by one of the authors (LV), which included a study protocol and four questionnaires. At the time of allocation, the assisting midwife picked the envelope with the lowest number on which she wrote the participant's name and social security number and then opened it.

Table 1: Participating midwives' previous education and experience with administrating acupuncture, prior to study training, $n = 38$.

	n
Education prior to study training	
One day course with a midwife at the delivery ward	10
Practical focus with acupuncture points chosen on TCM basis	
Included neither acupuncture physiology nor EA	
Five-day course with focus on TCM and WM	14
Practical and theoretical sessions.	
Acupuncture physiology based on both TCM and WM	
EA not included.	
Six-day course in WM	11
Practical and theoretical sessions	
Acupuncture physiology and research within the area of acupuncture and obstetrics	
EA included	
No previous education in acupuncture at all	3
Experience of administrating acupuncture during labour, prior to study	
None	12
<1 year	5
1-2 years	10
3–10 years	8
>10 years	3

TCM : traditional Chinese medicine, EA : electroacupuncture, WM : western medical acupuncture.

3.3. Education of Midwives. The participating midwives had varying training and experience of administering acupuncture treatment (Table 1). We therefore conducted a one-day study-specific course, which included theoretical sessions with a Western medical approach to acupuncture physiology, practical sessions in MA and EA, and lectures on research methodology with a focus on RCT. The course was repeated once every semester. The midwives at the antenatal clinics also received in-depth information about the study and the mechanisms of acupuncture. Furthermore, all midwives had access to the open website and, with the use of a password, access to a closed section that included instructional videos and written information about the study.

3.4. Interventions. All women in the trial received care from midwives throughout labour and birth and from obstetricians in cases of deviation from normal progress, according to Swedish clinical practice. All participants had access to all pharmacological and nonpharmacological analgesia available in Swedish maternity care with the exception of women in the SC group who did not have access to any form of acupuncture.

A list of acupuncture points based on literature and in collaboration with experienced clinicians and tutors was established (Table 2). Local points were selected in muscle tissue in the pain area with the same somatic innervations as the cervix and uterus. In addition, points in hands and feet, so-called distal points, were selected in order to strengthen and prolong the effect of local needles. From the list, 3 bilateral distal points and 4–8 bilateral local points were chosen individually depending on pain location. In total, women in the MA and EA groups were treated with 13–21 needles. Sterile acupuncture Hegu Xeno needles for single use were used, sized 0.30×30 mm and 0.35×50 mm. In the MA group, all needles were stimulated until DeQi was reached every ten minutes for a 40-minute period. In the EA group, all needles were stimulated manually until DeQi was reached, and then eight of the local needles were connected to an electrostimulator (Cefar Acus 4, CEFAR, Lund, Sweden) which was set at high-frequency stimulation (80 Hz) square wave pulses (0.18 ms duration) with alternating polarity. The woman adjusted the intensity of the electrical stimulation so it was just under their pain threshold. The remaining needles were stimulated manually by the midwife until DeQi was reached every ten minutes for a 40-minute period. The needles were removed after 40 minutes in both groups. Two hours later the treatment was repeated. Additional treatment with MA or EA was thereafter available on request. After the first treatment with MA or EA, all women had access to standard forms of analgesia, if needed.

Table 2: Acupuncture points used in the study.

Points	Segmental innervation	Tissue	Depth (cun)
Distal points			
GV 20	Nn. trigeminus (V), occipitalis minor (C2), occipitalis major (C2-3)	Aponeurosis epicranii	0.3–0.5
LI 4	Nn. medianus ulnaris (C8-Th1)	Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis	0.5–1
SP 6	N. tibialis (L4-S1)	Mm. flexor digitorum longus, tibialis posterior	0.5–1
LR 3	N. plantaris lateralis (S2-3)	M. interosseus dorsalis I	0.3–0.5
PC6	N. medianus (C8, Th1)	M. flexor digitorum superficialis	0.5–0.8
EX2	N. trigeminus	M. frontalis	0.3–0.5
LU7	N. cutaneus antebrachii lateralis (C5-6)	Fibrous tissue	0.3–0.5
Local points			
SP 12	N. thoracicus (Th7–12), lumbalis (L1)	Aponeurosis mm. obliquus externus, abdominis internus	0.5–1
BL 23	Nn. thoracodorsalis (C6–8), thoracicus (Th9–12), lumbalis (L1–3)	Mm. serratus posterior inferior, erector spinae, fascia thoracolumbalis	0.8–1
BL 24	Nn. thoracodorsalis (C6–8), thoracicus (Th9–12), lumbalis (L1–3)	Mm. erector spinae, fascia thoracolumbalis	0.8–1
BL 25	Nn. thoracodorsalis (C6-8), thoracicus (Th9–12), lumbalis (L1–3)	Mm. erector spinae, fascia thoracolumbalis	0.8–1
BL 26	Nn. thoracodorsalis (C6–8), thoracicus (Th9–12), lumbalis (L1–3)	Mm. erector spinae, fascia thoracolumbalis	0.8–1
BL 27	Nn. thoracodorsalis (C6–8), thoracicus (Th9–12), lumbalis (L1-3)	Mm. erector spinae, fascia thoracolumbalis	0.8–1
BL 28	Nn. thoracodorsalis (C6–8), thoracicus (Th9–12), lumbalis (L1–3)	Mm. erector spinae, fascia thoracolumbalis	0.8–1
BL 36	N. gluteus inferior (L5–S2)	M. gluteus maximus	1–1.5
BL 54	N. gluteus inferior	M. gluteus maximus	1.5–2
GB 25	N. thoracicus (Th7–12)	M. obliquus externus abdominis	0.3–0.5
GB 26	N. thoracicus (Th7–12)	M. obliquus externus abdominis	0.5–0.8
GB 27	Nn. thoracicus lumbalis (Th7–L1)	Aponeurosis mm. obliquus externus, internus abdominis	0.5–1
GB 28	M. obliquus externus abdominis	Aponeurosis mm. obliquus externus, internus abdominis	0.5–1
GB 29	N. gluteus superior (L4–S1)	M. tensor fasciae latae	0.5–1
LR 10	N. femoralis (L2-3)	M. pectineus	0.5–1
LR 11	N. femoralis (L2-3)	M. pectineus	0.5–1
KI 11	Nn. thoracicus (Th6–12), subcostalis (Th12)	Mm. pyramidalis, rectus abdominis. Vagina m. recti abdominis	0.5–1
ST 29	N. thoracicus (Th6–12)	M.rectus abdominis	0.7–1.2
CV3	N. iliohypogastric (L1)	Fibrous tissue	0.5–1
CV4	N. subcostalis (Th12)	Fibrous tissue	0.5–1

GV: governor vessel channel, LI: large intestine channel, SP: spleen channel, LR: liver channel, PC: pericardium channel, EX: extra channel, LU: lung channel, BL: bladder channel, GB: gall bladder channel, KI: kidney channel, ST: stomach channel, CV: conception Vessel, Cun: traditional Chinese unit of length, 1 cun: width of the distal interphalangeal joint of the thumb.

3.5. Data Collection. Established study protocols were filled in by midwives throughout the labour, including details on the labour, the intervention, and maternal and neonatal outcomes and by the women when making their assessments of labour pain and relaxation on VAS (Table 3). Data were also collected from patient records and by means of questionnaires (Table 4).

3.6. Measuring Pain and Relaxation. VAS was used for assessing experience of labour pain and relaxation. VAS is a 100 mm horizontal ungraded line with two endpoints: "no pain" (left) and "worst pain imaginable" (right) and "relaxed" (left) and "very tense" (right). Each woman assessed her level of pain/relaxation by making a vertical mark on the line with a sharp pencil [37]. VAS has been

Table 3: Content of study protocol, similar for all groups.

Concept	Measurement and response alternatives
Mother	
Pain during contraction	VAS, every 30 minutes for 5 hours, and then every hour until birth
Relaxation during contraction	VAS, every 30 minutes for 5 hours, and then every hour until birth
Pain localisation	Back/Abdomen/Groin. every 30 minutes for 5 hours, and then every hour until birth
Cervix dilatation and length	Cm 3 times during 5 hours
Contractions (duration/interval)	Seconds/Minutes 5 times during 5 hours
Details of intervention	Point selection from Table 2, duration of treatment, stimulation technique and stimulation frequency
Additional pain relief	Sterile water injections/TENS/Entonox/Opioid epidural and intrathecal analgesia/Pudendal nerve block/Paracervical block/Other
Midwives's evaluation of the treatment effect for pain relief and relaxation (MA and EA)	Very effective/Fairly effective/Not so effective/Not effective at all
Negative side effects (EA, MA)	Yes, if so, a description of the side effects/No
Augmentation of labour	Yes, if so, indication primary and secondary dystocia, other/No
Rupture of membranes/Amniotomy	Date/Time
Partus	Date/Time
Mode of delivery	Vaginal delivery/Vacuum extraction/Forceps/Emergency caesarean section
Perineal injury	Degree I–IV
Infant	
Apgar score	1, 5 and 10 min
Birth weight	Grams
Arterial and venous blood gases	pH/Base Excess (umbilical cord samples)
Neonatal transfer	Yes/No

VAS: visual analogue scale, TENS: transcutaneous electrical nerve stimulation, MA: manual acupuncture, EA: electroacupuncture.

shown to be valid in detecting changes in pain intensity [38, 39] and relaxation [5, 7, 9, 40], and most individuals have no difficulties using it [38, 41]. One problem, however, is that the suitability of using VAS for monitoring labour pain has been questioned, as it has an apparent problem with response shift. As the labour proceeds, the pain intensity increases, and there is a possibility that the meaning of a value on VAS is changed (recalibrated) due to the higher pain intensity [42]. Although there are problems with using VAS to assess labour pain, it is currently the most common and best-validated pain scale used in research into labour pain. The measurements were conducted before the first treatment, immediately after the first treatment and then every 30 minutes for five hours and thereafter every hour until birth or until an epidural was administered. A different person from the one who administered the intervention (help nurse or midwife) assisted the women in the procedure of measuring pain and relaxation, however; blinding was not possible.

3.7. Questionnaires. Questionnaires were answered before the first treatment (Q1), immediately after birth (Q2), the day after birth (Q3), and two months later (Q4) (Table 4). Q1 asked about social-demographic background, previous experience of acupuncture, experience of menstrual pain, and how painful the woman expected the upcoming birth to be. Q2 was answered by women in the MA and EA groups only and included questions regarding experience of the acupuncture treatment, effects of the treatment, and negative side effects, if any. Q3 and Q4 were answered by all participants and asked about experience of labour [5, 34, 43–45], pain relief, memory of labour pain measured by VAS, experience of the intervention, support during labour, and emotional health problems in terms of depressive symptoms, which were assessed by the Edinburgh Postnatal Depression Scale [35, 36]. All protocols and questionnaires were pilot tested.

3.8. Blood Sampling. Blood samples were collected in the MA and EA groups before the first treatment and directly after the first treatment and in the SC group, before the first analgesic treatment and 30 minutes later. An indwelling intravenous catheter was inserted, and from this 5 mL of blood was drawn and collected in a standard tube. The samples clotted for a minimum of 30 minutes and no longer than four hours before they were centrifuged at 3000 rpm for 15 minutes at 20°C. Four polypropylene tubes of 0.5 mL with aliquots of serum and plasma were frozen at minus

Table 4: Content of questionnaires, before treatment (Q1), and postnatal questionnaires (Q2–Q4).

Concept	Response alternative	Questionnaire
Previous acupuncture experience	Yes for pain/Yes for other than pain/No	Q1
Dysmenorrhea	Yes, if so, estimation on VAS/No	Q1
Worry of pain in daily life*	Not at all worried/Not very worried/Quite worried/Very worried	Q1
Worry of labour pain*	Not at all worried/Not very worried/Quite worried/Very worried	Q1
Sociodemographic background	Education/Ancestral homeland/Parents citizenship	Q1
Postnatal valuation of treatment effect on pain and relaxation (MA and EA)	Very effective/Rather effective/Not very effective/Not effective at all	Q2, Q3, Q4
Use this treatment again? (EA, MA)	Yes/No	Q2, Q3, Q4
Negative side effects (EA, MA)	Yes, if so, description of side effects/No	Q2, Q3, Q4
Prelabour worries for: (a) labour pain*, (b) not enough pain relief, (c) not enough support from midwife	Not at all worried/Not very worried/Quite worried/Very worried	Q3
Support from midwife during labour	Yes to a high extent/Yes to a rather high extent/No to a rather low extent/No not at all	Q3, Q4
Overall experience of pain during labour	VAS	Q3, Q4
Overall experience of relaxation during labour	VAS	Q3, Q4
Experienced labour pain in relation to expected**	Much more severe than expected/More severe than expected/As expected/Milder than expected/Much milder than expected	Q3, Q4
Assessment of midwife's acupuncture skills (EA, MA)	Very competent/Quite competent/Not very competent/Not competent at all	Q3, Q4
Overall assessment of pain relief	Very effective/Rather effective/Not very effective/Not effective at all	Q3, Q4
Sufficiency of pain relief	Enough/Not enough	Q3, Q4
Emotions during labour*	Strong/Weak/Happy/Sad/Calm/ Frightened/Alert/ Tired/Secure/Worried/Involved/Lonely/ Detached/Independent/Empowered/ Abandoned/Determined/Tense/Trust in my own capacity/Challenged/Focused/Panic/Disappointed/Present	Q3, Q4
Emotions during labour, overall	Positive/Negative	Q3, Q4
Perception of the midwife*	Calm/Rushed/Supportive/Unhelpful/Clear/ Incompetent/Rude/Humorous/Inconsiderate/Sensitive/ Bossy/Absent/Warm/Nonchalant/Secure/Condescending/ Considerate/Competent/Vague/Informative/ Insensitive/Supportive	Q3, Q4
Overall perception of the midwife	Positive/Negative	Q3, Q4
Why participate in this study?	Open-ended	Q3
Satisfaction with the allocation.	Yes satisfied/No, if so, which allocation would you have preferred?	Q3
Overall birth experience†	Very positive/Positive/Mixed feelings/Negative/Very negative	Q3, Q4
Depressive symptoms	Edinburgh Postnatal Depression Scale††	Q4
Experience of participating in this study	Positive/Negative	Q4

Q1–4: questionnaire 1–4, VAS: visual analogue scale, MA: manual acupuncture, EA: electroacupuncture.
*Schytt et al. [32], adjusted for this study, **Experience of pregnancy and delivery, the women's perspective [33], †Waldenström [34], ††Wickberg and Hwang [35], and Murray and Carothers [36].

70°C. Proinflammatory cytokines, for example, interleukin (IL)-1, IL-6, highly sensitive C-reactive protein (hs-CRP), and tumour necrosis (TNF)-alpha, will later be analysed by commercial assays at an accredited laboratory.

3.9. Sample Size Calculation. The sample size calculation was based on the primary outcome measure, experience of labour pain. The calculation used a Bonferroni adjusted significant level of 0.017 and a power of 0.80. A standard deviation of 20.4 mm on the VAS displayed in each group was brought from historical data [9]. To detect a difference of 15 mm on VAS between the three groups, 41 women in each group were needed. However, a previous study [5] shows that a high internal dropout could be expected

because of labour-related factors, such as an inability to carry through the measurements due to pain or that birth has occurred. Two hours after the first treatment, only 47% of the women had registered data on pain or relaxation (personal communication Dr. L. B. Mårtensson January 2008). To compensate for this expected dropout rate, 88 women in each group were needed. Finally, another 15% dropout due to women's dissatisfaction with the randomization or midwives' high workload could be expected. This required a total of 101 women in each group, that is, 303 women in total.

3.10. Statistical Analyses. A problem with analyses of longitudinal data, is that repeated observations for the same individual are often correlated. This correlation violates the assumption of independence necessary for more-traditional, repeated-measures analysis and leads to bias in regression parameters. To avoid this problem we intend to use a mixed effects models approach in the analysis. Typically, ignoring the correlation of observations leads to smaller standard errors (SEs) and increases type I errors which might lead to the wrong conclusion [46, 47]. Furthermore, the analysis of mixed effect models enables to handle missing data as well as the integration of time-varying factors, such as cervical dilatation, which are issues in the present study.

4. Ethical Considerations

The study has no foreseeable risks but may cause minor discomfort in the form of tiredness or minor bruising. The women were informed that (1) participation in the study was voluntary, (2) their decision whether or not to participate would not affect their current or future treatment, (3) if they decided to participate they were free to withdraw at any time, and (4) all questionnaires and blood samples would be unidentified. The women who agreed to participate in the study signed a consent form. The study was approved by the Regional Ethical Review Board, University of Gothenburg, 2008-05-15, Dnr: 136-08.

5. Summary

Since the evidence of the effect of acupuncture on labour pain is nonconclusive or lacking, possibly because of methodological reasons, there is a need for more well-designed studies, and this study intends to fill such a gap by avoiding some of the limitations of previous research. Many women would like to have nonpharmacological pain relief during labour, and acupuncture is one such treatment that is available in all delivery units in Sweden. However, if acupuncture is not proven to be effective, it should not be recommended for labour pain, which is possibly the most intensive pain a woman may ever experience.

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Research Article

Sino-European Transcontinental Basic and Clinical High-Tech Acupuncture Studies—Part 2: Acute Stimulation Effects on Heart Rate and Its Variability in Patients with Insomnia

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This second part of a series of Sino-European high-tech acupuncture studies describes the first clinical transcontinental teleacupuncture measurements in patients with insomnia. Heart rate (HR) and heart rate variability (HRV) measurements in 28 patients (mean age ± SD: 41.9 ± 14.6 years) were performed under standardized conditions in Harbin, China, and the data analysis was performed in Graz, Austria. Similar to the first part of the series, the electrocardiograms (ECGs) were recorded by an HRV Medilog AR12 system during acupuncture of the Shenmen point (HT7) on the left hand. HR decreased significantly ($P < 0.001$) during and after acupuncture stimulation of the HT7 acupuncture point. Total HRV increased significantly ($P < 0.05$) immediately after acupuncture stimulation, but there was no long-lasting effect. The values of the low-frequency (LF) and high-frequency (HF) band increased significantly after the stimulation compared to baseline values; however, the LF/HF ratio showed no significant changes. Together with the results of previous studies, the present results can serve as a solid basis for further development of acupressure or acupuncture stimulation equipment for complementary use in treating insomnia.

1. Introduction

Insomnia is a common condition in which the patient has trouble falling or staying asleep. It can range from mild to severe, depending on how often it occurs and for how long. It has become a global health problem. In the scientific database PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), there are more than 2,300 reviews on this topic. One of these articles, published recently in the Journal of Clinical Sleep Medicine [1], indicates the necessity for further research on the relationship between the effects of acupuncture on insomnia and autonomic regulation, which might guide better selective use of this treatment modality for insomnia [1].

This second part in a series of transcontinental high-tech acupuncture studies deals with the acute effects of manual needle acupuncture on heart rate (HR) and heart rate variability (HRV) in patients with insomnia. The aim of this study was to test the hypothesis that patients with insomnia will demonstrate decreased HR and increased HRV acute effects during and after acupuncture treatment as measured by electrocardiographic monitoring and spectral analysis techniques. Similar to previous studies on patients with depression [2] and poststroke patients [3] in Harbin, patients with burnout [4], and animal experimental investigations in Beijing [5], a transcontinental teleacupuncture design was used. This means that the data were recorded in patients in China and analyzed in Austria [4, 6].

2. Materials and Methods

2.1. Patients. In total, 28 patients (5 male, 23 female) with a mean age of 41.9 ± 14.6 (SD) years (range: 22–82) were investigated in this transcontinental study. They all presented

themselves at the hospital due to insomnia. The Athens Insomnia Scale (AIS) was used for classification of the disease [7]. The scores ranged from 6–21, resulting in a mean value of 12.4 ± 3.6 (SD). The subjects had no obvious history of heart disease or cerebrovascular disease, respiratory, or neurological problems. The patients were fully informed about the nature of the investigation, and they all provided their informed consent. The methodological procedure and registration of the noninvasive parameters were approved by the local ethics committee and in accordance with the Declaration of Helsinki of the World Medical Association.

2.2. Electrocardiographic Monitoring. Bioelectrical cardiographic (ECG) activity was recorded using an HRV Medilog AR12 (Huntleigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria) equipment. The data were analyzed using new "Fire of Life" software (Huntleigh Healthcare) [4, 8]. The sampling rate of the recorder is 4096 Hz, allowing R-waves to be detected extremely accurately, and a monitoring period of more than 24 hours is possible. All raw data are stored digitally on a 32 MB compact Flash memory card. After removing the card from the portable system, the data are read by an appropriate card reader connected to a standard computer and sent to the research unit in Graz. The dimensions of the HRV recorder are 70 × 100 × 22 millimeters, and the weight is approximately 95 grams with batteries [8]. ECG registration was performed in Harbin with three adhesive electrodes (Skintact Premier F-55; Leonhard Lang GmbH, Innsbruck, Austria) applied to the chest.

HR and HRV, which is the percentage change in sequential chamber complexes called RR-intervals, can be calculated from the ECG. HRV can be quantified in the time and frequency domains using ECG power spectra [8–11]. These parameters are recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [11]. The mean HR, total HRV, LF (low-frequency), and HF (high-frequency) bands, and the LF/HF ratio of the HRV were evaluated [11].

2.3. Acupuncture Stimulation and Procedure. The "Shenmen" (HT7) acupuncture point on the left arm was selected for stimulation. Shenmen is located on the wrist, at the ulnar end of the crease of the wrist, in the depression of the radial side of the tendon of the ulnar flexor muscle of the wrist (see Figure 1). This acupuncture point is indicated mainly in cases reporting cardiac pain, restlessness, and insomnia [12].

For manual acupuncture stimulation, sterile single-use needles (length: 30 mm, diameter: 0.3 mm; Huan Qiu, Suzhou, China) were inserted perpendicularly to the skin to a depth of approximately 15 mm at the acupoint. The needles were stimulated clockwise and counterclockwise for 15 seconds each, with two rotations per second, resulting in 30 rotations per stimulation. The stimulation was performed immediately after inserting the needle, 10 minutes later, and before removing the needle (see Figure 2). The measurement profile and measurement times (a–h) are shown schematically in Figure 2. Eight measurement periods were compared: two before stimulation (a, b), four during acupuncture (c–f), and two after acupuncture (g, h).

Figure 1: Shenmen (HT7) acupuncture point stimulated with a metal needle.

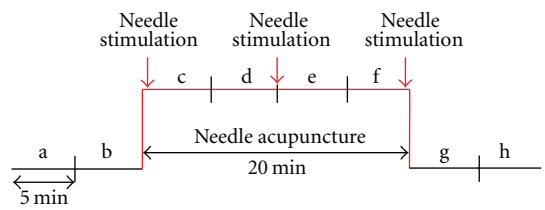

Figure 2: Experimental protocol for manual needle acupuncture at the Shenmen acupuncture point.

2.4. Statistical Analysis. The data were analyzed using one-way repeated measures analysis of variance (ANOVA) or Friedman repeated measures ANOVA on ranks (SigmaPlot 12.0, Systat Software Inc., Chicago, USA). Post-hoc analysis was performed using the Tukey and Holm-Sidak tests. The level of significance was defined as $P < 0.05$.

3. Results

Mean HR and total heart rate variability (HRVtotal) are shown in Figures 3 and 4. In these figures, the results from 28 patients for measurement phases a–h (before, during, and after stimulation of the Shenmen acupoint) are documented. There was a highly significant ($P < 0.001$) decrease in HR after the second needle stimulation (phase e) compared to the two control intervals (a, b) before stimulation. This effect remained manifest throughout the rest of the stimulation (phase f) and during the control intervals after stimulation (g, h).

In contrast to HR, total HRV increased significantly ($P < 0.05$) in two intervals immediately influenced by needle stimulation (c, g). However, this was not a long-lasting effect; at the end of the measurement period (approximately 10 minutes after the last stimulation), values had returned to baseline.

Figure 5 shows the values of the LF and HF bands within the different measurement phases. The values describing the LF and HF bands increased significantly in the interval immediately following the last needle stimulation compared to the interval before the first needle stimulation.

Furthermore, continuous HR-HRV monitoring showed no significant changes in the LF/HF ratio (Figure 6).

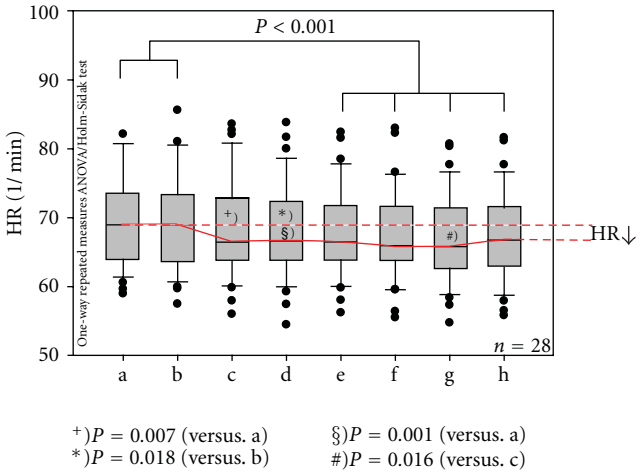

Figure 3: Box plots displaying the mean heart rate (HR) of the 28 patients. Note the highly significant decrease beginning in phase (e). The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles. The different measurement phases (a–h; compare with Figure 2) are indicated.

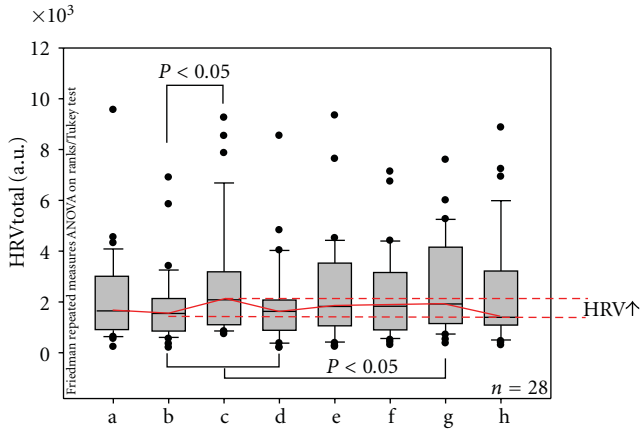

Figure 4: Changes in total heart rate variability (HRVtotal) before, during, and after needle stimulation at the Shenmen acupoint. For further explanation, compare with Figure 3.

4. Discussion

In recent years, computer analysis of heart rate and its variability has allowed for the identification of specific brain-modulated autonomic influences, which reflects the effects of individual mechanisms involved in cardiovascular regulation. New systems and tools for evaluating the features of cardiovascular control have been developed [8, 9]. The application of these tools in acupuncture research should lead to a deeper understanding of the regulation mechanisms and also to the quantitative assessment of the effects of acupuncture stimulation. Evidence has also been provided that HRV may have prognostic value in different diseases involving autonomic dysfunction [9, 11].

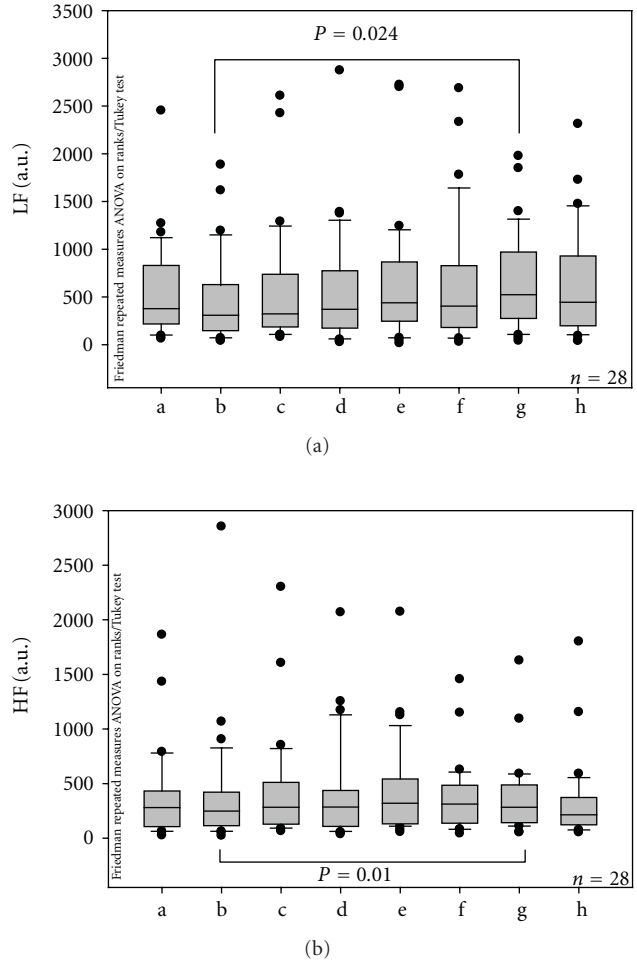

Figure 5: Values of the low-frequency (LF) and high-frequency (HF) bands. Note the significant increase in both bands. For further explanation, compare with Figure 3.

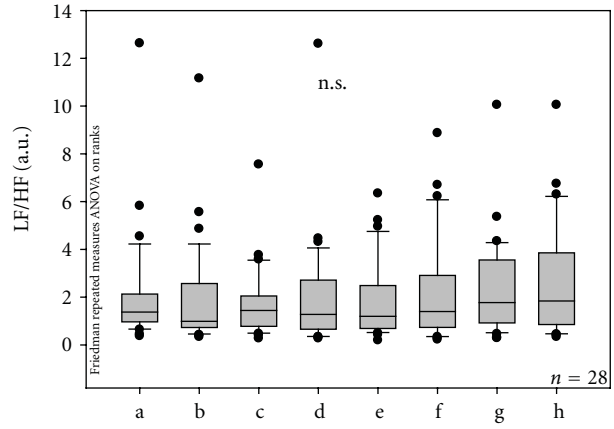

Figure 6: Ratio of the LF (low-frequency) and HF (high-frequency) band of HRV in the 28 patients before, during, and after needle stimulation. No significant alterations were found. For further explanation, see Figure 2.

Beat-to-beat variations of human heartbeat intervals (HRV) have also been investigated using spectral analysis during acupuncture [10]. Mean HR, total power of HRV, power in the LF and HF band, and a normalized power ratio of the LF and HF bands are parameters that somehow reflect sympathetic and/or vagal modulating influences on heart rhythms [10, 11]. In this study, the acute influence of manual needle acupuncture stimulation (Shenmen acupuncture point) on these parameters was investigated under standardized conditions in patients with insomnia. As already mentioned, acupuncture has been shown to modulate the activities of the sympathetic and the parasympathetic nervous systems, which are essential for cardiovascular function. This fact has led to its clinical use in the management of various diseases [9–11].

In 1995, Lin [13] stated in the journal *Psychiatry and Clinical Neurosciences* that acupuncture is a simple and useful treatment for insomnia. He reported a success rate of approximately 90% [13], stating that one of the most effective points is the Shenmen (HT7) body acupoint. To review trials on the efficacy of auricular acupuncture treatment for insomnia, 878 publications were included in a meta-analysis by Chen et al. in 2007 [14]. In all studies (100%), the most commonly used auricular acupoint was the Shenmen acupoint. The authors stated very critically that most trials were of low quality, and therefore, clinical trials with better design quality and a longer duration of treatment are necessary. In our study, we investigated the effects of the Shenmen (HT7) hand acupuncture point. It would be very interesting to perform a separate study investigating the effects of the corresponding or not corresponding Shenmen ear acupuncture point. This was also one of the reasons why we performed a one-point acupuncture study for the first time.

There are several studies concerning acupuncture and insomnia that randomly divided the patients into test groups and control groups [15–18]. Altogether, the rate of effectiveness was higher in the experimental groups. Therefore, all authors concluded that the symptoms induced by insomnia were significantly improved, which is why we did not choose a control point in our study. It has also been demonstrated in a paper published by Ruan in 2009 [19] that the sleep quality of insomnia patients can be significantly improved by acupuncture. Yeung et al. stated in *Sleep* [20] that there is an advantage of electroacupuncture over placebo acupuncture in the short-term treatment of primary insomnia. However, due to the small advantage and some shortcomings of their study, the five authors are not sure if there is a real benefit of electroacupuncture in the treatment of insomnia.

To clarify and elucidate the underlying mechanism involved in the use of acupuncture to treat insomnia, different parameters have to be investigated. Recent studies investigated serotonin and malondialdehyde levels, which are markers for oxidative stress in depressed patients with insomnia [21]. The authors found that the serotonin pathway is involved in the pathophysiological mechanism and that this could be influenced by acupuncture [21]. Other parameters investigated in this context were the blood flow velocity in the middle cerebral artery, basilar artery, and vertebral artery. The cerebral blood flow velocity was increased in this study in the control group, with a more obvious increase in the observation group [22]. In addition to the biosignal parameters, different scores can be used. The findings of recent Chinese investigations published in 2010 showed that acupuncture can improve insomnia patients' clinical symptoms [23, 24].

In an interesting prospective, randomized, placebo-controlled double blind cross-over study, polygraphic monitoring was performed during night sleep in six healthy volunteers [25]. Acupressure at the Shenmen (HT7) hand acupoint was used. After one night of adaptation, two PEBA cones (Polyether Block Amides; Isocones) were fixed bilaterally at HT7 or on the back of the hand (placebo application). Sleep efficiency increased in patients treated with verum acupressure as demonstrated by a decrease in wakefulness and increased their total sleep time as demonstrated by an increase in non-REM (rapid eye movement) sleep [25]. To the best of our knowledge, this is the only study using stimulation of a single acupoint (Shenmen, needled bilaterally) in the context of insomnia. The significance of our data indicates that maybe differences exist between those patients who respond to acupuncture insomnia therapy and those that do not. Further research into the use of electrocardiogram and other physiological parameters to stratify response to acupuncture therapeutic interventions is warranted. Together with the results of our present study, this report can serve as a solid basis for the further development of acupressure or acupuncture stimulation equipment for additional use in treating insomnia.

5. Conclusions

The following conclusions can be drawn from the results of the present transcontinental teleacupuncture study in patients with insomnia.

(i) Heart rate decreased significantly during and after acupuncture stimulation of the Shenmen acupuncture point on the left hand.

(ii) Total HRV increased significantly immediately after acupuncture stimulation, but there was no long-lasting effect.

(iii) The values of the LF and HF band increased significantly after the stimulation compared to baseline values; however, the LF/HF ratio showed insignificant changes.

Acknowledgments

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Medicine in Harbin, China. G. Litscher is also visiting professor at the Institute of Acupuncture and Moxibustion at the China Academy of Chinese Medical Sciences in Beijing.

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Research Article

Sino-European Transcontinental Basic and Clinical High-Tech Acupuncture Studies—Part 1: Auricular Acupuncture Increases Heart Rate Variability in Anesthetized Rats

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Evidence-based research concerning the effects of high-tech acupuncture on autonomic function was performed by two research teams from China and Austria. This study describes the first transcontinental teleacupuncture measurements in animals. Heart rate (HR) and heart rate variability (HRV) recordings in 10 male Sprague-Dawley anesthetized rats were performed under stable conditions in Beijing, China, and the data analysis was completed in Graz, Austria. The electrocardiograms (ECGs) were recorded by an HRV Medilog AR12 system during acupuncture of the ear and body (PC6 Neiguan, CV12 Zhongwan, ST36 Zusanli). The data were analyzed using specially adapted novel Austrian software. HR did not change significantly during any acupuncture stimulation in anesthetized rats (ear acupuncture, PC6, CV12, or ST36). Total HRV only changed significantly ($P = 0.025$) during auricular acupuncture (acupoint heart). The low-frequency/high-frequency ratio parameter decreased significantly ($P = 0.03$) during stimulation of ST36. This change was based on intensification of the related mechanism of blood pressure regulation that has been demonstrated in previous studies in humans. Modernization of acupuncture research performed as a collaboration between China and Austria has also been demonstrated.

1. Introduction

Acupuncture is being recognized as an effective treatment for various autonomic disorders; however, most of the mechanisms of this therapeutic method remain unclear. Evidence-based research and review studies on the effects of acupuncture on autonomic function have already been performed by the authors' research groups in the past [1–8]. The results obtained using high-tech methods are well documented and are important for general acceptance of this traditional Chinese medical treatment in the Eastern and Western world.

This study represents the first time that transcontinental teleacupuncture [3, 9] measurements have been performed in experimental animals. The main goal was to register and analyze the effects of different acupuncture stimulation on heart rate (HR) and heart rate variability (HRV) in anesthetized rats under stable conditions. The data were recorded for 10 rats in Beijing, China, and the data analysis was completed in Graz, Austria. A new HRV system partially developed in Austria was used to record the data in China, and the software that is normally used for human data analysis has been specifically adapted for this study in rats.

2. Materials and Methods

2.1. Animals. Experiments were conducted in accordance with *the Guide for Care and Use of Laboratory Animals* issued by the National Institutes of Health, and the procedures were approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences. Ten male Sprague-Dawley rats, weighing 300–350 g, were kept in an animal house maintained at 21 ± 2°C with a 12-hour light-dark cycle and were given free access to food and water. The animals were initially anesthetized

with an intraperitoneal injection of 10% urethane (1.0 g/kg, Sigma-Aldrich, St. Louis, USA). The left common carotid artery was cannulated with a polyethylene catheter filled with physiological saline containing heparin (200 IU/mL, Sigma-Aldrich, St. Louis, USA) to record arterial pressure (AP) via a blood pressure transducer (DA100, Biopac Systems Inc., Aero Camino Goleta, USA) and amplifier (MP150, Biopac Systems Inc., Aero Camino Goleta, USA). This signal was registered on Micro1401 and Spike2 (CED, Cambridge Electronic Design Limited, Cambridge, UK) data acquisition unit and software. The depth of anesthesia was monitored by changes in AP, and additional anesthetic (urethane 0.3 g/kg) was given if the animal showed large fluctuations in baseline AP or a withdrawal response to a pinch of the paw. After tracheal cannulation, the animals breathed spontaneously, and their core temperature was maintained at $37.0 \pm 0.5^{\circ}$C by a feedback-controlled electric blanket (FHC Inc., Bowdoin, USA). The animals were sacrificed after the investigation by an overdose of anesthetics.

2.2. Electrocardiographic Monitoring. The electrocardiograms (ECGs) were recorded by an HRV Medilog AR12 (Hunt-leigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria) system. The data were analyzed using specially adapted software (Huntleigh Healthcare). The system was designed for a monitoring period of more than 24 hours, and the sampling rate of the recorder is 4096 Hz, allowing R-waves to be detected extremely accurately. All raw data from the rat experiments were stored digitally on a 32 MB compact flash memory card. After removing the card from the portable system in the lab in Beijing, the data were read by an appropriate card reader connected to a standard computer and sent to the lab in Graz. The dimensions of the HRV recorder are $70 \times 100 \times 22$ millimeters, and the weight is approximately 95 grams with batteries [10].

To collect ECG data in rats, which have hairy skin, three electrode plates designed for use in humans were adapted by connecting them to three needle electrodes that were then placed separately in subcutaneous muscles (Figure 1).

HRV is measured as a percent change in sequential chamber complexes called RR-intervals in the ECG. It can be quantified in the time domain and in the frequency range by analyzing the ECG power spectra [1, 3, 4, 8, 10, 11]. The HRV parameters are recommended by the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [11]. Using new software (Huntleigh Healthcare, Cardiff, UK), the HRV is analyzed and displayed in a novel way to evaluate the function of the autonomic nervous system [10]. The mean HR, total HRV, and LF (low frequency)/HF (high frequency) ratio of the HRV were evaluated [11].

2.3. Acupuncture Stimulation and Procedure. The auricular point "Heart" and body points were selected, including PC6 (Neiguan) as the homotopic point that has the same segmental innervation as the heart and ST36 (Zusanli), as the heterotopic point that has different segmental innervation compared to the heart. Both points regulate cardiovascular functions. CV12 (Zhongwan) was selected as another heterotopic body point and is reported to regulate gastrointestinal function. All points were identified by anatomical marks based on descriptions in textbooks and previous reports [2, 12–14]. Briefly, PC6 is located proximal to the accessory carpal pad of the forelimb between the flexor carpi radialis and palmaris longus ligaments. CV12 is located in the medioventral line, 3 mm above the umbilicus. ST36 is located on the anterolateral side of the hindlimb near the anterior crest of the tibia below the knee under the tibialis anterior muscle. The auricular point "Heart" is located at the inferior concha [2, 12, 15].

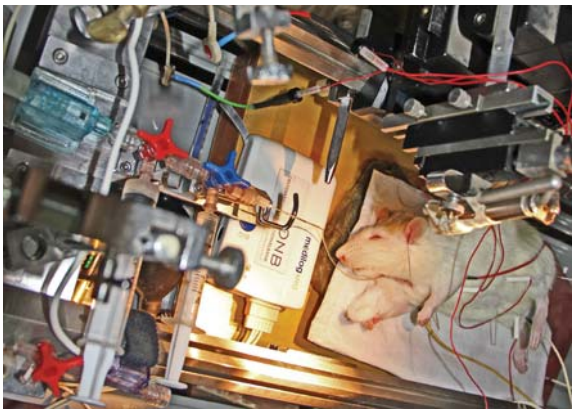

Figure 1: Transcontinental animal experiment using rats in Beijing at the Institute of Acupuncture and Moxibustion at China Academy of Chinese Medical Sciences. HRV equipment from the TCM Research Center Graz was used, and the data analysis was performed at the Medical University of Graz in Austria.

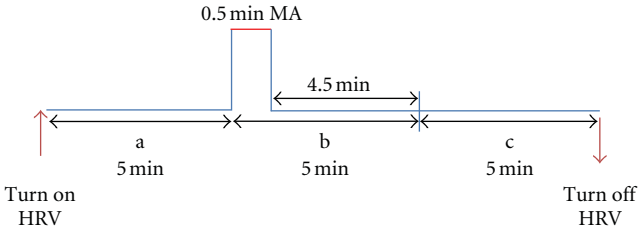

Figure 2: Experimental protocol for manual acupuncture (MA) at the auricular point Heart and body points.

For manual acupuncture stimulation, needles (length: 13 mm, diameter: 0.2 mm; Hwato, China) were inserted perpendicularly to the skin to a depth of 2 mm at the auricular point (Heart) and 4-5 mm at somatic points. When the fluctuations in arterial blood pressure were less than 5%, acupuncture stimulation was applied with neutral supplementation and draining manipulation by twisting the needle for 30 sec. The time course of each stimulation is shown in Figure 2. The order of point stimulation was randomized, and the time between the investigations of the different acupoints was at least 10 minutes.

The measurement profile and measurement times (a–c) are shown schematically in Figure 2. Three measurement periods were compared: one before stimulation (a) one immediately after 30 sec of acupuncture stimulation (b) and one as a second control (c).

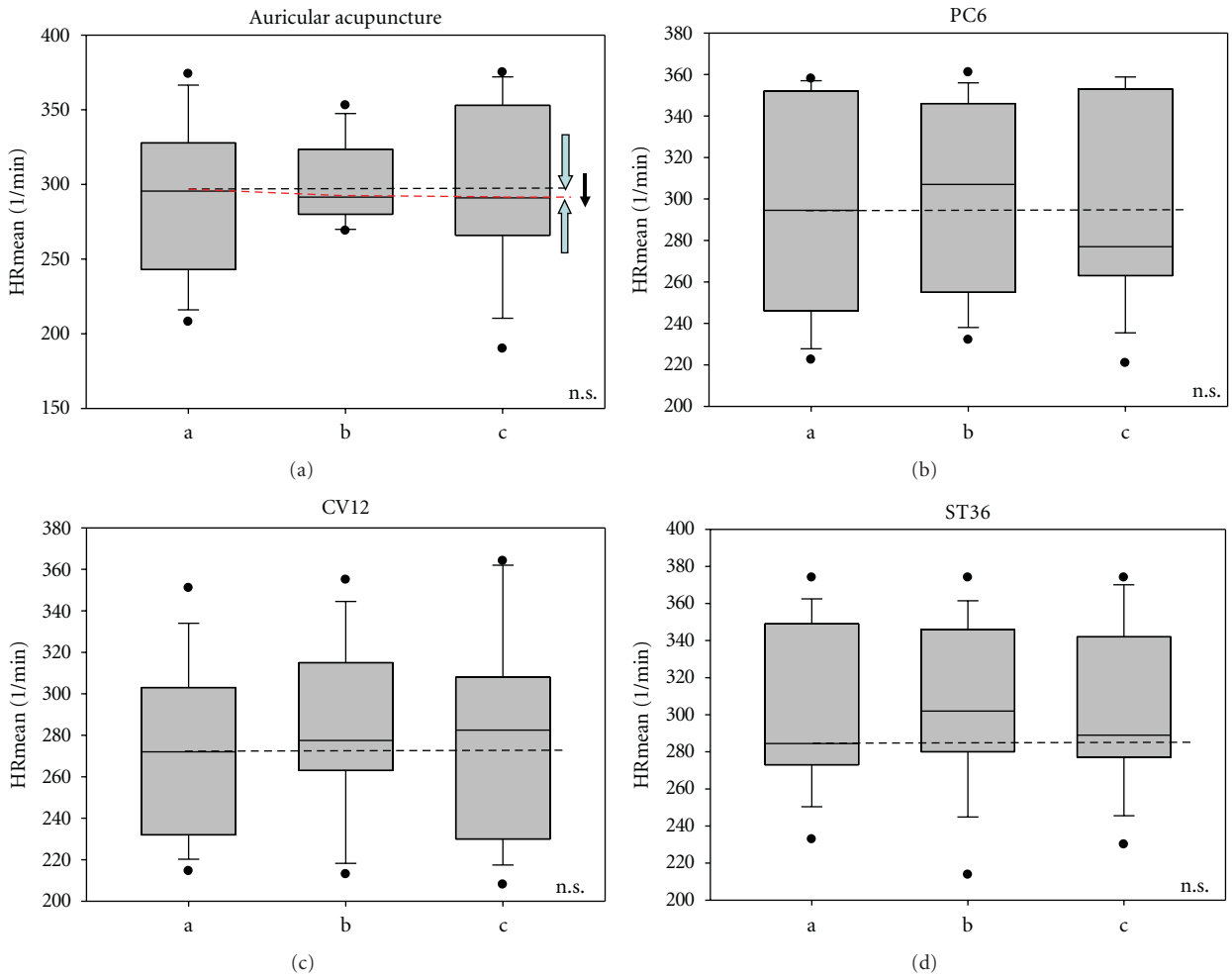

Figure 3: Box plots displaying the mean heart rate (HRmean) of the 10 rats. There are no significant differences. The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles. The different measurement phases (a–c; compare with Figure 2) and acupuncture points (auricular acupuncture: Heart point, PC6, CV12, and ST36) are indicated.

2.4. Statistical Analysis. The data were analyzed using one-way repeated measures analysis of variance (ANOVA) (SigmaPlot 11.0, Systat Software Inc., Chicago, USA). Post hoc analysis was performed using Tukey and Holm-Sidak tests. The level of significance was defined as $P < 0.05$.

3. Results

Figures 3 and 4 show the mean HR and HRV total (total heart rate variability) from the ECG recordings from the 10 rats during the three measurement phases (a, b, and c) as well as before, during, and after stimulation at the "heart" ear acupoint. The results from the stimulation of the body points are also shown (PC6, CV12, and ST36). There was no significant change in HR during the stimulation sessions (Figure 3).

HRV total increased significantly ($P = 0.025$) only after manual ear acupuncture at the point Heart (Figure 4).

Furthermore, continuous HR-HRV monitoring showed substantial and significant ($P < 0.03$) decreases in the LF/HF ratio after acupuncture stimulation at ST36 (Figure 5).

4. Discussion

In 1858, the first transatlantic telecable between Ireland and Newfoundland was installed. It was not successful and worked only for a short time period; however, this was one of the first communication connections between different countries. The first transatlantic connection was then realized in 1866. This connection was the beginning of transmitting data between continents and was the first step towards today's medical information technology.

Today, teleacupuncture between Europe and Asia is no longer a vision of the future. It has already become a reality, and we have described this new approach in previous studies [3, 9]. Up to now, we have performed these measurements in humans. This study is the first using teleacupuncture in an animal experiment.

Measurement of beat-to-beat HRV has been shown to provide a good estimation of autonomic control [1, 8, 11]. Power spectral analysis of HRV is a well-documented method in humans [1, 3, 4, 8–11]; however, there are only a few studies in rats [2, 12–20]. The HR of rats is much higher

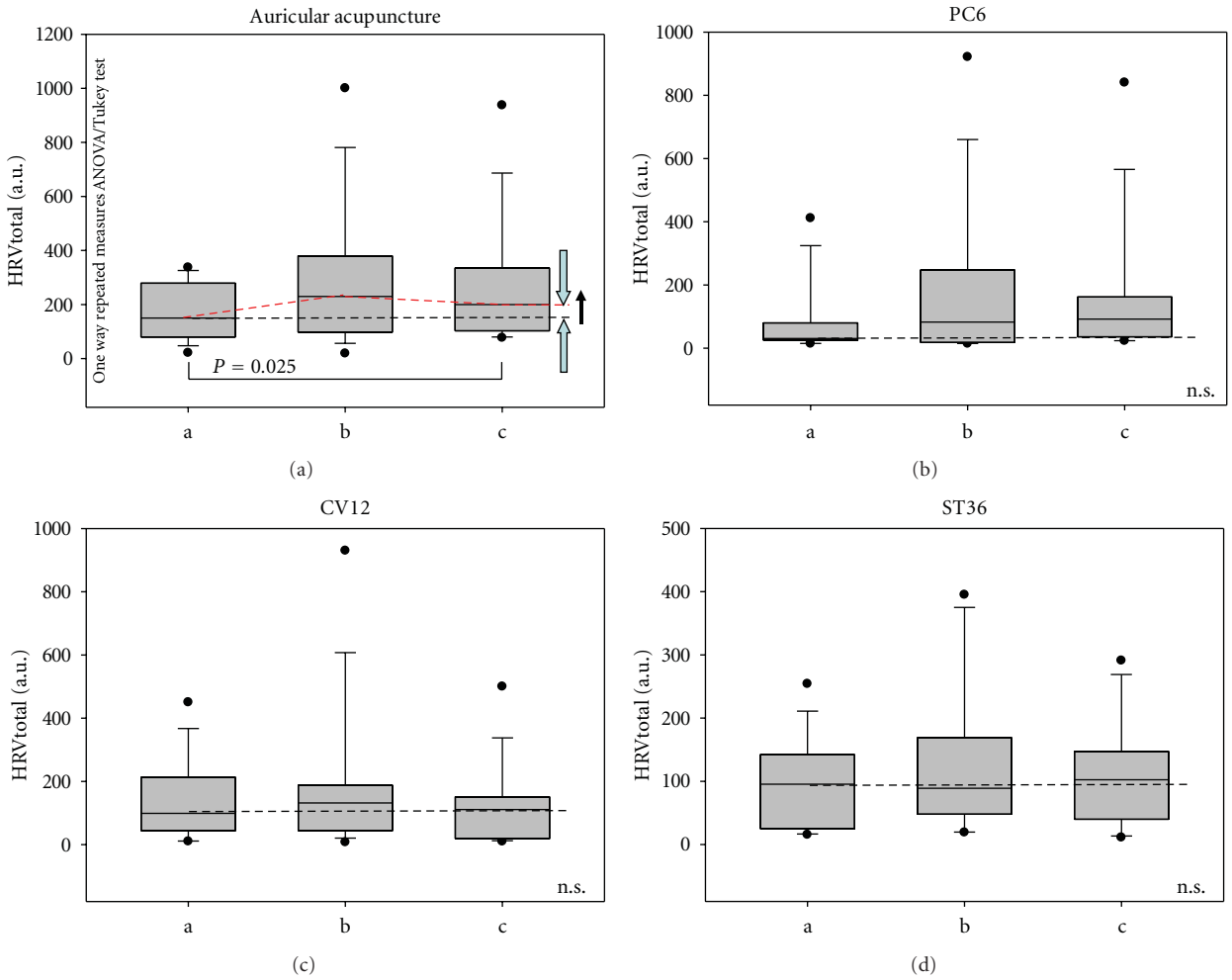

Figure 4: Box plots displaying total heart rate variability (HRV total) for the 10 rats. Note the significant increase in HRV total after auricular acupuncture. For further explanation, compare with Figure 3.

than that of humans, and therefore, the RR-intervals in rats are much smaller than those in humans or dogs. Thus, we developed and adapted the software to allow for adequate resolution of the ECG signals. The novel "fire of life" analysis program [10] from TOM Medical Development (GmbH, Graz, Austria) was used.

Standardization of the evaluated parameters in rats has not yet been performed, so results from different studies cannot be compared. For example, different methods are used to determine the LF/HF ratio, as different studies use different frequency bands. Hashimoto et al. [16] defined the LF with 0.04–1 Hz and the HF with 1.0–3.0 Hz. In contrast, Gao et al. [17] defined the LF band with 0.04–0.15 Hz and the HF band with 0.15–0.40 Hz. Kuwahara et al. [18] also used the LF/HF ratio but employed yet another set of ranges: LF (0.04–1.0 Hz) and HF (1.0–3.0 Hz). The study conducted by Kuwahara et al. is from the same group in Japan as the study conducted by Hashimoto et al. [16]. In the studies by Shen et al. [19] and Li et al. [14], no numerical values for the LF and HF edge frequencies can be found. We defined our ranges according to previous HRV studies in rats [20], with LF < 0.5 Hz and HF ≥ 0.5 Hz up to the Nyquist frequency as determined by the mean RR-interval of the tachogram [20]. From each unconscious rat, ECG signals were recorded continuously during steady state conditions over a period of 15 minutes (see Figure 2). In addition to HRV total, Kuwahara et al. [18] stated that the LF/HF ratio seems to be a convenient index of parasympathetic and sympathetic interactions in the rat. In our investigation, this parameter decreased significantly after stimulating the acupoint, ST36.

One major finding of this study was a significant increase in HRV total after auricular acupuncture at the ear point Heart. This increase is very interesting as HR did not increase at the same time; on the contrary, HR decreased slightly (insignificantly) during and after auricular acupuncture (compare Figure 3). In agreement with these results, biomedical studies on 14 human subjects in Graz have indicated that the ear acupuncture point heart is a very important point in the regulation of the cardiocirculatory mechanism [4]. The results from the Graz study showed a significant decrease in HR and a significant increase in HRV total after manual ear acupressure at this ear acupuncture point, "Heart" [4].

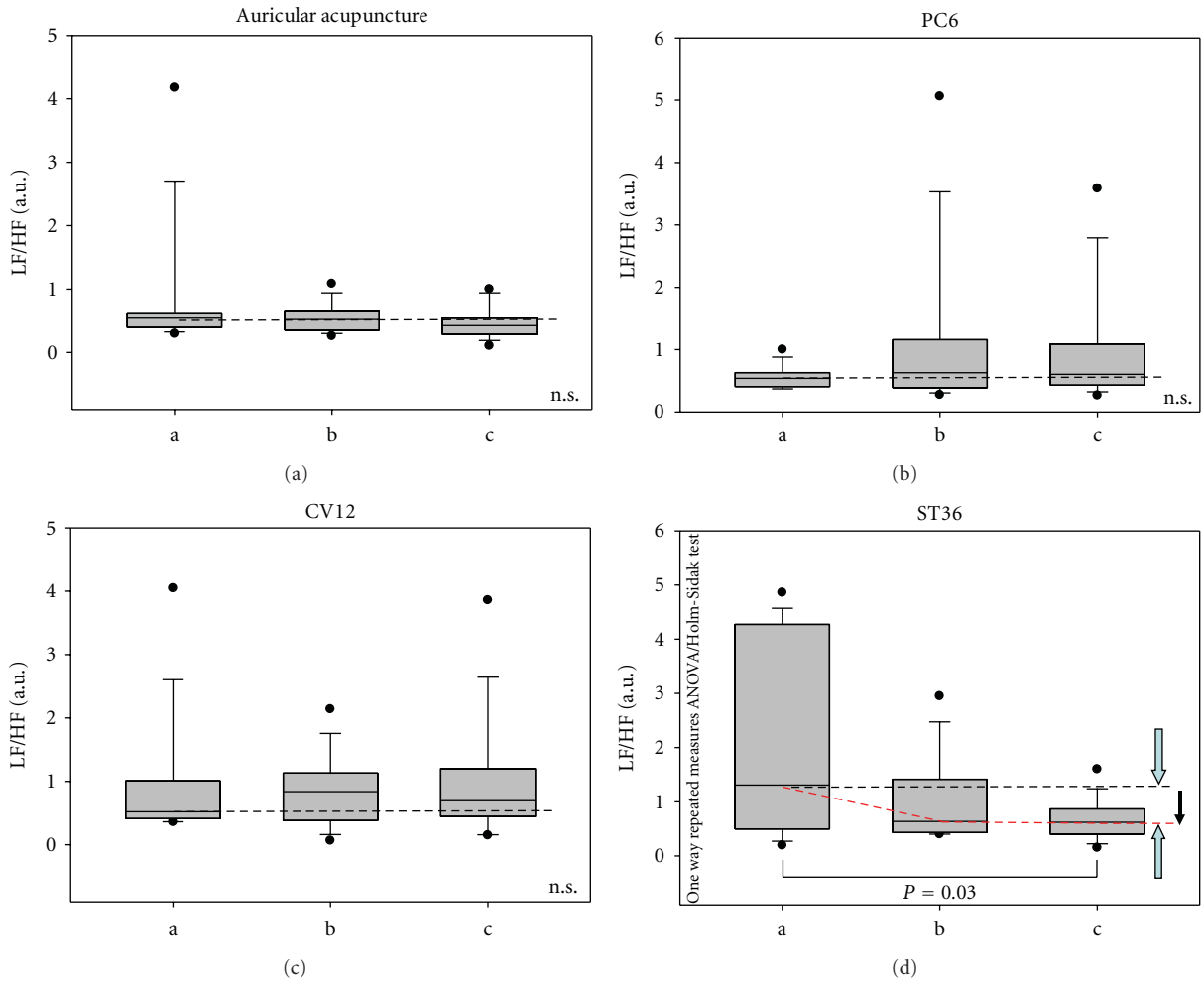

Figure 5: LF (low frequency)/HF (high frequency) ratio. Note that the median of the LF/HF parameter decreases after acupuncture in the ten rats. For further explanation, see Figures 4 and 5.

Basic animal research concerning how acupuncture and acupuncture-like stimulations affect cerebral autonomic function has been performed by Gao et al. in two important previous studies [2, 12] on animal models. One study [2] aimed to examine the effects of acupuncture stimulation at different auricular areas on cardiovascular and gastric responses. Similar to this experiment, stimulation with manual acupuncture was performed in anesthetized Sprague-Dawley rats. They found that the largest depressor response was evoked from an area that corresponds to the "heart" stimulation point in humans that was also used in our study. Similar patterns of cardiovascular and gastric responses could be evoked by stimulating different areas of the auricle [2]. This does not support the theory of a highly specific functional map on the ear; rather, there is a similar pattern of autonomic changes in response to auricular acupuncture with variable intensity depending on the area of stimulation [2]. In light of these previous results, we used active manual stimulation methods applied at the same acupoint and did not perform acupuncture or acupuncture-like stimulation at a control point close to the stimulation area [2, 4].

The second important previous study from Gao et al. was published recently in 2011 in *Brain Research* [12] and demonstrated that auricular acupuncture induces cardiovascular inhibition, increases the response of cardiac-related neurons in the nucleus tractus solitarius, and evokes cardiovascular inhibition via the baroreceptor reflex. In that study, acupuncture-like stimulation was repeated in 58 male Sprague-Dawley rats in the area of the auricular point, "heart". The authors clearly showed that acupuncture at this point regulates cardiovascular function by activating the cardiac-related and depressor neurons in the nucleus tractus solitarius in a manner similar to the baroreceptor reflex [12].

There are only a few experimental studies concerning acupuncture-like stimulation of the ear at the "heart" acupoint in humans [4, 21, 22]. In addition to the aforementioned Graz study [4], one study [21] demonstrated a marked hypotensive effect associated with stimulation of the "heart" point. The results of another investigation [22] indicated that auricular acupuncture plus needle-embedding at the "heart" acupoint could improve left cardiac function in patients with heart failure complicated by dilated cardiomyopathy and that

the function of an acupoint is distinctly different from that of a nonacupoint.

The RR-interval is controlled by the system that regulates blood pressure, which is in turn influenced by the hypothalamus and, particularly, the vagal cardiovascular center in the lower brainstem [4, 11]. Some of the frequency bands in the ECG spectrum of the HRV can be interpreted as markers of physiological relevance. Several of the associated mechanisms are involved in regulating temperature (found in the very low-frequency band), blood pressure, and respiration [4, 11]. The following influences can be distinguished for different ranges of HRV in humans: (a) respiratory sinus arrhythmia (approximately 0.15–0.5 Hz), including central nervous system respiratory impulses and interactions with pulmonary afferents; (b) the so-called "10-s-rhythm" (approx. 0.05–0.15 Hz), which describes the natural rhythm of active cardiovascular neurons in the lower brainstem (the circulatory center and its modulation by feedback with natural vasomotor rhythms via baroreceptor feedback); and (c) longer wave HRV-rhythms (approx. <0.05 Hz), such as effects from the renin-angiotensin system and temperature regulation as well as metabolic processes [4, 11]. Although there are not many studies on ECG power spectral analysis in rats, similar frequency ranges can be determined as described previously [16–20].

In this study, HR decreased insignificantly at the same time that HRV total increased significantly during acupuncture of the ear (Figures 3 and 4). Manual ear acupuncture had a greater effect on HRV than acupuncture at points on the body (PC6, CV12, or ST36). The LF/HF ratio decreased significantly during acupuncture stimulation of ST36. This decrease could be mainly attributed to intensification of the related mechanism of blood pressure regulation (10-s-rhythm) as described in previous investigations in humans [4].

There are some limitations of this pilot study. The number of rats was small ($n = 10$), and there was no control group with a control nonacupuncture point. As already mentioned in a previous study by our two teams [4] and in the discussion, previous results from a study by Gao et al. [2] showed that it is difficult to identify a placebo point on the ear for such investigations. Our study design also does not allow conclusions concerning the underlying mechanism. This could be a topic for future investigations.

Progress can be made in high-tech acupuncture research by using modern biomedical techniques like teleacupuncture and analysis techniques like HRV "fire of life analysis" in animal experiments. Furthermore, modernization of acupuncture research performed as a collaborative effort between the Institute of Acupuncture and Moxibustion at the China Academy of Chinese Medical Sciences in Beijing and the TCM Research Center at the Medical University of Graz in Austria has been demonstrated in this study.

5. Conclusions

The following conclusions can be drawn from the results of this transcontinental experimental animal teleacupuncture study.

(i) Heart rate does not change significantly during acupuncture stimulation of the ear, PC6, CV12, or ST36 in anesthetized rats.

(ii) Total HRV changes significantly during auricular acupuncture (acupoint heart), but not during stimulation of the other acupuncture points (PC6, CV12 or ST36). HRV total increases during auricular acupuncture, which improves the neurovegetative condition. This is interesting as the decrease in HR was not significant during this time.

(iii) The LF/HF ratio decreases significantly only after stimulation of ST36 based on intensification of the blood pressure regulation, which is a related mechanism, as has been demonstrated in previous studies [4].

Acknowledgments

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Research Article

An Innovative High-Tech Acupuncture Product: SXDZ-100 Nerve Muscle Stimulator, Its Theoretical Basis, Design, and Application

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We introduce the theoretical basis, design, and application of a patented innovative high-tech product, SXDZ-100 nerve and muscle stimulator. This product is featured with a built-in chip containing transcoding information from different acupuncture manipulation collected from the wide dynamic neurons (WDR) in the spinal dorsal horn in animal experiments, which is bioinformation feedback therapy. The discharges of WDR neurons excited by different manipulations are analyzed using chaos theory in this study. It combines the advantages of manual acupuncture (MA) like no receptor adaptation and treatment individualization and that of electroacupuncture (EA) such as relatively low stimulation intensity and good quantification and thus makes it more effective than common stimulators in acupuncture clinic.

1. Introduction

Acupuncture is a somatic stimulation therapy. Needling signal is a spatiotemporal grouped sequence of input neuroinformation [1]. Previous reports demonstrate that acupuncture is an effective approach which regulates the human body through encoding external stimulation [2]. Acupuncture manipulation, as one of the major components of acupuncture, is an essential skill for clinic practitioners. Previous studies show that the effect of manipulation of manual acupuncture (MA) is better than that of electroacupuncture (EA) with fixed parameters [3, 4], whereas others still think that EA is more effective, indicating that each stimulation refers to different disease states [5, 6]. However, MA is not easy to be explained and learnt by practitioners. In addition, to ensure the comparability among researchers or subjects in acupuncture research, EA is usually selected by investigators because of the well-quantified stimulation parameters [7, 8]. The problem is that the EA instruments with fixed pulses by settled intensity and frequency are easily adapted to [9]. Adaptation also occurs in the other sensory receptors [10]. EA stimulates mechanoreceptors that respond to mechanical pressure or distortion. This response to a continuous stable stimulation with fixed frequency and intensity will soon be adapted to and lead to an attenuation of the effect of EA in clinic. Thus, it is necessary to develop a novel EA instrument which can scientifically combine acupuncture manipulation and electric stimulation but avoid the adaption issue of EA simultaneously. Through combining chaos and acupuncture manipulation theories, we developed this patented instrument SXDZ-100 nerve and muscle stimulator (patent no. 200710133656.0, State Intellectual Property Office of the People's Republic of China), manufactured by Suzhou Hua Tuo Medical Instruments Co., Ltd., and applied it in clinic and basic research (Figures 1(a), 1(b), and 1(c)). Here, we would like to introduce our first findings.

2. Theoretical Basis

In mammalians, there exist commonly known peripheral sensory fibers as Aα, Aβ, and Aδ. Nociceptive stimulation excites C fiber receptors, whereas nonnociceptive stimulation excites Aα- and Aβ-fiber endings [11]. Wide dynamic range (WDR) neurons in lamina IV–VI of spinal dorsal horn, especially lamina V, respond to both nociceptive and nonnociceptive stimuli of any kind (thermal, chemical, and

Figure 1: Introduction of SXDZ-100 and SXDZ-50. (a) SXDZ-100 nerve and muscle stimulator; (b) SXDZ-50 nerve and muscle stimulator; (c) certification of invention patent from State Intellectual Property Office of the People's Republic of China. SXDZ-100 is of bigger size and more expensive than SXDZ-50.

mechanical) imposed on the middle of the receptive field. This response is intensity dependent [12].

Acupoints are usually some excitable muscle or skin nerve complexes with high density of nerve endings, and the distribution of receptive fields of Aα, Aβ, Aδ, and C fibers is closely associated with the acupoints both in the skin and muscles [13]. Receptors excited by acupuncture or EA are mechanoreceptors and polymodal receptors [14]. Needling sensation receptors include touch unit, pressure unit, muscle spindles, or Golgi tendon receptor unit according to the location of the acupoint, and the receptors and the afferent fibers of the acupoint play a critical role in forming and maintaining the needling sensations [15]. Studies about the relationship between acupuncture manipulations and responsive discharges of deep receptors recorded in medial gastrocnemius nerve in rabbits demonstrated that every type of deep receptor can react to any manipulation, and that the discharge patterns of different receptors are alike when stimulated with the same manipulation, while different manipulations acting on the same receptor can induce different discharge patterns [16].

EA and MA are two different stimulations. During EA, repeated external currents or voltages are delivered to acupoints via the needles. Currents which are intense enough to excite Aβ and part of Aδ fibers can produce an analgesic effect [17]. MA is the insertion of an acupuncture needle into an acupoint followed by different manual technique stimulations, and all types of afferent fibers (Aβ, Aδ, and C) are activated [17]. Excited afferent fibers and evoked sensations are different between EA and MA. Aβ fibers are mainly excited, and numb sensation is produced by EA, whereas for MA, Aδ fibers are excited, and sour and distending sensations are thus produced [15]. To obtain the same effect induced by MA, a certain amount of intensity will be requisite for EA [18].

De qi is the sensation following acupuncture needle placement and subsequent manipulation of the needle, which is important for treatment efficacy [19]. The complex pattern of sensations in the de qi response involves a wide spectrum of myelinated and unmyelinated nerve fibers, particularly the slower conducting fibers in the tendinomuscular layers [20]. De qi sensation was reported qualitatively and quantitatively different between manual and electrical stimulation; the most predominant de qi sensation with electrical stimulation appears to be tingling in nature. However, in manual stimulation, an aching sensation appeared to be the most predominant de qi sensation, followed by sharp pain and tingling sensations [21]. It is hypothesized that de qi is more easily obtained by a stimulation with combined acupuncture manipulations and electric stimulation in the present study. Pacinian corpuscles, for example, are pressure receptors located in the skin and various internal organs. Each pacinian corpuscle is a nerve ending of a sensory neuron. Mechanical pressure of varying strength and frequency induces corpuscle deformation, which creates a generator potential in the sensory neuron arising within it [22]. While reaching its threshold, generator potential induces action potentials, also called nerve impulses. Nerve impulses or action potentials are formed by pressure-sensitive sodium channels at the first node of Ranvier, the first node of the myelinated sensory neuron. The magnitude of the stimulus is encoded by the frequency of impulses generated in the neurons. The more massive or rapid the deformation of a single receptor corpuscle, the higher the frequency of nerve impulses generated in its neuron. This information is encoded by the frequency of impulses since a bigger

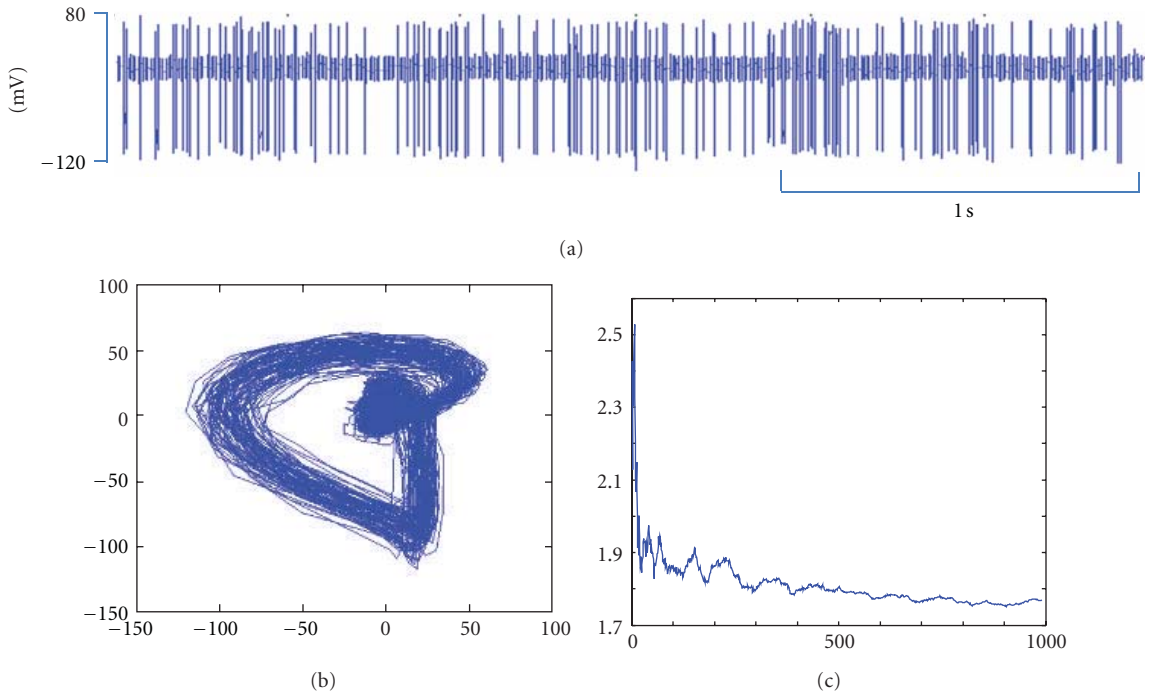

Figure 2: Chaos analysis for the signal recorded in the spinal dorsal horn neuron evoked by manual manipulation of neutral supplementation and draining on ST 36. (a) Time series of neuronal discharge; (b) the reconstructed attractors; (c) the LLE (1.7691).

or faster deformation induces a higher impulse frequency. Action potentials are formed when the skin is rapidly distorted but not when pressure is continuous. Therefore, with continuous pressure, the frequency of action potentials decreases quickly and stops soon. This is the phenomenon of receptor adaptation [10, 23]. The evidence shows that there exist four receptors and afferents responding differently to twisting and lifting-inserting manipulation, and both manipulations stimulate adaptive receptors [24].

It has been found in our study that the 12 most common acupuncture manipulations, including 6 monotypes and 6 multitypes, have individualized group bioinformation encodings. Receptor adaptation, featured with decaying neuronal discharges, existed during EA, but it was not found during MA stimulation [9]. Also it was found that different manipulations have different analgesic effects on somatic acute pain, inflammatory pain, and visceral pain, allowing the possibility for the practitioner to select the optimal stimulation to meet the need of individualized therapy [25].

In general, the monotype manipulations include twirling, handle waggling, handle scraping, trembling, handle flicking, and handle flying, and multitype manipulations include twirling supplementation and draining, lifting-thrusting supplementation and draining, quick-slow supplementation and draining, neutral supplementation and draining, blue dragon wagging tail, and dark tortoise seeking hole [26]. Acupuncture manipulations evoke afferent pulses as various electrical signal time series, called discharges in spinal dorsal horn neurons, which can be analyzed by nonlinear dynamics method to determine the optimal embedding dimension. The time series are considered chaotic by plotting the attractor, qualitatively. The largest Lyapunov exponents (LLEs) are computed based on the Takers' embedding theorem. The LLEs of the time series are positive and obviously vary in different acupuncture manipulations [27, 28].

The research concerning chaos theory has been made in many related fields such as power system and biomedical engineering as well as applications to the human brain and heart [29–31]. In the 1980s, Japanese investigators studied the repetitive firing of the action potential in squid giant axons stimulated by sinusoidal current and found various motions including periodic, quasiperiodic, and chaotic through theoretical computations [32, 33]. In our previous work, we successfully analyzed each time series of neuronal discharge in the dorsal horn neurons evoked by twisting, reinforcing-reducing by slow-quick needling, and reinforcing-reducing by lifting-inserting manipulations on ST36 using chaos theory [28]. In this paper, the time series as neuronal discharge signals of two manipulations as mild reinforcing-reducing and blue dragon wagging tail were analyzed also using chaos for the attractors of the reconstructed phase space and as examples of LLE. Of these two manipulations, mild reinforcing-reducing is easier than blue dragon wagging tail to be performed on patients (Figures 2 and 3). Therefore, this time series information of each manipulation is encoded in the built-in chips in SXDZ-100, as one of the most important steps.

3. Design

Based on the above theory, we designed the mechanism diagram of SXDZ-100 as seen in Figure 4. In this diagram,

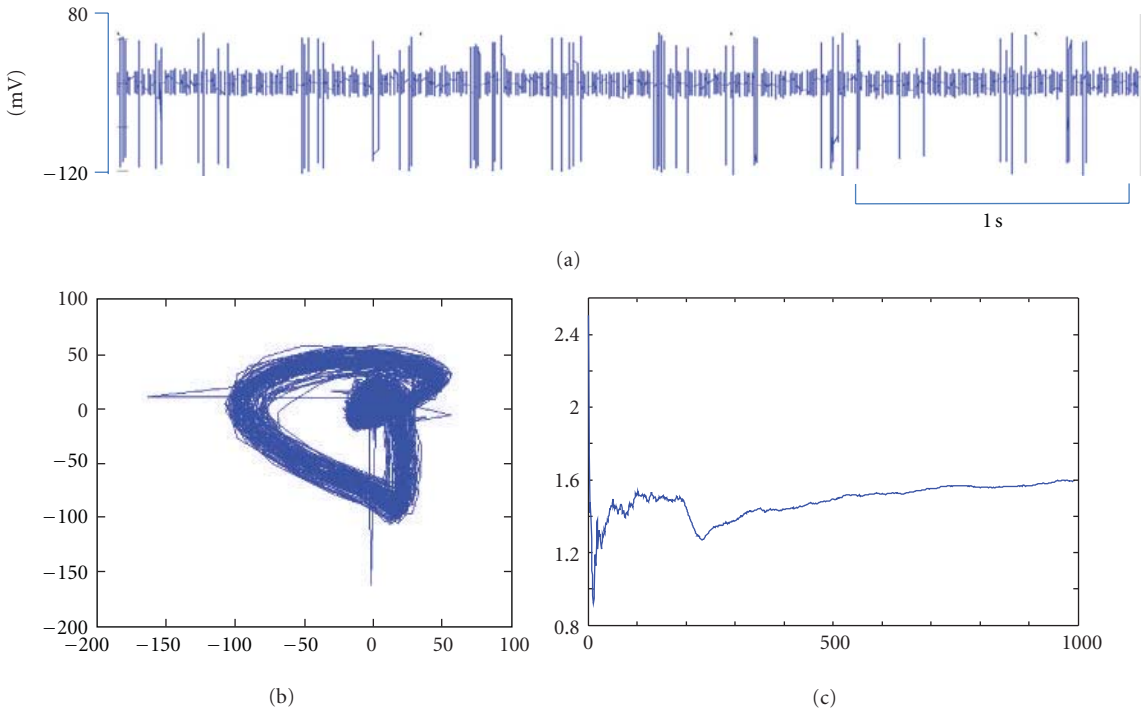

Figure 3: Chaos analysis for the signal recorded in the spinal dorsal horn neuron evoked by manual manipulation of blue dragon wagging tail on ST 36. (a) Time series of neuronal discharge; (b) the reconstructed attractors; (c) the LLE (1.5934).

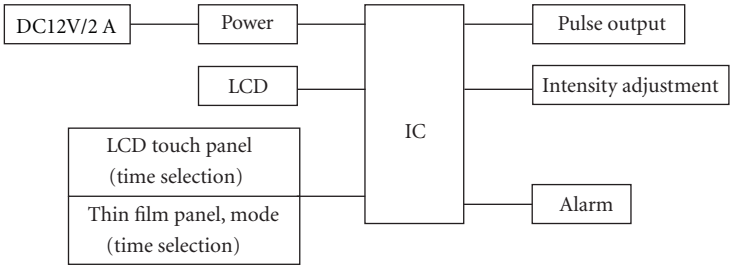

Figure 4: Design diagram of SXDZ-100. LCD: liquid crystal display; DC: direct current; IC: integrated circuit.

the chip is the core of the instrument. The built-in chips of SXDZ-100 contain the time series information of each manipulation. The pulses generated by SXDZ-100 are not just simply external currents, but a kind of encoded currents containing neurobiological messages collected from the human body, in accordance with the concept of bioinformation feedback therapy. Due to the built-in bioinformation group encoding system, SXDZ-100 is significantly different from commonly used electronic pulse generators. Its output is a continuous multivariate waveform which may not be easily adapted to by cutaneous and intramuscular receptors. It combines the advantages of both MA with uneasily adapted manual stimulation and EA with comparatively low stimulation intensity required. We designed and developed SXDZ-100 by selecting characteristic manipulations from numerous acupuncturists and acupuncture academicians. The manual skill signals between fingers of acupuncture specialists are cloned in spinal WDR neurons where decoding of the afferent stimulation signals happens through laboratory approach. The electronic signals produced by the built-in chip containing the afferent signal are not simple external currents, but neuroinformation encoding currents, meeting the needs of bio-information feedback therapy. Therefore, its output currents on needles mimic manual techniques. Figure 4 shows the circuit diagram of SXDZ-100.

4. Application and Discussion

SXDZ-100 has been widely utilized in acupuncture clinic. It has been reported that SXDZ-100 has a higher cure rate than the G6805 electric stimulator did for facial paralysis, indicating that it is a promising high-tech product and can be widely used and would be able to substitute old style electric stimulators in acupuncture clinic [34]. In acupuncture research, SXDZ-100 can also be used as a transcoding stimulator in the research whose common EA stimulator is used and possesses both the advantages of MA

and EA. Considering wider applicability and lower cost, we also designed and developed a smaller version, the SXD-50 nerve and muscle stimulator, which is easier to operate and suitable for home use (Figure 1(b)).

Chaos analysis is a powerful nonlinear dynamic method enabling the extraction of characteristic quantities. It has been found that the phase space of the time series corresponding to the electrical activity of neurons is embedding parameters, and that the largest Lyapunov exponents are calculated and turned out to be positive, based on which, it is concluded that the neuronal signals are chaotic [28]. In this patented SXDZ-100 stimulator, the skillful manipulation signals produced by the fingers of acupuncture specialists as reflected in spinal WDR neurons are cloned. Thus, currents produced from the built-in chip containing the afferent signal codes are not simple biphasic currents, but neuro-information encoded currents, meeting the needs of bio-information feedback therapy. Acupuncture signal may be input into the central nervous system for final integration and organization [35]. In the present study, firings were recorded in the WDR neurons rather than those of the peripheral nerve fibers. Analysis of the input signal encoding of acupuncture helps us to understand the acupuncture information quantification and processing well.

Combined study of the acupuncture mechanism and encoding and the organization of neuro-information will definitely propel the profound understanding of both. Acupuncture manipulations are essential skills for practitioners in clinic, however, not easy to be expressed and learnt by successors. Notably, the effect of different manipulation of MA is better than EA with fixed parameters. However, in acupuncture research, to ensure the comparability among researchers and subjects, EA is mostly selected as the stimulation parameters can easily be quantified by stimulator setting. Although the discrepancy between basic research and clinical practice is obvious, it provides ideas for the invention of a novel electronic stimulator. On the other hand, studies about the mechanism of acupuncture and the improvement of the effect of EA have been conducted nationally and internationally. It is a promising trend to seek out a product with features as labor saving, substituting for MA with long-time needle manipulation, and easily set and assessed as electroacupuncture.

SXDZ-100 is an original scientific research product. It is a more significant innovation of Traditional Chinese Medicine acupuncture combined with electronic stimulation therapy. Compared to other therapeutic instruments both in the national and international markets, SXDZ-100 possesses several major advantages: (1) high-tech: it has built-in chips containing encoded group bio-information from different acupuncture manipulations and provides individualized EA stimulation with varied modes and intensities; (2) easy to operate: it can be easily operated by selecting solidified acupuncture manipulation module from specialists, and by tuning output intensity according to patients' constitution; (3) adjustable parameters: parameter is optional within the ranges ≤ 120 Hz, $\leq 300\,\mu$s, and ≤ 70 V according to different manipulations; (4) endorsement of International Electrotechnical Commission (IEC) and Medical Device Directive (MDD): the circuit board and the whole machine are measured rigidly according to IEC-IEC60601-1, IEC60601-1-2 and IEC60601-2-10 of the US, and the European MDD is also executed to ensure that the criteria of SXDZ-100 are higher than the current national one, such as high- and low-temperature circulation, vibration and shocking, and electronic neutrality, electricity and construction safety, electromagnetic compatibility.

Supported by the above qualification, superiorities of SXDZ-100 are distinct compared to products like both EA apparatus and transcutaneous electrical stimulation (TENS) in the national and international markets. Firstly, merged functions of EA and MA perfectly lead to an innovation and development of both through combining the advantage of both MA with uneasily adapted manual stimulation and EA with comparatively low stimulation intensity required. Its output currents on needles mimic manual techniques well. SXDZ-100 with proprietary intellectual property rights in China is a novel product in Traditional Chinese Medicine acupuncture field. Secondly, SXDZ-100 has fixed stimulation module set by group bioinformation encoding and can be tuned individually to avoid the adaptation of the body. Thirdly, the output of SXDZ-100 is bipolar positive and negative currents. Ideally, bio-electronic stimulation should not be net direct current (DC), and it will be accessible to introduce train, symmetric current, or asymmetric counter-current, which is another innovative point of SXDZ-100.

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Review Article

Auricular Acupuncture May Suppress Epileptic Seizures via Activating the Parasympathetic Nervous System: A Hypothesis Based on Innovative Methods

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Auricular acupuncture is a diagnostic and treatment system based on normalizing the body's dysfunction. An increasing number of studies have demonstrated that auricular acupuncture has a significant effect on inducing parasympathetic tone. Epilepsy is a neurological disorder consisting of recurrent seizures resulting from excessive, uncontrolled electrical activity in the brain. Autonomic imbalance demonstrating an increased sympathetic activity and a reduced parasympathetic activation is involved in the development and progress of epileptic seizures. Activation of the parasympathetic nervous system such as vagus nerve stimulation has been used for the treatment of intractable epilepsy. Here, we propose that auricular acupuncture may suppress epileptic seizures via activating the parasympathetic nervous system.

1. Introduction

Epilepsy is a neurological disorder consisting of recurrent seizures resulting from excessive, uncontrolled electrical activity in the brain. Despite active pharmacological and neurosurgical treatments used for the treatment of epileptic disorders, the management of medically intractable epilepsy remains a difficult problem.

Over the past two decades, concerns regarding the side effects of pharmacological and neurosurgical approaches have increased interest in the use of complementary and alternative medicine (CAM) [1–3].

Autonomic imbalance is involved in the development and progress of epileptic seizures. Auricular acupuncture can treat diseases by increasing parasympathetic tone. Here, we propose that auricular acupuncture may suppress epileptic seizures via activating the parasympathetic nervous system.

2. Auricular Acupuncture Can Increase Parasympathetic Tone

Auricular acupuncture is a diagnostic and treatment system based on normalizing the body's dysfunction which is suggested to stimulate the peripheral reflexes, then activate these central brain pathways, and thus inhibit the maladaptive reflexes that contribute to neuropsychic disorders [4]. Auricular acupuncture was utilized to treat postoperative pain [5], improve neurorehabilitation [6], insomnia [7], and obesity [8] via modifying endorphinergic systems and the autonomic nervous system (ANS).

An increasing number of studies have demonstrated that auricular acupuncture has a significant effect on inducing parasympathetic tone. Manual ear acupressure at "heart" auricular acupoint induced a significant decrease in heart rate and a significant increase in heart rate variability total [9, 10]. Acupuncture on auricular acupoint "Shenmen" might

calm the mind, slow down the heart rate, activate the parasympathetic nerves, and inhibit the sympathetic nerves [11]. Acupuncture conducted on the concha of the ear induces an increase in vagal activity [12]. During needling vision-related acupoints of ear acupuncture, mean blood flow velocity of the ophthalmic artery was significantly increased which may be induced by parasympathetic tone [13]. Another clinical study showed that stimulation of the ear induced a significant increase in the parasympathetic activity during the stimulation period of 25 min and during the poststimulation period of 60 min [14]. The external ear is innervated by several nerves, including vagus nerve, glossopharyngeal nerve, trigeminal nerve, facial nerve, and branches (the second and third) of the cervical spinal nerves [15]. The auricular branch of vagus nerve (ABVN) innervates the auricular concha and the external auditory meatus. Parasympathetic tone such as Arnold's reflexes has been clinically observed after stimulating innervation regions of the ABVN [16, 17], which is considered as a bridge between the external ear and the internal organs [18]. In Traditional Chinese Medicine, auricular acupoints related to internal organs are located at the auricular concha [4]. Except for the ABVN, the glossopharyngeal nerve, the trigeminal nerve, and the facial nerve, all carry parasympathetic nerve fibers. Most nerves innervating the external ear carry parasympathetic components.

3. Epilepsy Is Associated with Decreased Parasympathetic Tone

Autonomic symptoms accompany all generalized tonic-clonic seizures (GTC) and one-third of simple partial seizures. The ANS centers can be involved in complex partial, absence, and generalized tonic seizures. Measurements of ANS functions may be helpful in differentiating between epileptic seizures and nonepileptic psychogenic seizures [19]. The autonomic imbalance of epileptic seizures probably results from the hypersynchronized electrical impulse from the temporal and frontal areas to the limbic system, then to autonomic central nuclei in medulla including the nucleus tractus solitarius (NTS) and ambiguus nuclei. Both sympathetic and parasympathetic efferent discharges are then generated.

There is ample experimental and clinical proof that epilepsy goes along with autonomic imbalance demonstrating an increased sympathetic activity and a reduced parasympathetic activation. Novak et al. documented rapid parasympathetic withdrawal approximately 30 seconds before seizure onset and a sympathetic activation peak at seizure onset [20]. Temporal lobe epilepsy is known to be associated with ictal and interictal autonomic dysregulation, predominantly with sympathetic overactivity [21]. Higher sympathetic function and lower parasympathetic function have been demonstrated to be significant risk factors for sudden unexplained death in epilepsy subjects [22, 23].

Activation of the parasympathetic nervous system (PNS) has shown therapeutic benefits in brain diseases. Examples include vagus nerve stimulation (VNS) for epilepsy. VNS has been successfully applied for more than 20 years to treat drug-resistant epilepsy [24]. The antiseizure effect of VNS is considered to be mediated via vagal afferent projections to the NTS, then from the NTS to different brain regions which correlate with the pathogenesis of epilepsy [25]. Recently, VNS has also been applied for treatment of drug-resistant depression [26] and was suggested as a new approach for the treatment of heart failure [27] and stroke [28] by increasing the parasympathetic tone.

4. Hypothesis

Auricular acupuncture appears to modify the autonomic dysfunction by increasing parasympathetic activity. Thus, we hypothesize that auricular acupuncture may suppress epilepsy by increasing parasympathetic tone. We have done clinical trials and animal experiments on the effect and mechanism of auricular electroacupuncture for the treatment of epilepsy. In clinical trials, auricular electroacupuncture reduced seizure frequency and attenuated seizure severity. Animal results showed that auricular electroacupuncture suppressed epileptic discharges in electroencephalogram traces. All the results support our hypothesis.

5. The Mechanism of Auricular Acupuncture for Epilepsy

Acupuncture has been used to treat epilepsy. Acupoints selected to treat epilepsy included "GV 14" [29, 30], "ST 36" [31], and auricular acupoints such as "Pizhixia, Nao, and Shenmen" [32, 33]. Most nerves innervating the external ear carry parasympathetic nerve fibers. Moreover, the ABVN is the only peripheral branch of the vagus nerve. Acupuncture at auricular acupoints especially in the area of auricular concha may induce vagal tone to suppress epileptic seizures. As the main vagal afferent, the NTS is considered as a neuroanatomical center for pathways of the antiseizure effect of auricular acupuncture [34]. Amelioration of illness by auricular acupuncture is believed to be through the reticular formation which is found to be histopathologically connected with focal-cortical seizure-induced generalized convulsive status epilepticus [35]. Recent findings highlight the possibility of inflammation in seizures and epileptogenesis [36]. Prototypical inflammatory cytokines such as IL-1β, TNF-α, and IL-6 have been shown to be overexpressed prominently by glia. Cytokines receptors are also upregulated, and the related intracellular signalling is activated in brain areas of seizure generation and propagation in experimental models of seizures [37]. The anti-inflammation effect perhaps is the mechanism of auricular acupuncture for epilepsy [38]. Possible mechanism of auricular acupuncture for the treatment of epilepsy is shown in Figure 1.

6. Potential Application of Auricular Acupuncture for Other Diseases

The ANS is the primary neural mediator of physiological responses to internal and external stimuli [39]. Functions of many or perhaps all visceral organs can be modulated by somatosympathetic or somatoparasympathetic reflex activity induced by an appropriate somatic afferent stimulation [40].

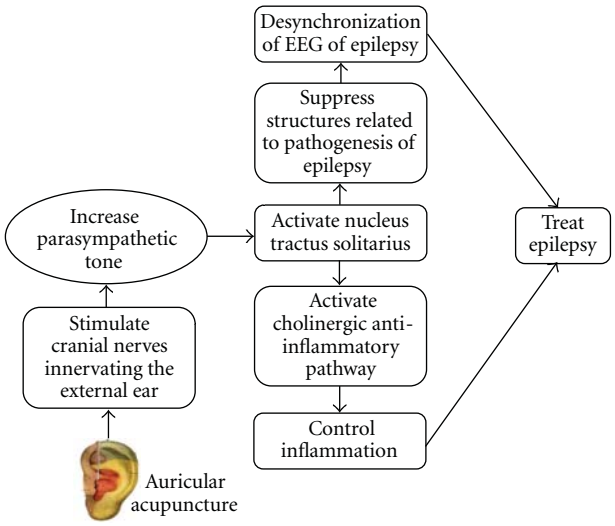

Figure 1: Possible mechanism of auricular acupuncture for the treatment of epilepsy.

According to the theory of Traditional Chinese Medicine, acupuncture has the function of bidirection-regulative effect. In addition to the dysfunction of parasympathetic system, auricular acupuncture can also modulate the dysfunction of sympathetic system [41]. Therefore, auricular acupuncture provides a somatic stimulation to treat diseases being induced or accompanied by an imbalance of the autonomic system.

VNS has been proposed to have the potential for the treatment of neuropsychiatric illnesses [42]. Yet it is an invasive procedure that may have potential side effects and complications. Its application in developing countries is limited for high costs. We can use auricular electroacupuncture, which is less invasive, of less cost, and convenient to treat diseases by setting up suitable parameters. Recently, kinds of acupuncture treatment instruments such as radio electric stimulator device [43] and P-Stim auricular electroacupuncture stimulation device [44] have been developed for the treatment of stress-related disorders and pain relief. Auricular vagal nerve stimulator is expected to be explored.

Abbreviations

CAM: Complementary and alternative medicine
GTC: Generalized tonic-clonic seizures
ANS: Autonomic nervous system
NTS: Nucleus tractus solitarius
VNS: Vagus nerve stimulation
PNS: Parasympathetic nervous system
ABVN: Auricular branch of vagus nerve.

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Research Article

Transcutaneous Electrical Nerve Stimulation on the PC-5 and PC-6 Points Alleviated Hypotension after Epidural Anaesthesia, Depending on the Stimulus Frequency

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Neuraxial blockade causes arterial hypotension. Transcutaneous electrical nerve stimulation (TENS) at the Neiguan (PC-6) and Jianshi (PC-5) reduces the severity of hypotension after spinal anaesthesia, but did not clarify the optimal stimulus frequency. We hypothesized that the stimulus frequency of TENS at the PC-6 and PC-5 points would influence the severity of hypotension after epidural anaesthesia. 65 ASA I or II male patients presenting for inguinal hernia repair were randomized to five groups: the control group received no treatment; the 2 Hz, 10 Hz, 20 Hz, and 40 Hz groups received TENS at a frequency of 2 Hz, 10 Hz, 20 Hz, and 40 Hz, respectively. The lowest SBP was significantly higher in the 40 Hz group [the control, 84 (74–110) mmHg; the 2 Hz, 96 (62–116) mmHg; the 10 Hz, 100 (68–110) mmHg; the 20 Hz, 96 (64–115) mmHg; the 40 Hz, 104 (75–140) mmHg: $P = 0.004$]. Significantly less patients experienced hypotension in the 40 Hz group [the control, 78%; the 2 Hz, 43%; the 10 Hz, 38%; the 20 Hz, 38%; the 40 Hz, 8%: $P = 0.008$]. TENS on the PC-6 and PC-5 points reduced the severity and incidence of hypotension after epidural anaesthesia, depending on the stimulus frequency.

1. Introduction

Neuraxial blockade, epidural anaesthesia, causes arterial and venous vasodilation and decrease in venous return due to blockade of the sympathetic nervous system, which results in arterial hypotension [1, 2].

In animal studies, electroacupuncture at the Neiguan (PC-6) and Jianshi (PC-5) points affects the circulatory and sympathetic nervous system [3, 4], especially electroacupuncture at the PC-6 point increases haemodynamics [5] and makes bleeding-induced hypotension less severe [6]. Our previous study showed that a frequency of 50 Hz of transcutaneous electrical nerve stimulation (TENS) at the PC-5 and PC-6 reduces the severity of hypotension after spinal anaesthesia, but did not test the appropriate frequency of electrical stimulation of TENS [7]. However, several studies showed that electroacupuncture evokes pressor or depressor response, depending on the stimulus frequency. That is, the autonomic nervous system might be influenced by the stimulus frequency of TENS.

We thus hypothesized that the stimulus frequency of TENS at the PC-6 and PC-5 points would affect the activity of the autonomic nervous system, thereby differentially influencing the severity of hypotension after epidural anaesthesia. The purpose of the present study was to test the effect of four different frequencies of TENS at the PC-5 and PC-6 points on haemodynamics after epidural anaesthesia in patients undergoing inguinal hernia repair.

Table 1: Demographic and anaesthetic data. Data are median (range). Wound-based time: baseline measurements to wound closure time.

	Control ($n = 14$)	2 Hz ($n = 13$)	10 Hz ($n = 12$)	20 Hz ($n = 13$)	40 Hz ($n = 13$)	P
Age (yr)	69 (61–74)	66 (43–82)	74.5 (49–88)	73 (23–84)	69 (36–886)	0.442
Height (cm)	160 (148–173)	163 (148–175)	159 (148–174)	162 (150–170)	159 (137–179)	0.773
Weight (kg)	61 (50–72)	62 (39–76)	57 (41–64)	58 (45–66)	54 (47–94)	0.578
Wound-based time (min)	45 (28–55)	40 (28–55)	42 (38–46)	42 (38–45)	40 (35–55)	0.932

2. Methods

After obtaining approval from the Ethics Committees of our institutions and written informed patient's consent, 67 ASA I or II male patients presenting for inguinal hernia repair under epidural anaesthesia were enrolled in the present study. Patients suffering from hypertension, diabetes, or obesity were excluded.

Patients were randomized into five groups, using sealed envelopes: the control group received no treatment; the 2 Hz group received TENS at a frequency of 2 Hz bilaterally at the PC-5 and PC-6 points (on the palmar side of both arms, between the tendon of the long palmar muscle and radial flexor muscle of the wrist) [7, 8] (Figure 1) by a TENS stimulator (NeuroTrax TENS & AcuStim; Verity Medical LTD, Hampshire, uk); the 10 Hz, 20 Hz, and 40 Hz groups received TENS at 10, 20, and 40 Hz bilaterally at the PC-5 and PC-6 points by the TENS stimulator, respectively. All patients fasted for a minimum of 6 h preoperatively. At the operation room, all patients had standard monitoring in place (noninvasive arterial pressure, electrocardiogram (ECG), and pulse oximetry) and these baseline values were recorded. In the 2 Hz, 10 Hz, 20 Hz, and 40 Hz groups, then, small-sized (1.5 cm) cutaneous electrode pads were put bilaterally at the PC-6 and PC-5 points. The intensity of the electrical stimulation was adjusted to produce the most intense tolerable electrical sensation without muscle contractions at a frequency of 2, 10, 20, or 40 Hz and a duration of 100 μs until the end of surgery. After intravenous access, acetated Ringer's solution (10 mL kg^{-1}) was administered before the induction of anaesthesia. With the patient on the right side, 10 mL of 2% lidocaine was injected after identification of the epidural space at the L1-L2 interspace using a 17-gauge Tuohy needle, and then a 20-gauge epidural catheter was placed.

The anaesthetic level was measured by pinprick at the right mid-clavicular line every two minutes. Surgery started when an adequate dermatome level of anaesthesia from Th8 to L2 was ensured. In the case of inappropriate cephalad spread of anaesthesia, incremental epidural supplements of 2% lidocaine were given, starting with 4 mL. When necessary, additional 2 mL boluses were given no earlier than 5 min after the preceding top-up. Haemodynamics were recorded every minute for 30 minutes after the injection of 10 mL of 2% lidocaine and then every 2.5 minutes until the end of surgery. In the case of hypotension, ephedrine of 4 mg was given intravenously. If necessary, additional ephedrine of 4 mg was given every two minutes. We defined hypotension as a decrease in systolic blood pressure (SBP) 30% below baseline values or to less than 90 mmHg in the present study.

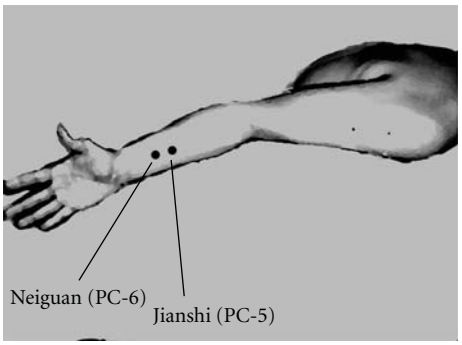

Figure 1: The locations of small-sized cutaneous electrode pads for transcutaneous electrical nerve stimulation.

Atropine of 0.5 mg was given intravenously to treat bradycardia, defined as a decrease in heart rate to less than 50 beats min^{-1}.

Using empirical data from our daily clinical practice, the mean (SD) of the lowest SBP was 85 [9] after epidural injection of 10 mL of 2% lidocaine. Thus, a group size of at least 12 patients was needed to show a difference of 15 (SD 10) in the lowest SBP with a significant level of 0.05 ($\alpha = 0.05$) and a power of 80% ($\beta = 0.20$). Data are expressed as median (range). Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's method for multiple comparisons. A $P < 0.05$ value was considered to be significant.

3. Results

Demographic data and baseline measurements to wound closure time were comparable among the five groups (Table 1). Baseline systolic blood pressures (SBPs) were similar among these groups (Table 2).

The lowest SBP was significantly higher in the 40 Hz group compared to that of the control, 2 Hz and 20 Hz groups (the control, 84 (74–110) mmHg; the 2 Hz, 96 (62–116) mmHg; the 10 Hz, 100 (68–110) mmHg; the 20 Hz, 96 (64–115) mmHg; the 40 Hz, 104 (75–140) mmHg, $P = 0.004$) (Table 2). Significantly less patients experienced hypotension in the 40 Hz group (the control, 11 (78%); the 2 Hz, 6 (43%); the 10 Hz, 5 (38%); the 20 Hz, 5 (38%); the 40 Hz, 1 (8%), $P = 0.008$). Two, one, and four patients experienced severe hypotension (SBP less than 70 mmHg) in the 2 Hz, 10 Hz, and 20 Hz groups, respectively. Less ephedrine was required to maintain arterial blood pressure in the 40 Hz group (the control, 8 (0–16) mg; the 2 Hz, 0 (0–20) mg; the 10 Hz,

Table 2: Haemodynamic data and dose of ephedrine. Data are median (range) or number (percentage). SBP: systolic blood pressure. †: significantly different from the control group. *: significantly different from the 40 Hz group.

	Control (n = 14)	2 Hz (n = 14)	10 Hz (n = 13)	20 Hz (n = 13)	40 Hz (n = 13)	P
Intensity of TENS (mA)	—	13 (9–17)	12 (9–15)	13 (9–15)	13 (9–15)	0.721
Baseline SBP (mmHg)	130 (104–150)	135 (115–145)	130 (110–145)	130 (114–145)	132 (116–158)	0.994
Lowest SBP (mmHg)	84 (74–110)*	96 (62–116)*	100 (68–110)	96 (64–115)*	104 (75–140)	0.004
Baseline HR (beats min^{-1})	72 (56–98)	76 (56–105)	70 (60–85)	70 (54–85)	75 (55–105)	0.343
HR at lowest SBP (beats min^{-1})	62 (45–80)	64 (40–76)	61 (44–86)	55 (45–75)	65 (50–95)	0.522
Incidence of hypotension (n)	11 (78%)	6 (43%)	5 (38%)	5 (38%)	1 (8%)†	0.008
Ephedrine (mg)	0 (0–16)	8 (0–20)	0 (0–8)	0 (0–12)	0 (0–8)†	0.013

0 (0–8) mg; the 20 Hz, 0 (0–12) mg; the 40 Hz, 0 (0–8) mg, $P = 0.013$).

4. Discussion

The present study showed that a frequency of 40 Hz of TENS on the PC-5 and PC-6 points significantly reduced the severity and incidence of hypotension after epidural anaesthesia in patients undergoing inguinal hernia repair, compared to frequencies of 2, 10, and 20 Hz of TENS on the two points.

Acupuncture, acupressure, and TENS on the traditional acupuncture points have been administered for peri-operative management [9–11]. Electroacupuncture at an acupuncture point affects haemodynamics and the sympathetic nervous system [8, 12, 13]. Electroacupuncture at the PC-5 increases stroke volume and cardiac output and furthermore reduces the severity of bleeding-induced hypotension [5, 6].

Since TENS was administered at the most intense tolerable electrical sensation in the present study, there is a possibility that TENS itself had some activating effects on the sympathetic nervous system [7]. While administering the same level of electrical stimulation, however, TENS at the specific frequency, 40 Hz, significantly sustained blood pressure. Some studies showed that electroacupuncture on the PC-5 at 40 Hz enhances myocardial function and prevents bleeding-induced hypotension [5, 6], which are consistent with the present study. In contrast, electoacupuncture at 2–4 Hz suppresses cardiovascular sympathetic reactions [3, 4]. In fact, 2, 10, and 20 Hz stimuli induced severe hypotension in some patients in the present study. A stimulation of somatic afferent fibres at 5 Hz causes a depressor effect, while a stimulation of 40 Hz leads to a pressor effect [14]. Also, a study showed that stimulation of myelinated fibres alone or myelinated and unmyelinated fibres together leads to a depressor effect [15]. In contrast, stimulation of unmyelinated fibres alone causes a pressor effect. We thus speculate that 40 Hz stimulus might have provoked the activation of unmyelinated fibres, compared with 2, 10, and 20 Hz stimuli in the present study.

In conclusion, TENS on the PC-5 and PC-6 points reduced the severity and incidence of hypotension after epidural anaesthesia in patients undergoing inguinal hernia repair, depending on the stimulus frequency.

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