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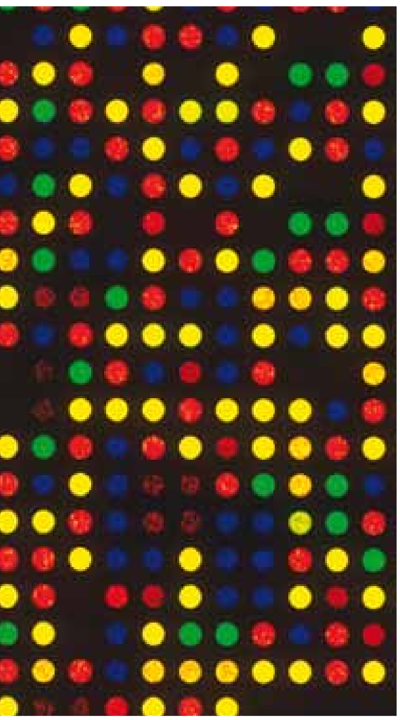
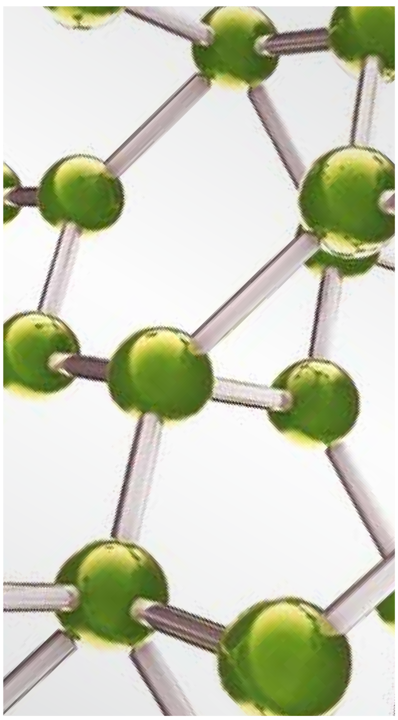
Heart Rate Variability and Complementary Medicine

Guest Editors: Gerhard Litscher, Wei He, Seung-Ho Yi, and Lu Wang



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Editorial Board

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Editorial

Heart Rate Variability and Complementary Medicine

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Heart rate variability (HRV) is a parameter of the neurocontrol of the heart and is used more and more in recent scientific research and practice, not only in Western medicine, but also in evidence-based traditional medicine. Today innovative research including the latest recording technology and also artificial intelligence techniques are used for data acquisition and data analysis of HRV in acupuncture and herbal medicine research.

The scope of HRV is not yet completely clear, but it is known that there are intraindividual and interindividual variances and that heart rate variation depends on age. It becomes less random with the aging process and the appearance of age-related diseases. 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 and so-called lifestyle diseases like diabetic neuropathy, renal failure, essential hypertension, cardiac disorders, coronary artery disease, or intracranial lesions. In all cases, different medications have to be taken into account.

HRV can be used as a globally reliable indicator of the state of health. However, it could be demonstrated in this special issue that in special syndromes like stress or burnout one can counteract this process using different preventive methods like acupuncture.

This special issue focuses on the latest innovative aspects concerning HRV and complementary medicine. Altogether, 25 manuscripts underwent a review process. Sixteen articles were accepted for publication. Some of the papers report results of HRV in combination with different kinds of acupuncture (manual needle acupuncture, laser acupuncture, and electroacupuncture).

Important topics of this special issue are basic and clinical HRV research in complementary medicine, the development of innovative HRV-related concepts for assessing the state of health, new methods for the quantification of HRV, HRV and practical implications in traditional medicine, and telemedicine and HRV. This special issue contains basic research studies and clinical studies concerning menstrual pain and menstrual distress, burnout syndrome, depression, and insomnia, animal experimental studies in rats and dogs, and a review article.

Conflict of Interests

The authors state that there is no conflict of interests regarding the publication of this editorial.

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Review Article

Effect of Acupuncture on Heart Rate Variability: A Systematic Review

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Aim. To summarize all relevant trials and critically evaluate the effect of acupuncture on heart rate variability (HRV). *Method.* This was a systematic review with meta-analysis. Keyword search was conducted in 7 databases for randomized controlled trials (RCTs). Data extraction and risk of bias were done. *Results.* Fourteen included studies showed a decreasing effect of acupuncture on low frequency (LF) and low frequency to high frequency ratio (LF/HF ratio) of HRV for nonhealthy subjects and on normalized low frequency (LF norm) for healthy subjects. The overall effect was in favour of the sham/control group for high frequency (HF) in nonhealthy subjects and for normalized high frequency (HF norm) in healthy subjects. Significant decreasing effect on HF and LF/HF ratio of HRV when acupuncture was performed on ST36 among healthy subjects and PC6 among both healthy and nonhealthy subjects, respectively. *Discussion.* This study partially supports the possible effect of acupuncture in modulating the LF of HRV in both healthy and nonhealthy subjects, while previous review reported that acupuncture did not have any convincing effect on HRV in healthy subjects. More published work is needed in this area to determine if HRV can be an indicator of the therapeutic effect of acupuncture.

1. Introduction

Acupuncture has been described as a philosophy-based, not science-based, medicine [1]. Probably, this is because the philosophy underpinning Chinese medicine reflects heavily oriental culture and philosophy. It emphasizes a holistic view embracing both internal and external environment of an individual. Acupuncture is a commonly practiced Chinese medicine therapeutic modality. The mechanism of acupuncture is the restoration of a harmonious flow of *qi* (life energy) by manipulating the complementary and opposing elements of yin and yang so as to maintain its balance throughout the body. The balance and harmonious flow of *qi* are believed to be the basis of good health [2].

The Western medical community now accepts that acupuncture works, at least for the relief of pain, but it still struggles to translate the mechanism of acupuncture into the Western paradigm. In recent decades, the increasing popularity of Chinese medicine in general and acupuncture in particular among the public around the world has motivated the scientific community to undertake intensive investigation of acupuncture's efficacy, as well as the physiological basis behind it. After decades if not centuries of treating individual problems, the Western medical community is coming to understand that human health is a holistic phenomenon reflecting balance—or imbalance—in the bio-psycho-social-spiritual aspects of life. While this balance reflects complex factors, there are parameters we can measure—there are physical indications—of how an individual responds to a disease state in order to maintain the bio-psycho-social-spiritual balance within our body. Heart rate variability (HRV) is a measure of this kind.

HRV can be measured by time domain analysis or frequency domain analysis as a power spectrum produced by a selected chain of heart to heart beat. Using power spectral analysis [3], Figure 1 shows an estimate of power spectral density obtained from the entire 24-hour interval of a long-term Holter recording. Only the low frequency (LF) and

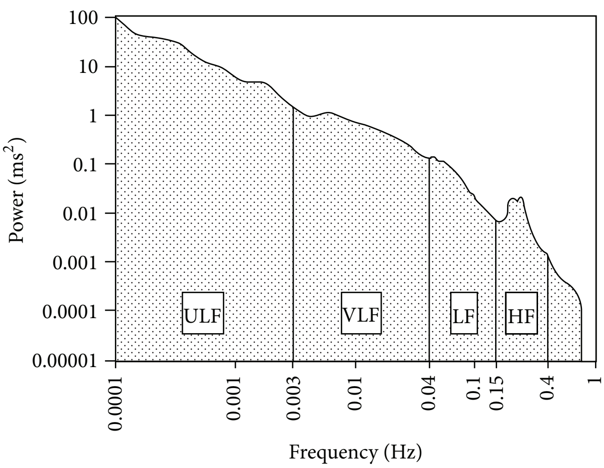

Figure 1: Diagram of the power spectral density heart rate variability (HRV) (adapted from [4]).

high frequency (HF) components correspond to peaks of the spectrum, while the very low frequency (VLF) and ultra-low frequency (ULF) can be approximated by a line in this plot with logarithmic scales on both axes. The slope of such a line is the α measure of HRV [4].

According to [4], VLF, LF, HF, and LF/HF ratios are the common parameters used in assessing the autonomic system (Table 1).

HRV measures the balance of our autonomic nervous system which reflects physiological, hormonal, and emotional balance. The autonomic nervous system is composed of two branches, namely, sympathetic nervous system and parasympathetic nervous system. Sympathetic nervous system is responsible for flight and stress situation while parasympathetic nervous system is dominant when relaxed [5]. Hence, HRV measures the balance of the activities of the sympathetic nervous system and the parasympathetic nervous system. Researchers have hypothesized that acupuncture modulates the autonomic nervous system, thereby revitalizing the balance of metabolism [6–9]. These modulations and revitalizing effects can be measured by HRV.

The autonomic nervous system dynamically controls the response of the body to a range of external and internal stimuli, providing physiological stability [10]. HRV is a measure of the naturally occurring beat-to-beat changes in heart rate [11]. It serves as a critical method for gauging human health and resiliency. HRV measures regulation of the heart by the autonomic nervous system [9]. In other words, HRV analysis reveals the interaction between the sympathetic and parasympathetic activities by modulation of heart beat interval [7].

HRV is an established tool in cardiology research and is being increasingly used for a range of clinical applications [10]. For example, HRV decreases in congestive heart failure [12]. HRV power spectral analysis shows that the LF component diminishes in patients with congestive heart failure, and the decrease is related to an increased risk of sudden death [12]. This was supported by [12] in which they reported that dogs with a reduction in LF power and LF/HF ratio observed in heart failure dogs had diminished sinus node responsiveness to autonomic modulation.

Both timedomain heart rate variability and respiration rate in various positions—for example, lying down in a laboratory, cycling in a laboratory, and sleeping in an ambulatory surrounding—showed high intraclass correlation coefficients [13]. Therefore, HRV is a reliable tool for tracking changes of clinical state.

Acupuncture is generally documented to be effective in treating chronic and acute pain conditions by inserting needles into specific "acupuncture points" (acupoints) on the patient's body [14]. Acupuncture analgesia can be described in technical terms as a manifestation of integrative processes at different levels in the central nervous system between afferent impulses from pain regions and impulses from acupoints. Recent research has suggested different mechanisms for acupuncture. For instance, HRV results from the regulation of the heart by the autonomic nervous system. However, the interrelationship between these mechanisms and how acupuncture affects complex physiological systems is still not understood [9].

Previous studies have showed a relationship between acupuncture and heart rate variability. Wang et al. reported that acupuncture enhances cardiac vagal activity and suppresses sympathetic activities in healthy humans [15]. In other words, the LF decreased. This is consistent with another study also conducted with healthy adults. The study reported that acupuncture significantly increased HF and decreased both the LF/HF ratio and heart rate [16]. The result supports the hypothesis that acupuncture affects parasympathetic activity.

Several studies reported the effect of acupuncture on HRV using specified acupoints. For instance, acupuncture on Neiguan (PC6) and Gongsun (SP4) decreased sympathetic activity and balanced the autonomic nervous system for those who were under stress [17]. Similarly, acupuncture on Neiguan (PC6) and Shenmen (HT7) among patients with poststroke onset insomnia was reported to be effective in that there was a reduction in sympathetic hyperactivities after the acupuncture [18]. A study comparing different acupuncture methods (needle, laser acupuncture, moxibustion, and teleacupuncture) on 72 patients to evaluate the influence of acupuncture on heart rhythm over the short-term and long-term measurements [19]. Significant decreases in heart rate after *verum* intervention at Yintang, Neiguan, and Guanyuan were found. Improvements in state of health following teleacupuncture were shown. HRV analysis was demonstrated to be effective in evaluating the status of health during acupuncture [19]. However, Wright and Aickin reported essentially no support for a relationship between HRV and acupuncture intervention [20]. In another study with 16 acupoints, acupuncture intervention did not show any generalized depressor effect on the autonomic nervous system [21]. To date, only one systematic review evaluated the effect of acupuncture on HRV [8]. Results, yet, were contradictory.

To understand the mechanism of acupuncture, we need to understand how clinical scientific studies were done and how acupuncture established its effects for some specific

Table 1: Summary of the normal values of HRV.

Component	Outcome parameter	Clinical indication
Very low frequency (VLF)	Frequency (0.0033–0.04 Hz)	Indicator of overall activity of various slow sympathetic activities
Low frequency (LF)	Frequency (0.04–0.15 Hz)	Indicator of sympathetic activities with parasympathetic influence when the respiration rate is lower than 7 per minute
High frequency (HF)	Frequency (0.15–0.4 Hz)	Indicator of parasympathetic activities
LF/HF ratio	1.5–2.0	Indicator of sympathetic activities; a higher number means increased sympathetic activities or reduced parasympathetic activities
sNormalized LF and HF (LF norm, HF norm)	Frequency (Hz)	The monotonic nonlinear transformation of LF/HF ratio. The LF/HF ratio, LF norm, and HF norm indicate the same aspects of sympathovagal balance of autonomic nervous system

Table 2: Inclusion criteria of the RCTs.

Component	Criteria
Participant	Adults (aged ≥18) were treated with needle acupuncture with or without electric stimulation
Intervention	Trials employed acupuncture as the sole treatment
Comparator	Trials compared needle acupuncture with any type of sham acupuncture or no treatment (control)
Outcome measures	Trials used spectral analysis of HRV as the outcome measure

health conditions. A well-designed trial, therefore, isolates the specific treatment variable to see what effect it has. To this end, a systematic review investigating (a) the effect of acupuncture on HRV and (b) the potential for using HRV as an indicator of the therapeutic effect of acupuncture was conducted in this study.

2. Methods and Materials

2.1. Study Design. It was a systematic review with meta-analysis.

2.2. Inclusion and Exclusion Criteria. In this research, randomized control trials (RCTs) were included. Table 2 shows the inclusion criteria of the RCTs that were selected.

There was no language restriction and only papers with fulltext were included for analysis. Studies were excluded if trials testing forms of acupuncture other than needle acupuncture, for example, laser acupuncture or moxibustion; and, if there was only one acupuncture session reported in the studies.

2.3. Search Strategy: Keywords and Database. The following keywords were used in the database search: acupuncture OR meridian OR acupoint AND heart rate variability or HRV or heart rate. For Chinese databases, keywords in Chinese used were *acupuncture OR meridian OR acupoint OR electroacupuncture AND Heart rate OR heart rate variability.*

Databases searched were CINAHL (1937-), EMbase (1980-), PsycInfo (1597-), Cochrane Central Register of Controlled Trials (1898-), Ovid Medline (1950-), Korean Studies Info Services System, and China Academic Journals Fulltext Database (CAJ) (1915-).

2.4. Study Identification. The initial search yielded 94, 848, 30, 155, 89, 3, and 1 paper from CINAHL, EMbase, PsycInfo, Cochrane Central Register of Controlled Trials, Ovid Medline, Korean Studies Info Services System, and CAJ, respectively. Then, the researcher screened the title and the abstract to determine the relevancy. As a result, 1,220 articles were found and comprised the initial data extraction.

2.5. Data Extraction. The researcher designed a data extraction form, and the two independent reviewers completed the data extraction according to the topics predetermined in the form (Table 3). These topics were (1) first author and year, (2) design, sample size, conditions, (3) intervention, (4) heart rate variability, (5) main results and intergroup differences, (6) respiratory regulation, measuring position, measuring time, and (7) risk of bias [8].

2.6. Assessment of the Risk of Bias. Cochrane criteria were used to assess the risk of bias of the studies [34]. The assessment comprised evaluation of randomization, blinding, withdrawals, and blinding and allocation concealment. As pointed out by [8], it is technically difficult to blind the therapist in the application of acupuncture; therefore, the researcher divided "blinded" into "blinded patients" (which is possible) and "blinded therapist" (which is nearly impossible).

Hard copies of all the articles with full text were printed and studied by two independent reviewers. The two independent reviewers agreed on the assessment of risk of bias. The results of the two reviewers were the same, and the results are shown in Table 3.

2.7. Data Synthesis. Chi-squared test and Higgins I^2 tests were used to evaluate heterogeneity. To compare the mean changes in the HRV parameters, the researcher computed the weighted mean differences (WMDs) for outcome on the same scale and standardized mean differences (SMDs) for outcomes on a different scale. A summary of estimates of the treatment effect was calculated using the more conservative approach of a random effects model. Forest plots were done

Table 3: Summary of extracted RCTs.

Authors (year) and origin	Design sample size Conditions	Intervention (regimen)	Heart rate variability	Main results of intergroup differences	Risk of bias
Kwak et al. [22], South Korea	Parallel; 42 healthy students with examination stress	(A) AT ($n = 14$); (B) sham Tx (relaxation therapy, $n = 14$); (C) Co (no Tx, $n = 14$)	(1) HRT (beats/min); (2) SDNN (ms); (3) SDSD (ms); (4) LF norm (n.u.); (5) HF norm (n.u.); (6) LF/HF	(1) A$\rightarrow$, B$\rightarrow$, C$\downarrow$ when comparing between examination and normal time: $P < 0.05$; (1), (4), and (6) A$\downarrow$, B$\rightarrow$, C$\rightarrow$ before and after examination: $P < 0.05$; (2) A$\uparrow$, B$\rightarrow$, C$\rightarrow$ at normal time: $P < 0.05$; (5) A$\uparrow$, B$\rightarrow$, C$\rightarrow$ before and after examination: $P < 0.05$	U, U, U, U, U
Huang et al. [23], Taiwan	Parallel; 111 healthy subjects	(A) AT (P6, 20 min on both forearms, $n = 39$); (B) sham AT (sham acupuncture at dummy points, 20 min, $n = 38$); (C) Co: no Tx, $n = 34$)	(1) Mean RR interval (ms); (2) SD of RR interval (ms); (3) coefficient of variation (%); (4) HF norm (nu); (5) LF norm (nu); (6) LF/HF	(1) A$\uparrow$, B$\uparrow$, C$\rightarrow$: $P < 0.05$; (4) A$\uparrow$, B$\rightarrow$, C$\rightarrow$: $P < 0.05$	U, N, N, N, N
Chang et al. [24], Taiwan	Crossover, 15 healthy subjects	(A) EA (2 Hz, 30 min, $n = 15$); (B) Sham EA (minimal penetration on nonacupuncture point, no electric stimulation, $n = 15$); (C) EA plus atropine injection; sessions A, B, C in a randomized order with 3 days between sessions	(1) LF/HF; (2) LF; (3) HF	(1) A$\uparrow$, B$\rightarrow$: NS; (2), (3) A$\rightarrow$, B$\rightarrow$: NS	U, N, Y, U, U
Chang et al. [25], Taiwan	Crossover, 15 healthy subjects	(A) EA (2 Hz at Zusanli, 30 min, $n = 15$); (B) EA (2 Hz at Shousanli, 30 min, $n = 15$); (C) EA (100 Hz at Zusanli, 30 min, $n = 15$); (D) EA (100 Hz at Shousanli, 30 min, $n = 15$); (E) sham EA (minimal penetration on nonacupuncture point, no electric stimulation, $n = 15$); Sessions in a randomized order with 3 days between sessions	(1) LF/HF; (2) LF; (3) HF	(1) A$\uparrow$ during and after EA, B$\rightarrow$, C$\rightarrow$, D$\rightarrow$, E$\rightarrow$: NS; (2) A$\uparrow$ during and after EA, B$\rightarrow$, C$\rightarrow$, D$\rightarrow$, E$\rightarrow$: NS; (3) A$\downarrow$ during and after EA, B$\rightarrow$, C$\rightarrow$, D$\rightarrow$, E$\rightarrow$: NS	U, N, Y, U, U
Hsu et al. [26], Taiwan	Crossover, 10 healthy subjects	(A) EA (2 Hz at BL15, 10 min, $n = 10$); (B) sham EA ($n = 10$)	(1) LF norm; (2) HF norm; (3) LF/HF; (4) HRT	(1)–(4) A$\downarrow$, B$\rightarrow$: $P < 0.05$	U, N, U, U, U
Hsu et al. [27], Taiwan	Crossover, 10 healthy subjects	(A) AT (scalp-Sishencong, 10 min, $n = 10$); (B) AT (Auricular-Shenmen, 10 min, $n = 10$); (C) sham AT ($n = 10$)	(1) LF norm; (2) HF norm; (3) LF/HF; (4) HRT	(1)–(4) A$\downarrow$, B$\uparrow$, C$\rightarrow$: $P < 0.05$	U, N, U, U, U

Table 3: Continued.

Authors (year) and origin	Design sample size Conditions	Intervention (regimen)	Heart rate variability	Main results of intergroup differences	Risk of bias
Kim et al. [28], South Korea	Crossover, 38 female subjects with regular menstrual cycle	(A) AT (LI4 and SP6, 15 min, $n = 38$); (B) sham AT (minimal penetration on the same acupoints, 15 min, $n = 38$); One-month washout between Tx	(1) LF; (2) HF; (3) LF/HF	(1) A↑, B↑: NS; (2) A↑, B↑: $P < 0.001$ and <0.005; (3) A↓, B↓: $P = 0.001$ and NS	U, N, Y, U, U
Streitberger et al. [16], Germany	Crossover, 20 healthy subjects	(A) AT (LI4, 5 min and 15 sec manual stimulation, $n = 20$); (B) sham AT (blunted placebo needle with no penetration on the same acupoints, 5 min and 15 sec manual stimulation, $n = 20$)	(1) LF; (2) HF; (3) LF/HF	(1) and (2) A →, B →: NS; (3) A →, B →: NS, but A↑ during stimulation: NS	Y, N, Y, Y, U
Li et al. [29], China	Parallel, 29 male healthy subjects (after 3 hr driving workout)	(A) AT (15 min, $n = 10$); (B) sham AT (minimal penetration on nonacupuncture points, $n = 10$); (C) Co (with driving, 15 min, $n = 9$)	(1) LF/HF; (2) LF norm; (3) HF norm	(1)-(2) A↓, B →: $P < 0.05$; (3) A↑, B →: $P < 0.05$	U, N, Y, U, U
Chang et al. [30], Taiwan	Parallel, 12 male healthy subjects with no neurological diseases	(A) AT (PC6, 3 sessions with one-week washout, 30 min, $n = 6$); (B) sham AT (minimal penetration on nonacupuncture points, 3 sessions with one-week washout, 30 min, $n = 6$)	(1) VLF; (2) LF; (3) HF; (4) LF/HF	NS for all cases	U, N, U, U, U
Kurono et al. [31], Japan	Crossover, 14 male healthy subjects	(A) AT (epifascial stimulation at CV17 and CV16, needle inserted for 1s only, $n = 14$); (B) sham AT (AT on nonacupoints, $n = 14$)	(1) LF; (2) HF; (3) LF/HF	(1)-(2) A↑ in CV17, but not CV16; (3) NS for both CV17 and CV16	U, N, Y, U, U
Liu et al. [32], USA	1st phase (acute); Crossover, 27 patients with functional dyspepsia (Rome II)	(A) TEA (25 Hz, PC6 and ST36, 30 min, 2 sessions, $n = 27$); (B) Sham TEA (TEA was performed on sham acupoints, 30 min, 2 sessions, $n = 27$); (C) Co (electrodes were placed in the acupoints but not turned on, $n = 27$)	(1) HF; (2) LF/HF	(1) A↑ during 1st 30 min Tx, B →, C →: $P = 0.01$; (2) A↓, B →, C →: $P < 0.05$	Y, N, U, U, U
Liu et al. [32], USA	2nd phase (chronic); Crossover, 27 patients with functional dyspepsia (Rome II)	(A) TEA for 2 weeks (twice daily, 30 min), 1 week-wash-out, sham TEA for 2 weeks; (B) viceversa	(1) HF; (2) LF/HF	(1) A↑ after 2 weeks, B →, C →: $P = 0.05$; (2) A↓, B →, C →: $P < 0.05$	Y, N, Y, Y, U
Shi et al. [33], China	Crossover, 20 patients with coronary heart disease	(A) AT (30 min, $n = 20$); (B) EA (30 min, $n = 20$); (C) Co (no Tx, $n = 20$)	(1) LF; (2) HF; (3) LF/HF	(1) A↓, B↓, C →: $P < 0.05$; (2) NS for all groups; (3) A →, B↓, C →: $P < 0.05$	U, N, U, U, U

AT: acupuncture treatment; EA: electroacupuncture; TEA: transcutaneous electroacupuncture; Co: control; Tx: treatment.
Risk of bias (1) sequence generation; (2) incomplete data; (3) patient-blinded; (4) assessor blinded; (5) allocation concealment performed.

to display the treatment effect visually. The analysis was conducted using Revman 5.0 software.

3. Results

3.1. Study Description. Using the keyword search, the researcher identified 1220 articles initially. Upon reading the abstracts, 1133 articles were excluded because they were not related to acupuncture or HRV. The remaining 87 articles in full text were then reviewed by the two reviewers. Seventy-three articles were excluded because of non-RCTs and incomplete outcome measures. Fourteen studies were included in the analysis (Figure 2). Among the 14 studies, 2 were from South Korea, 6 from Taiwan, 2 from USA, 1 from Germany, 2 from China, and 1 from Japan. Slightly less than a quarter of them (10/14 = 71.42%) were of crossover design, and 4 were of parallel design. Eleven studies involved healthy subjects, 2 involved patients with functional dyspepsia, and 1 involved patients with coronary heart disease. All studies used LF, HF, and LF/HF as their outcome measures; some had LF norm and HF norm. Therefore, these measures were used in the meta-analysis as long as data were sufficient for computation.

3.2. Risk of Bias. For risk of bias evaluation, the researcher used the following criteria: sequence generation, incomplete data, patient-blinded, assessor-blinded, and allocation concealment (Table 3). These criteria were evaluated and rated as unknown (U), criteria met (Y), and criteria not met (N). For sequence generation, 11 (78.57%) studies rated U and 3 rated Y (21.43%). For incomplete data, 11 studies rated U (78.57%) and 3 rated Y (21.43%). For patient-blinded, 11 studies rated U (78.57%) and 3 rated Y (21.43%). For assessor blinded, 11 studies rated U (78.57%), 1 rated N (7.14%), and 2 rated Y (14.29%). For allocation concealment, 3 studies (21.43%) rated U, 1 rated N (7.14%) and 10 rated Y (71.49%). None of the studies scored Y for all criteria while 1 study got all U. Figure 3 shows the results of risk of bias analysis. The studies without patient-blinded, assessor-blinded or allocation concealment were subjected to high risk of bias.

3.3. Outcome Measures. Fourteen studies meeting the selection criteria were included in the study. It is noted that some of the included studies had more than one group, that is, acupuncture, sham, and control. In view of the fact that all the tests in the groups had the same outcome measures, the researcher entered the outcome measures of the respective group as one study ID in the Revman 5.0. Six outcome measures were input for analysis, namely, HF, LF and LF/HF ratio, HF norm, and LF norm. Thus, there may be more than one study ID with the same author and year entry in the subsequent meta-analysis for one included study.

3.4. Healthy Subject Studies

3.4.1. High Frequency (7 Studies). Seven studies were included in this part of the analysis, corresponding to 16 study IDs, as explained in the previous section (Figure 4). There were 183 subjects in acupuncture group and 183 in sham/control group. The heterogeneity (χ^2 = 16.99, df = 11, P = 0.11) was not significant while the I^2 was 35% which means low heterogeneity. The overall effect was insignificant (Z = 1.02, P = 0.31). All the 95% CI of the WMDs of the studies and the overall effect (diamond) crossed the line of no effect.

3.4.2. Low Frequency (6 Studies). Six studies were evaluated in this section of the study, while there were 13 study IDs in this analysis (Figure 5). There were 163 subjects in acupuncture group and 163 in sham/control group. The heterogeneity (χ^2 = 47.44, df = 10, P < 0.00) was significant while the I^2 was 79% which means high heterogeneity. Though the heterogeneity was high, the overall effect of the acupuncture in the included studies was insignificant (Z = 0.72, P = 0.47). All the 95% CI of the weighted mean difference of the studies and the overall effect (diamond) crossed with line of no effect.

3.4.3. LF/HF Ratio (9 Studies). Nine studies were evaluated in this section of the study; for these 9 studies, there were 13 study IDs (Figure 6). There were a total of 206 subjects in the acupuncture groups of these studies and a total of 205 in the sham/control groups. The heterogeneity (χ^2 = 913.49, df = 12, P < 0.00) was significant while the I^2 was 99% which was high heterogeneity. The overall effect was not significant (Z = 1.26, P = 0.21). Results in Chang et al. [24] and Streitberger et al. [16] did not support that effect of acupuncture while Hsu et al. [27], Hsu et al. [27], Kim et al. [28], and Li et al. [29] did. However, the overall effect (diamond) was insignificant and crossed the line of no effect.

3.4.4. HF Norm (5 Studies). Five studies were evaluated in this section of the study, corresponding to 8 study IDs (Figure 7). There were 121 subjects in acupuncture group and 120 in sham/control group. The heterogeneity (χ^2 = 32.35, df = 7, P < 0.00) was significant while the I^2 was 78% which means high heterogeneity. The overall effect was significant (Z = 5.00, P < 0.00). Hsu et al. [26], Hsu et al. [27], Hsu et al. [27] did not support the effect of acupuncture on HF norm, and the overall effect was significant and was in favour of the sham/control group with decreased HF norm as shown in the WMDs.

3.4.5. LF Norm (5 Studies). Five studies were included while there were 8 study IDs in this analysis (Figure 8). There were 121 subjects in acupuncture group and 120 in sham/control group. The heterogeneity (χ^2 = 15.39, df = 7, P = 0.03) was significant while the I^2 was 55% which means moderate heterogeneity. The overall effect was significant (Z = 4.03, P < 0.00). Results showed a decreased effect of acupuncture on LF norm [26, 27] while the rest showed no effect. The overall effect (diamond) supported the conclusion that acupuncture had a significant effect on LF norm with a decreased magnitude in the WMDs.

3.4.6. ST36 on HF (2 Studies). Two studies were included while there were 3 study IDs in this analysis (Figure 9). There were 45 subjects in acupuncture group and 45 in

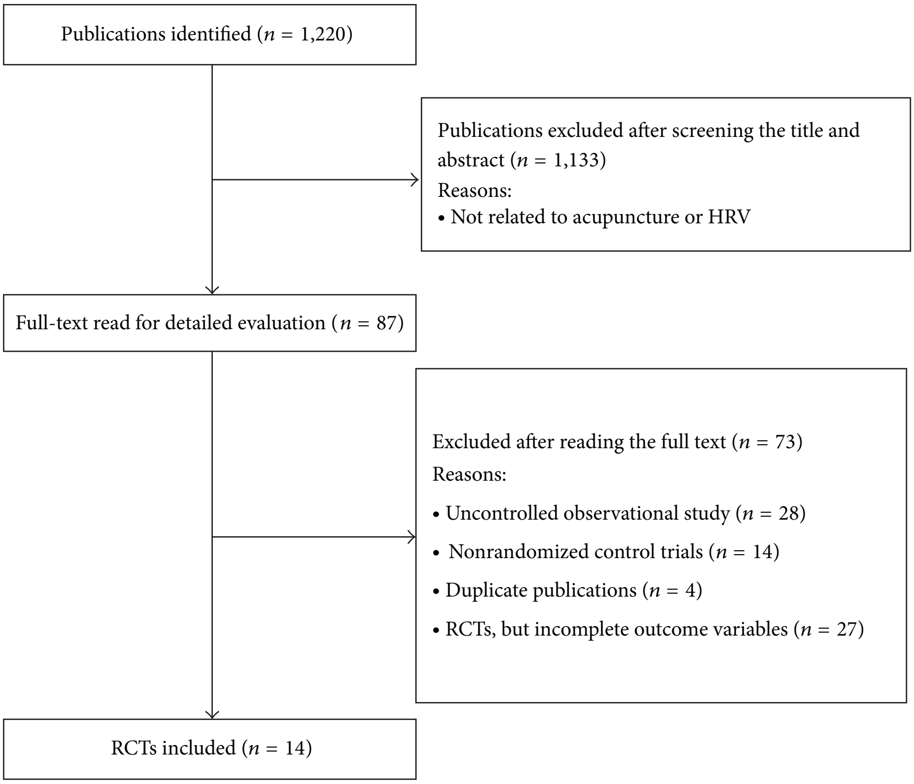

Figure 2: Process of studies selection.

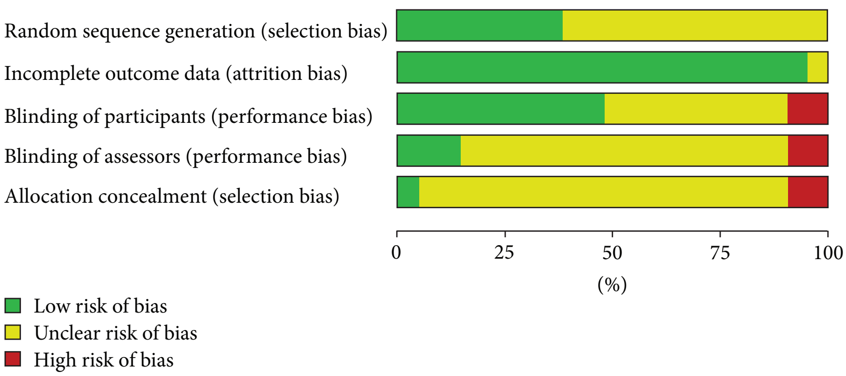

Figure 3: Results of risk of bias analysis.

sham/control group. The heterogeneity (χ^2 = 0.49, df = 2, P = 0.78) was not significant while the I^2 was 0% which means no heterogeneity. The overall effect was significant (Z = 3.00, P = 0.003). Results showed a decreased effect of acupuncture at ST36 on HF while the rest showed no effect. The overall effect (diamond) supported significant effect acupuncture of ST36 on HF and showed a decreased magnitude in the WMDs.

3.5. Nonhealthy Subject Studies

3.5.1. High Frequency (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 10). There were 94 subjects in acupuncture group and 94 in sham/control group. The heterogeneity (χ^2 = 24.31, df = 3, $P < 0.00$) was significant while the I^2 was 88% which means high heterogeneity. The overall effect was significant (Z = 5.25, $P < 0.00$). Results of the WMDs showed a significant decrease of HF in the sham/control group [32, 33]. The overall effect (diamond) favoured the control; thus, effect of acupuncture on HF was not supported.

3.5.2. Low Frequency (1 Study). One study was included while there were 2 study IDs in this analysis (Figure 11). There were 40 subjects in acupuncture group and 40 in sham/control group. The heterogeneity (χ^2 = 0.15, df = 1, P = 0.70) was not significant while the I^2 was 0% because there was only one

Study or subgroup	Acupuncture Mean (ms)	Acupuncture SD (ms)	Acupuncture Total	Sham treatment/control Mean (ms)	Sham treatment/control SD (ms)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [24]	1,019.5	140.2	15	1,145.2	163.3	15	0.0%	−125.70 [−234.62, −16.78]
Chang et al. [30]	0.2025	0.1347	6	0.169	0.097	6	35.3%	0.03 [−0.10, 0.17]
Chang et al. [30]	0.2208	0.2312	6	0.1647	0.1348	6	28.9%	0.06 [−0.16, 0.27]
Chang et al. [25]	1,017.4	138.2	15	1,088.5	163.8	15	0.0%	−71.10 [−179.55, 37.35]
Chang et al. [25]	978.1	137.9	15	1,088.5	163.8	15	0.0%	−110.40 [−218.76, −2.04]
Chang et al. [25]	985.9	177.4	15	1,088.5	163.8	15	0.0%	−102.60 [−224.79, 19.59]
Chang et al. [25]	1,020.6	175.5	15	1,088.5	163.8	15	0.0%	−67.90 [−189.39, 53.59]
Kim et al. [28]	806.73	119.12	38	806	150.26	38	0.0%	0.73 [−60.24, 61.70]
Kurono et al. [31]	5.1	1.2	14	5	1	14	5.7%	0.10 [−0.72, 0.92]
Kurono et al. [31]	5.3	1.3	14	4.8	1.1	14	4.9%	0.50 [−0.39, 1.39]
Li et al. [29]	569	133	10	578	244	10	0.0%	−9.00 [−181.24, 163.24]
Streitberger et al. [16]	0.2	0.18	20	−0.01	0.57	20	25.2%	0.21 [−0.05, 0.47]
Total (95% CI)			**183**			**183**	**100.0%**	**0.11 [−0.10, 0.32]**

Heterogeneity: $\tau^2 = 0.03$; $\chi^2 = 16.99$, df = 11 ($P = 0.11$); $I^2 = 35\%$

Test for overall effect: $Z = 1.02$ ($P = 0.31$)

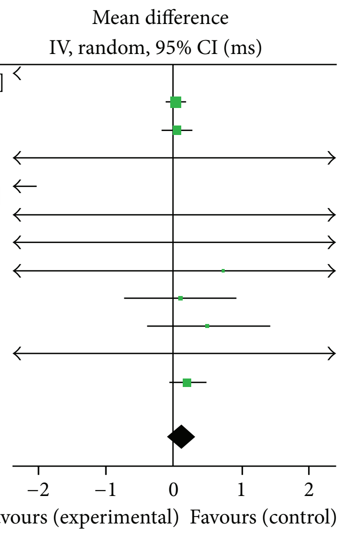

Figure 4: Forest plot of the effects of acupuncture on HF for healthy subjects.

Study or subgroup	Acupuncture Mean (ms)	Acupuncture SD (ms)	Acupuncture Total	Sham treatment/control Mean (ms)	Sham treatment/control SD (ms)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [24]	1,446.8	360.2	15	1,396.2	380.1	15	0.0%	50.60 [−214.40, 315.60]
Chang et al. [30]	0.3592	0.1229	6	0.4513	0.1611	6	29.4%	−0.09 [−0.25, 0.07]
Chang et al. [30]	0.3907	0.1092	6	0.3495	0.0357	6	30.4%	0.04 [−0.05, 0.13]
Chang et al. [25]	1,450.9	359.9	15	1,491.7	371.9	15	0.0%	−40.80 [−302.70, 221.10]
Chang et al. [25]	1,341.6	372.7	15	1,491.7	371.9	15	0.0%	−150.10 [−416.55, 116.35]
Chang et al. [25]	1,094.4	253.6	15	1,491.7	371.9	15	0.0%	−397.30 [−625.10, −169.50]
Chang et al. [25]	1,510.5	371.9	15	1,491.7	371.9	15	0.0%	18.80 [−247.36, 284.96]
Kim et al. [28]	694.07	111.32	38	609.41	60.99	38	0.0%	84.66 [44.30, 125.02]
Kurono et al. [31]	6	0.8	14	5.9	0.6	14	20.5%	0.10 [−0.42, 0.62]
Kurono et al. [31]	6.2	0.8	14	5.5	0.7	14	19.7%	0.70 [0.14, 1.26]
Li et al. [29]	851	510	10	1,692	694	10	0.0%	−841.00 [−1374.79, −307.21]
Total (95% CI)			**163**			**163**	**100.0%**	**0.15 [−0.26, 0.56]**

Heterogeneity: $\tau^2 = 0.14$; $\chi^2 = 47.44$, df = 10 ($P < 0.00001$); $I^2 = 79\%$

Test for overall effect: $Z = 0.72$ ($P = 0.47$)

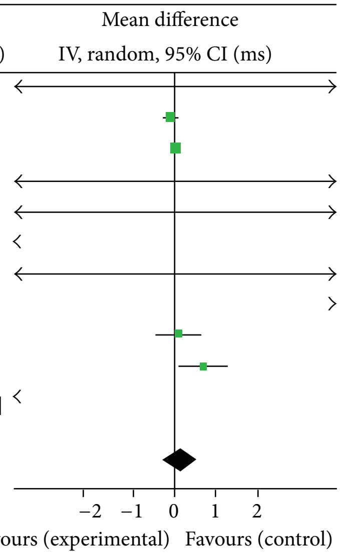

Figure 5: Forest plot of the effects of acupuncture on LF for healthy subjects.

study. The overall effect was significant ($Z = 2.04$, $P = 0.04$). The results of the WMDs [33] showed effect of a decreased LF. The overall effect (diamond) was marginally significant.

3.5.3. LF/HF Ratio (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 12). There were 94 subjects in acupuncture group and 94 in sham/control group. The heterogeneity ($\chi^2 = 0.75$, df = 3, $P = 0.86$) was not significant while the I^2 was 0%. The overall effect was significant ($Z = 15.82$, $P < 0.00$). Results of the WMDs and the overall effect (diamond) showed a significant effect of acupuncture on LF/HF ratio as in Liu et al. [32]. The LF/HF ratio was decreased.

3.5.4. PC6 on HF (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 13). There were 94 subjects in acupuncture group and 94 in sham/control group. The heterogeneity ($\chi^2 = 24.31$, df = 3, $P < 0.00$) was significant while the I^2 was 88%. It showed high heterogeneity. The overall effect was significant ($Z = 5.25$, $P < 0.00$). With reference to WMDs, the significant effect of sham at PC6 on decreased HF was reported, not on the acupuncture group.

3.5.5. PC6 on LF/HF (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 14). There were 94 subjects in acupuncture group and 94 in

Study or subgroup	Acupuncture Mean	Acupuncture SD	Acupuncture Total	Sham treatment/control Mean	Sham treatment/control SD	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI
Chang et al. [24]	1.51	0.28	15	1.17	0.32	15	9.0%	0.34 [0.12, 0.56]
Chang et al. [30]	2.8614	1.8839	6	2.6314	1.3708	6	3.1%	0.23 [−1.63, 2.09]
Chang et al. [30]	3.879	4.7606	6	3.9538	2.8528	6	0.7%	−0.07 [−4.52, 4.37]
Hsu et al. [26]	0.31	0.01	10	2.84	0.27	10	9.1%	−2.53 [−2.70, −2.36]
Hsu et al. [27]	0.22	0.12	10	0.52	0.02	10	9.2%	−0.30 [−0.38, −0.22]
Hsu et al. [27]	0.21	0.23	10	0.52	0.02	10	9.1%	−0.31 [−0.45, −0.17]
Huang et al. [23]	1.7	2.3	39	1.3	0.9	38	6.9%	0.40 [−0.38, 1.18]
Kim et al. [28]	1.05	0.12	38	1.12	0.11	38	9.2%	−0.07 [−0.12, −0.02]
Kwak et al. [22]	0.62	0.3	14	0.58	0.21	14	9.1%	0.04 [−0.15, 0.23]
Kwak et al. [22]	0.72	0.35	14	0.6	0.43	14	8.8%	0.12 [−0.17, 0.41]
Kwak et al. [22]	0.61	0.27	14	0.54	0.21	14	9.1%	0.07 [−0.11, 0.25]
Li et al. [29]	1.49	0.42	10	2.92	0.86	10	7.7%	−1.43 [−2.02, −0.84]
Streitberger et al. [16]	0.52	0.37	20	−0.14	0.25	20	9.0%	0.66 [0.46, 0.86]
Total (95% CI)			**206**			**205**	**100.0%**	**−0.26 [−0.66, 0.14]**

Heterogeneity: $\tau^2 = 0.45$; $\chi^2 = 913.49$, df = 12 ($P < 0.00001$); $I^2 = 99\%$
Test for overall effect: $Z = 1.26$ ($P = 0.21$)

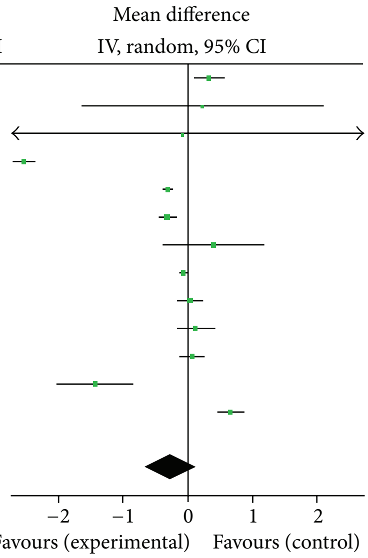

Figure 6: Forest plot of the effects of acupuncture on LF/HF ratio for healthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	Acupuncture SD (n.u.)	Acupuncture Total	Sham treatment/control Mean (n.u.)	Sham treatment/control SD (n.u.)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Hsu et al. [26]	0.7	0.15	10	0.18	0.07	10	32.7%	0.52 [0.42, 0.62]
Hsu et al. [27]	0.64	0.14	10	0.18	0.06	10	33.1%	0.46 [0.37, 0.55]
Hsu et al. [27]	0.68	0.12	10	0.18	0.06	10	33.7%	0.50 [0.42, 0.58]
Huang et al. [23]	30.7	14.1	39	31.8	13.8	38	0.1%	−1.10 [−7.33, 5.13]
Kwak et al. [22]	60.05	9.59	14	66.45	12.62	14	0.1%	−6.40 [−14.70, 1.90]
Kwak et al. [22]	63.62	9.52	14	66.48	8.74	14	0.1%	−2.86 [−9.63, 3.91]
Kwak et al. [22]	64.3	10.78	14	65.09	8.02	14	0.1%	−0.79 [−7.83, 6.25]
Li et al. [29]	38	5.76	10	24.34	5.44	10	0.2%	13.66 [8.75, 18.57]
Total (95% CI)			**121**			**120**	**100.0%**	**0.51 [0.31, 0.70]**

Heterogeneity: $\tau^2 = 0.03$; $\chi^2 = 32.35$, df = 7 ($P < 0.0001$); $I^2 = 78\%$
Test for overall effect: $Z = 5.00$ ($P < 0.00001$)

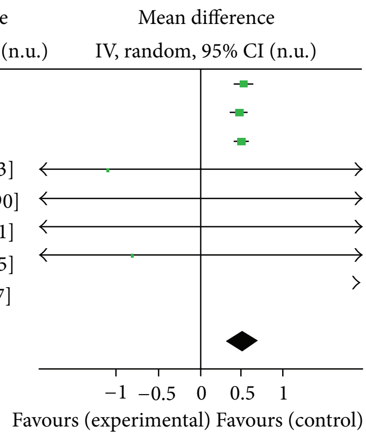

Figure 7: Forest plot of the effects of acupuncture on HF norm for healthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	Acupuncture SD (n.u.)	Acupuncture Total	Sham treatment/control Mean (n.u.)	Sham treatment/control SD (n.u.)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Hsu et al. [26]	0.22	0.23	10	0.5	0.03	10	29.6%	−0.28 [−0.42, −0.14]
Hsu et al. [27]	0.22	0.07	10	0.52	0.02	10	40.4%	−0.30 [−0.35, −0.25]
Hsu et al. [27]	0.21	0.23	10	0.52	0.02	10	29.7%	−0.31 [−0.45, −0.17]
Huang et al. [23]	34.4	12.1	39	31.5	10.4	38	0.1%	2.90 [−2.14, 7.94]
Kwak et al. [22]	40.01	10.58	14	34.84	12.17	14	0.0%	5.17 [−3.28, 13.62]
Kwak et al. [22]	36.61	11.01	14	36.42	7.68	14	0.0%	0.19 [−6.84, 7.22]
Kwak et al. [22]	36.76	10.56	14	34.33	7.33	14	0.0%	2.43 [−4.30, 9.16]
Li et al. [29]	58.92	6.33	10	72.5	10.65	10	0.0%	−13.58 [−21.26, −5.90]
Total (95% CI)			**121**			**120**	**100.0%**	**−0.30 [−0.44, −0.15]**

Heterogeneity: $\tau^2 = 0.01$; $\chi^2 = 15.39$, df = 7 ($P = 0.03$); $I^2 = 55\%$
Test for overall effect: $Z = 4.03$ ($P < 0.0001$)

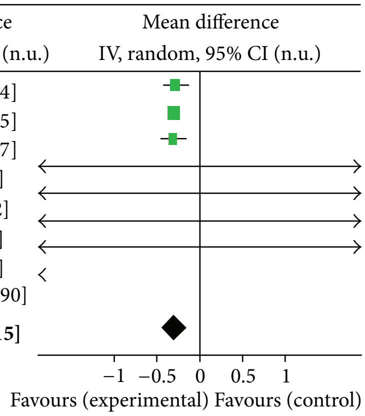

Figure 8: Forest plot of the effects of acupuncture on LF norm for healthy subjects.

Study or subgroup	Acupuncture			Sham treatment/control			Weight	Mean difference
	Mean (ms)	SD (ms)	Total	Mean (ms)	SD (ms)	Total		IV, random, 95% CI (ms)
Chang et al. [24]	1,019.5	140.2	15	1,145.2	163.3	15	35.7%	−125.70 [−234.62, −16.78]
Chang et al. [25]	1,017.4	138.2	15	1,088.5	163.8	15	36.0%	−71.10 [−179.55, 37.35]
Chang et al. [25]	985.9	177.4	15	1,088.5	163.8	15	28.3%	−102.60 [−224.79, 19.59]
Total (95% CI)			**45**			**45**	**100.0%**	**−99.51 [−164.56, −34.45]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.49$, df = 2 ($P = 0.78$); $I^2 = 0\%$

Test for overall effect: $Z = 3.00$ ($P = 0.003$)

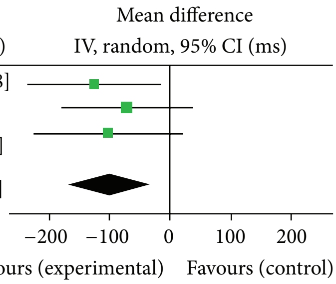

Figure 9: Forest plot of the effects of acupuncture at ST36 on HF for healthy subjects.

Study or subgroup	Acupuncture			Sham treatment/control			Weight	Mean difference
	Mean (ms)	SD (ms)	Total	Mean (ms)	SD (ms)	Total		IV, random, 95% CI (ms)
Liu et al. [32]	0.08	0.02	27	0.02	0.01	27	49.7%	0.06 [0.05, 0.07]
Liu et al. [32]	0.13	0.02	27	0.04	0.01	27	49.7%	0.09 [0.08, 0.10]
Shi et al. [33]	1.02	0.84	20	0.93	0.4	20	0.5%	0.09 [−0.32, 0.50]
Shi et al. [33]	1.03	1.49	20	0.93	0.4	20	0.2%	0.10 [−0.58, 0.78]
Total (95% CI)			**94**			**94**	**100.0%**	**0.08 [0.05, 0.10]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 24.31$, df = 3 ($P < 0.0001$); $I^2 = 88\%$

Test for overall effect: $Z = 5.25$ ($P < 0.00001$)

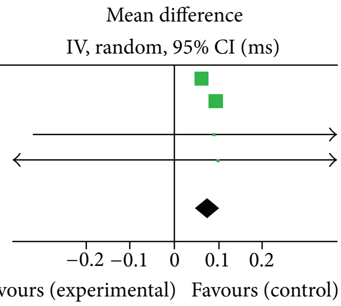

Figure 10: Forest plot of the effects of acupuncture on HF for nonhealthy subjects.

sham/control group. The heterogeneity ($\chi^2 = 0.75$, df = 3, $P = 0.86$) was not significant while the I^2 was 0%. It showed no heterogeneity. The overall effect was significant ($Z = 15.83$, $P < 0.00$). With reference to WMDs, the significant effect of acupuncture at PC6 on decreased LF/HF was reported.

3.6. Combined Analysis for Healthy and Nonhealthy Subjects Studies

3.6.1. LI4 on High Frequency (2 Studies). Two studies were included while there were 2 study IDs in this analysis (Figure 15). There were 58 subjects in acupuncture group and 58 in sham/control group. The heterogeneity ($\chi^2 = 0.00$, df = 1, $P = 0.99$) was not significant while the I^2 was 0%. It showed no heterogeneity. The overall effect was significant ($Z = 1.57$, $P = 0.12$) on sham/control only which was contributed by Kim et al. [28] and Streitberger et al. [16]. Results did not support the effect of acupuncture at LI4 on decreased HF.

3.6.2. LI4 on LF/HF Ratio (4 Studies). Four studies were included while there were 6 study IDs in this analysis (Figure 16). There were 110 subjects in acupuncture group and 110 in sham/control group. The heterogeneity ($\chi^2 = 73.89$, df = 5, $P < 0.00$) was significant while the I^2 was 93%. It showed high heterogeneity. The overall effect was not significant ($Z = 0.04$, $P = 0.97$). Results did not support the effect of acupuncture at LI4 on decreased LF/HF ratio.

3.6.3. LI4 on HF Norm (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 17). There were 52 subjects in acupuncture group and 52 in sham/control group. The heterogeneity ($\chi^2 = 26.62$, df = 3, $P < 0.00$) was significant while the I^2 was 89%. It showed high heterogeneity. The overall effect was not significant ($Z = 0.24$, $P = 0.81$). Results did not support the effect of acupuncture at LI4 on decreased HF norm.

3.6.4. LI4 on LF Norm (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 18). There were 52 subjects in acupuncture group and 52 in sham/control group. The heterogeneity ($\chi^2 = 13.43$, df = 3, $P = 0.004$) was significant while the I^2 was 78%. It showed moderate heterogeneity. The overall effect was not significant ($Z = 0.36$, $P = 0.72$). Results did not support the effect of acupuncture at LI4 on decreased LF norm.

3.6.5. PC6 on HF (3 Studies). Three studies were included while there were 6 study IDs in this analysis (Figure 19). There were 106 subjects in acupuncture group and 106 in sham/control group. The heterogeneity ($\chi^2 = 24.71$, df = 5, $P = 0.0002$) was significant while the I^2 was 80%. It showed high heterogeneity. The overall effect was significant ($Z = 5.47$, $P < 0.00$). With reference to WMDs, the significant effect of acupuncture at PC6 on sham decreased HF was contributed by Chang et al. [30], Liu et al. [32], and Shi et al. [33].

Study or subgroup	Acupuncture Mean (ms)	Acupuncture SD (ms)	Acupuncture Total	Sham treatment/control Mean (ms)	Sham treatment/control SD (ms)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (ms)
Shi et al. [33]	2.2	1.42	20	2.99	2.79	20	47.4%	−0.79 [−2.16, 0.58]
Shi et al. [33]	1.83	1.03	20	2.99	2.79	20	52.6%	−1.16 [−2.46, 0.14]
Total (95% CI)			**40**			**40**	**100.0%**	**−0.98 [−1.93, −0.04]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.15$, df = 1 ($P = 0.70$); $I^2 = 0\%$

Test for overall effect: $Z = 2.04$ ($P = 0.04$)

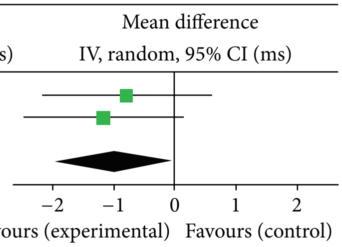

Figure 11: Forest plot of the effects of acupuncture on LF for nonhealthy subjects.

Study or subgroup	Acupuncture Mean	Acupuncture SD	Acupuncture Total	Sham treatment/control Mean	Sham treatment/control SD	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI
Liu et al. [32]	0.58	0.15	27	1.09	0.18	27	49.4%	−0.51 [−0.60, −0.42]
Liu et al. [32]	0.71	0.1	27	1.2	0.21	27	50.2%	−0.49 [−0.58, −0.40]
Shi et al. [33]	2.79	1.96	20	3.6	2.47	20	0.2%	−0.81 [−2.19, 0.57]
Shi et al. [33]	2.61	2.1	20	3.6	2.47	20	0.2%	−0.99 [−2.41, 0.43]
Total (95% CI)			**94**			**94**	**100.0%**	**−0.50 [−0.56, −0.44]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.75$, df = 3 ($P = 0.86$); $I^2 = 0\%$

Test for overall effect: $Z = 15.82$ ($P < 0.00001$)

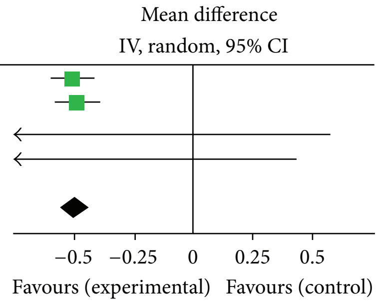

Figure 12: Forest plot of the effects of acupuncture on LF/HF ratio for nonhealthy subjects.

3.6.6. PC6 on LF (2 Studies). Two studies were included while there were 4 study IDs in this analysis (Figure 20). There were 52 subjects in acupuncture group and 52 in sham/control group. The heterogeneity ($\chi^2 = 6.33$, df = 3, $P = 0.10$) was not significant while the I^2 was 53%. It showed low heterogeneity. The overall effect was not significant ($Z = 0.57$, $P = 0.57$). Results did not support the effect of acupuncture at PC6 on decreased LF.

3.6.7. PC6 on LF/HF Ratio (6 Studies). Six studies were included while there were 11 study IDs in this analysis (Figure 21). There were 197 subjects in acupuncture group and 196 in sham/control group. The heterogeneity ($\chi^2 = 85.78$, df = 10, $P < 0.00$) was significant while the I^2 was 88%. It showed high heterogeneity. The overall effect was significant ($Z = 2.23$, $P = 0.03$). With reference to WMDs, the significant effect of acupuncture at PC6 on acupuncture group decreased LF/HF ratio was contributed by Chang et al. [30], Huang et al. [23], Kwak et al. [22], Li et al. [29], and Shi et al. [33].

3.6.8. PC6 on HF Norm (3 Studies). Three studies were included while there were 5 study IDs in this analysis (Figure 22). There were 91 subjects in acupuncture group and 90 in sham/control group. The heterogeneity ($\chi^2 = 67.18$, df = 4, $P < 0.00$) was significant while the I^2 was 86%. It showed high heterogeneity. The overall effect was not significant ($Z = 0.20$, $P = 0.84$). Results did not support the effect of acupuncture at PC6 on decreased HF norm.

3.6.9. PC6 on LF Norm (3 Studies). Three studies were included while there were 5 study IDs in this analysis (Figure 23). There were 91 subjects in acupuncture group and 90 in sham/control group. The heterogeneity ($\chi^2 = 15.22$, df = 4, $P = 0.004$) was significant while the I^2 was 74%. It showed moderate heterogeneity. The overall effect was not significant ($Z = 0.15$, $P = 0.88$). Results did not support the effect of acupuncture at PC6 on decreased LF norm.

3.6.10. ST36 on High Frequency (3 Studies). Three studies were included while there were 5 study IDs in this analysis (Figure 24). There were 99 subjects in acupuncture group and 99 in sham/control group. The heterogeneity ($\chi^2 = 33.34$, df = 4, $P < 0.00$) was significant while the I^2 was 88%. It showed high heterogeneity. The overall effect was significant ($Z = 4.47$, $P < 0.00$). With reference to WMDs, the significant effect of acupuncture at ST36 on sham group decreased HF was contributed by Chang et al. [24], Chang et al. [25], and Liu et al. [32].

3.6.11. ST36 on Low Frequency (2 Studies). Two studies were included while there were 3 study IDs in this analysis (Figure 25). There were 45 subjects in acupuncture group and 45 in sham/control group. The heterogeneity ($\chi^2 = 7.39$, df = 2, $P = 0.02$) was significant while the I^2 was 73%. It showed moderate heterogeneity. The overall effect was not significant ($Z = 0.96$, $P = 0.34$). Results did not support the effect of acupuncture at ST36 on decreased LF.

3.6.12. ST36 on LF/HF Ratio (3 Studies). Three studies were included while there were 6 study IDs in this analysis (Figure 26). There were 111 subjects in acupuncture group and 111 in sham/control group. The heterogeneity ($\chi^2 = 108.59$, df = 5, $P < 0.00$) was significant while the I^2 was 95%. It showed high heterogeneity. The overall effect was not

Study or subgroup	Acupuncture Mean (ms)	SD (ms)	Total	Sham treatment/control Mean (ms)	SD (ms)	Total	Weight	Mean difference IV, random, 95% CI (ms)
Liu et al. [32]	0.13	0.02	27	0.04	0.01	27	49.7%	0.09 [0.08, 0.10]
Liu et al. [32]	0.08	0.02	27	0.02	0.01	27	49.7%	0.06 [0.05, 0.07]
Shi et al. [33]	1.03	1.49	20	0.93	0.4	20	0.2%	0.10 [−0.58, 0.78]
Shi et al. [33]	1.02	0.84	20	0.93	0.4	20	0.5%	0.09 [−0.32, 0.50]
Total (95% CI)			**94**			**94**	**100.0%**	**0.08 [0.05, 0.10]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 24.31$, df $= 3$ ($P < 0.0001$); $I^2 = 88\%$

Test for overall effect: $Z = 5.25$ ($P < 0.00001$)

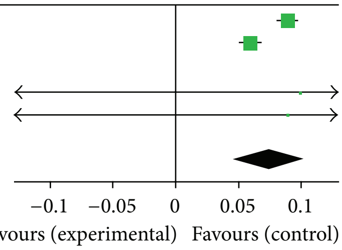

Figure 13: Forest plot of the effects of acupuncture at PC6 on HF for nonhealthy subjects.

Study or subgroup	Acupuncture Mean	SD	Total	Sham treatment/control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Liu et al. [32]	0.58	0.15	27	1.09	0.18	27	49.4%	−0.51 [−0.60, −0.42]
Liu et al. [32]	0.71	0.1	27	1.2	0.21	27	50.2%	−0.49 [−0.58, −0.40]
Shi et al. [33]	2.61	2.1	20	3.6	2.47	20	0.2%	−0.99 [−2.41, 0.43]
Shi et al. [33]	2.79	1.96	20	3.6	2.47	20	0.2%	−0.81 [−2.19, 0.57]
Total (95% CI)			**94**			**94**	**100.0%**	**−0.50 [−0.56, −0.44]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.75$, df $= 3$ ($P = 0.86$); $I^2 = 0\%$

Test for overall effect: $Z = 15.82$ ($P < 0.00001$)

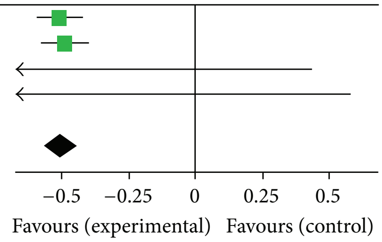

Figure 14: Forest plot of the effects of acupuncture at PC6 on LF/HF for nonhealthy subjects.

Study or subgroup	Acupuncture Mean (ms)	SD (ms)	Total	Sham treatment/control Mean (ms)	SD (ms)	Total	Weight	Mean difference IV, random, 95% CI (ms)
Kim et al. [28]	806.73	119.12	38	806	150.26	38	0.0%	0.73 [−60.24, 61.70]
Streitberger et al. [16]	0.2	0.18	20	−0.01	0.57	20	100.0%	0.21 [−0.05, 0.47]
Total (95% CI)			**58**			**58**	**100.0%**	**0.21 [−0.05, 0.47]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.00$, df $= 1$ ($P = 0.99$); $I^2 = 0\%$

Test for overall effect: $Z = 1.57$ ($P = 0.12$)

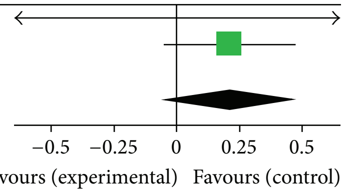

Figure 15: Forest plot of the effects of acupuncture at LI4 on HF for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean	SD	Total	Sham treatment/control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Kim et al. [28]	1.05	0.12	38	1.12	0.11	38	19.5%	−0.07 [−0.12, −0.02]
Kwak et al. [22]	0.72	0.35	14	0.6	0.43	14	16.2%	0.12 [−0.17, 0.41]
Kwak et al. [22]	0.61	0.27	14	0.54	0.21	14	18.1%	0.07 [−0.11, 0.25]
Kwak et al. [22]	0.62	0.3	14	0.58	0.21	14	17.9%	0.04 [−0.15, 0.23]
Li et al. [29]	1.49	0.42	10	2.92	0.86	10	10.4%	−1.43 [−2.02, −0.84]
Streitberger et al. [16]	0.52	0.37	20	−0.14	0.25	20	17.9%	0.66 [0.46, 0.086]
Total (95% CI)			**110**			**110**	**100.0%**	**−0.01 [−0.29, 0.27]**

Heterogeneity: $\tau^2 = 0.10$; $\chi^2 = 73.89$, df $= 5$ ($P < 0.00001$); $I^2 = 93\%$

Test for overall effect: $Z = 0.04$ ($P = 0.97$)

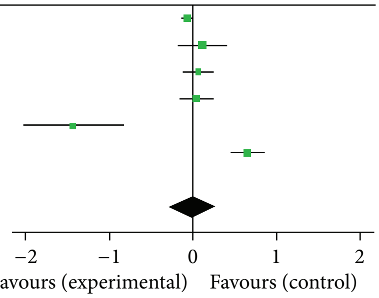

Figure 16: Forest plot of the effects of acupuncture at LI4 on LF/HF ratio for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	SD (n.u.)	Total	Sham treatment/control Mean (n.u.)	SD (n.u.)	Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Kwak et al. [22]	60.05	9.59	14	66.45	12.62	14	23.6%	−6.40 [−14.70, 1.90]
Kwak et al. [22]	63.62	9.52	14	66.48	8.74	14	25.0%	−2.86 [−9.63, 3.91]
Kwak et al. [22]	64.3	10.78	14	65.09	8.02	14	24.8%	−0.79 [−7.83, 6.25]
Li et al. [29]	38	5.76	10	24.34	5.44	10	26.5%	13.66 [8.75, 18.57]
Total (95% CI)			**52**			**52**	**100.0%**	**1.20 [−8.64, 11.04]**

Heterogeneity: $\tau^2 = 88.76$; $\chi^2 = 26.62$, df = 3 ($P < 0.00001$); $I^2 = 89\%$

Test for overall effect: $Z = 0.24$ ($P = 0.81$)

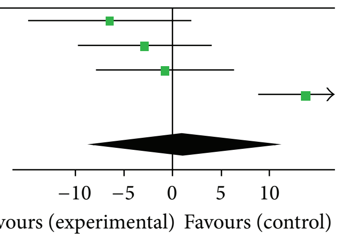

FIGURE 17: Forest plot of the effects of acupuncture at LI4 on HF norm for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	SD (n.u.)	Total	Sham treatment/control Mean (n.u.)	SD (n.u.)	Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Kwak et al. [22]	36.76	10.56	14	34.33	7.33	14	26.1%	2.43 [−4.30, 9.16]
Kwak et al. [22]	40.01	10.58	14	34.84	12.17	14	23.5%	5.17 [−3.28, 13.62]
Kwak et al. [22]	36.61	11.01	14	36.42	7.68	14	25.7%	0.19 [−6.84, 7.22]
Li et al. [29]	58.92	6.33	10	72.5	10.65	10	24.7%	−13.58 [−21.26, −5.90]
Total (95% CI)			**52**			**52**	**100.0%**	**−1.45 [−9.32, 6.41]**

Heterogeneity: $\tau^2 = 49.88$; $\chi^2 = 13.43$, df = 3 ($P = 0.004$); $I^2 = 78\%$

Test for overall effect: $Z = 0.36$ ($P = 0.72$)

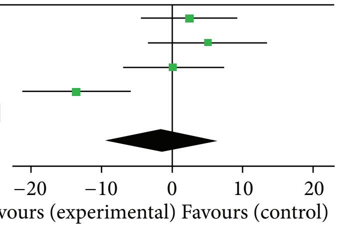

FIGURE 18: Forest plot of the effects of acupuncture at LI4 on LF norm for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (ms)	SD (ms)	Total	Sham treatment/control Mean (ms)	SD (ms)	Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [30]	0.2025	0.1347	6	0.169	0.097	6	3.6%	0.03 [−0.10, 0.17]
Chang et al. [30]	0.2208	0.2312	6	0.1647	0.1348	6	1.5%	0.06 [−0.16, 0.27]
Liu et al. [32]	0.13	0.02	27	0.04	0.01	27	47.2%	0.09 [0.08, 0.10]
Liu et al. [32]	0.08	0.02	27	0.02	0.01	27	47.2%	0.06 [0.05, 0.07]
Shi et al. [33]	1.02	0.84	20	0.93	0.4	20	0.4%	0.09 [−0.32, 0.50]
Shi et al. [33]	1.03	1.49	20	0.93	0.4	20	0.2%	0.10 [−0.58, 0.78]
Total (95% CI)			**106**			**106**	**100.0%**	**0.07 [0.05, 0.10]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 24.71$, df = 5 ($P = 0.0002$); $I^2 = 80\%$

Test for overall effect: $Z = 5.47$ ($P < 0.00001$)

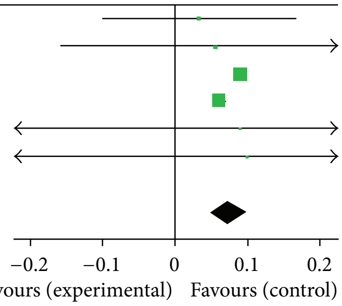

FIGURE 19: Forest plot of the effects of acupuncture at PC6 on HF for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (ms)	SD (ms)	Total	Sham treatment/control Mean (ms)	SD (ms)	Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [30]	0.3592	0.1229	6	0.4513	0.1611	6	42.3%	−0.09 [−0.25, 0.07]
Chang et al. [30]	0.3907	0.1092	6	0.3495	0.0357	6	54.0%	0.04 [−0.05, 0.13]
Shi et al. [33]	2.2	1.42	20	2.99	2.79	20	1.8%	−0.79 [−2.16, 0.58]
Shi et al. [33]	1.83	1.03	20	2.99	2.79	20	2.0%	−1.16 [−2.46, 0.14]
Total (95% CI)			**52**			**52**	**100.0%**	**−0.05 [−0.24, 0.13]**

Heterogeneity: $\tau^2 = 0.01$; $\chi^2 = 6.33$, df = 3 ($P = 0.10$); $I^2 = 53\%$

Test for overall effect: $Z = 0.57$ ($P = 0.57$)

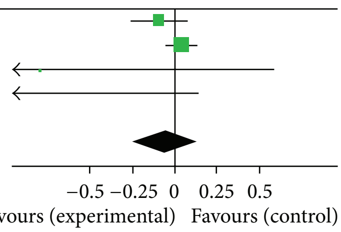

FIGURE 20: Forest plot of the effects of acupuncture at PC6 on LF for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean	Acupuncture SD	Acupuncture Total	Sham treatment/control Mean	Sham treatment/control SD	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI
Chang et al. [30]	3.879	4.7606	6	3.9538	2.8528	6	0.3%	−0.07 [−4.52, 4.37]
Chang et al. [30]	2.8614	1.8839	6	2.6314	1.3708	6	1.5%	0.23 [−1.63, 2.09]
Huang et al. [23]	1.7	2.3	39	1.3	0.9	38	6.1%	0.40 [−0.38, 1.18]
Kwak et al. [22]	0.62	0.3	14	0.58	0.21	14	15.5%	0.04 [−0.15, 0.23]
Kwak et al. [22]	0.72	0.35	14	0.6	0.43	14	13.8%	0.12 [−0.17, 0.41]
Kwak et al. [22]	0.61	0.27	14	0.54	0.21	14	15.7%	0.07 [−0.11, 0.25]
Li et al. [29]	1.49	0.42	10	2.92	0.86	10	8.4%	−1.43 [−2.02, −0.84]
Liu et al. [32]	0.71	0.1	27	1.2	0.21	27	16.9%	−0.49 [−0.58, −0.40]
Liu et al. [32]	0.58	0.15	27	1.09	0.18	27	16.9%	−0.51 [−0.60, −0.42]
Shi et al. [33]	2.61	2.1	20	3.6	2.47	20	2.4%	−0.99 [−2.41, 0.43]
Shi et al. [33]	2.79	1.96	20	3.6	2.47	20	2.6%	−0.81 [−2.19, 0.57]
Total (95% CI)			**197**			**196**	**100.0%**	**−0.27 [−0.51, −0.03]**

Heterogeneity: $\tau^2 = 0.09$; $\chi^2 = 85.78$, df = 10 ($P < 0.00001$); $I^2 = 88\%$

Test for overall effect: $Z = 2.23$ ($P = 0.03$)

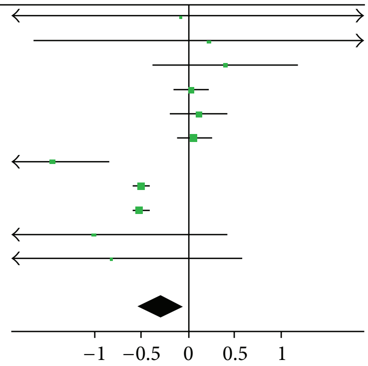

Figure 21: Forest plot of the effects of acupuncture at PC6 on LF/HF ratio for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	Acupuncture SD (n.u.)	Acupuncture Total	Sham treatment/control Mean (n.u.)	Sham treatment/control SD (n.u.)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Huang et al. [23]	30.7	14.1	39	31.8	13.8	38	20.4%	−1.10 [−7.33, 5.13]
Kwak et al. [22]	64.3	10.78	14	65.09	8.02	14	19.7%	−0.79 [−7.83, 6.25]
Kwak et al. [22]	60.05	9.59	14	66.45	12.62	14	18.5%	−6.40 [−14.70, 1.90]
Kwak et al. [22]	63.62	9.52	14	66.48	8.74	14	19.9%	−2.86 [−9.63, 3.91]
Li et al. [29]	38	5.76	10	24.34	5.44	10	21.5%	13.66 [8.75, 18.57]
Total (95% CI)			**91**			**90**	**100.0%**	**0.80 [−6.99, 8.58]**

Heterogeneity: $\tau^2 = 67.18$; $\chi^2 = 28.68$, df = 4 ($P < 0.00001$); $I^2 = 86\%$

Test for overall effect: $Z = 0.20$ ($P = 0.84$)

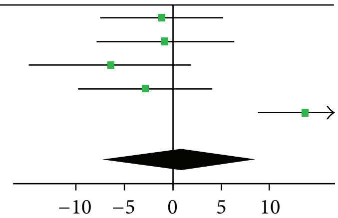

Figure 22: Forest plot of the effects of acupuncture at PC6 on HF norm for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (n.u.)	Acupuncture SD (n.u.)	Acupuncture Total	Sham treatment/control Mean (n.u.)	Sham treatment/control SD (n.u.)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (n.u.)
Huang et al. [23]	34.4	12.1	39	31.5	10.4	38	23.0%	2.90 [−2.14, 7.94]
Kwak et al. [22]	36.61	11.01	14	36.42	7.68	14	19.9%	0.19 [−6.84, 7.22]
Kwak et al. [22]	36.76	10.56	14	34.33	7.33	14	20.4%	2.43 [−4.30, 9.16]
Kwak et al. [22]	40.01	10.58	14	34.84	12.17	14	17.7%	5.17 [−3.28, 13.62]
Li et al. [29]	58.92	6.33	10	72.5	10.65	10	18.9%	−13.58 [−21.26, −5.90]
Total (95% CI)			**91**			**90**	**100.0%**	**−0.45 [−6.42, 5.52]**

Heterogeneity: $\tau^2 = 33.72$; $\chi^2 = 15.22$, df = 4 ($P < 0.004$); $I^2 = 74\%$

Test for overall effect: $Z = 0.15$ ($P = 0.88$)

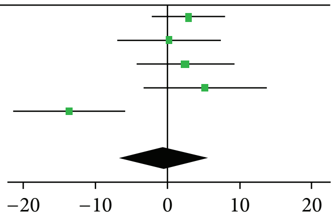

Figure 23: Forest plot of the effects of acupuncture at PC6 on LF norm for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (ms)	Acupuncture SD (ms)	Acupuncture Total	Sham treatment/control Mean (ms)	Sham treatment/control SD (ms)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [24]	1,019.5	140.2	15	1,145.2	163.3	15	0.0%	−125.70 [−234.62, −16.78]
Chang et al. [25]	985.9	177.4	15	1,088.5	163.8	15	0.0%	−102.60 [−224.79, 19.59]
Chang et al. [25]	1,020.6	175.5	15	1,088.5	163.8	15	0.0%	−67.90 [−189.39, 53.59]
Liu et al. [32]	0.13	0.02	27	0.04	0.01	27	50.0%	0.09 [0.08, 0.10]
Liu et al. [32]	0.08	0.02	27	0.02	0.01	27	50.0%	0.06 [0.05, 0.07]
Total (95% CI)			**99**			**99**	**100.0%**	**0.07 [0.04, 0.11]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 33.34$, df = 4 ($P < 0.00001$); $I^2 = 88\%$

Test for overall effect: $Z = 4.47$ ($P < 0.00001$)

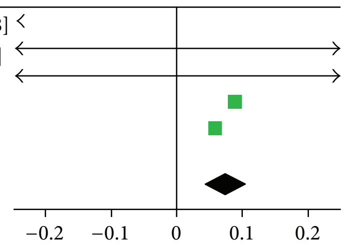

Figure 24: Forest plot of the effects of acupuncture at ST36 on HF for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean (ms)	Acupuncture SD (ms)	Acupuncture Total	Sham treatment/control Mean (ms)	Sham treatment/control SD (ms)	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI (ms)
Chang et al. [24]	1,446.8	360.2	15	1,396.2	380.1	15	32.4%	50.60 [−214.40, 315.60]
Chang et al. [25]	1,450.9	359.9	15	1,491.7	371.9	15	32.6%	−40.80 [−302.70, 221.10]
Chang et al. [25]	1,094.4	253.6	15	1,491.7	371.9	15	35.0%	−397.30 [−625.10, −169.50]
Total (95% CI)			**45**			**45**	**100.0%**	**−136.15 [−414.89, 142.59]**

Heterogeneity: $\tau^2 = 44206.06$; $\chi^2 = 7.39$, df $= 2$ ($P = 0.02$); $I^2 = 73\%$

Test for overall effect: $Z = 0.96$ ($P = 0.34$)

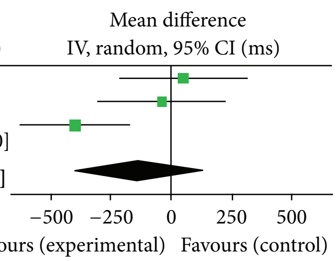

FIGURE 25: Forest plot of the effects of acupuncture at ST36 on LF for all healthy and nonhealthy subjects.

Study or subgroup	Acupuncture Mean	Acupuncture SD	Acupuncture Total	Sham treatment/control Mean	Sham treatment/control SD	Sham treatment/control Total	Weight	Mean difference IV, random, 95% CI
Chang et al. [24]	1.51	0.28	15	1.17	0.32	15	16.2%	0.34 [0.12, 0.56]
Kwak et al. [22]	0.61	0.27	14	0.54	0.21	14	16.8%	0.07 [−0.11, 0.25]
Kwak et al. [22]	0.62	0.3	14	0.58	0.21	14	16.6%	0.04 [−0.15, 0.23]
Kwak et al. [22]	0.72	0.35	14	0.6	0.43	14	15.0%	0.12 [−0.17, 0.41]
Liu et al. [32]	0.58	0.15	27	1.09	0.18	27	17.7%	−0.51 [−0.60, −0.42]
Liu et al. [32]	0.71	0.1	27	1.2	0.21	27	17.7%	−0.49 [−0.58, −0.40]
Total (95% CI)			**111**			**111**	**100.0%**	**−0.09 [−0.36, 0.19]**

Heterogeneity: $\tau^2 = 0.11$; $\chi^2 = 108.59$, df $= 5$ ($P < 0.00001$); $I^2 = 95\%$

Test for overall effect: $Z = 0.61$ ($P = 0.54$)

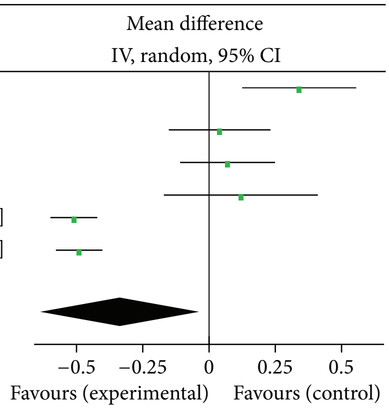

FIGURE 26: Forest plot of the effects of acupuncture at ST36 on LF/HF ratio for all healthy and non-healthy subjects.

significant ($Z = 0.61$, $P = 0.54$). Results did not support the effect of acupuncture at ST36 on decreased LF/HF ratio.

3.7. Summary of Effect of Acupuncture on HRV. Table 4 provides a summary of results with regard to heterogeneity of the studies. All the studies on related HRV outcome measures showed moderate to high heterogeneity. This supported the use of a random effect model in meta-analysis. Table 5 shows the effect of acupuncture on LF and LF/HF ratio for nonhealthy subjects and its effect on LF norm for healthy subjects. Considering the WMDs, we find that the overall effect was in favour of the sham/control, not the acupuncture group, for HF in nonhealthy subjects and for HF norm in healthy subjects. For effect of HRV when acupoints were stimulated, significant decreasing effect on HF component of HRV when acupuncture was performed on ST36 among healthy subjects and on PC6 among both healthy and nonhealthy subjects. For nonhealthy subjects, studies found a decreasing effect on HF component of HRV when sham acupuncture was performed on PC6 and a decreasing effect on LF/HF component of HRV when actual acupuncture was performed on PC6.

4. Discussion

4.1. Comparison with Previous Findings. To date, there has been one published systematic review which studied the effect of acupuncture on HRV [8]. The study reported that acupuncture did not have any convincing effect on HRV including the HF, LF and its related components in healthy subjects. Some of the findings in the current studies are contradictory to those of Lee and colleague's study [8] (refer to Section 3.7 for details).

Since this systematic review adopted the same inclusion and exclusion criteria as Lee and colleague's study [8], the researcher suggested that the difference in findings may be due to the difference in the number of studies included. It was 12 for Lee and colleague's study [8] and 14 for the current review. This review may have included studies with higher effect sizes. Apart from this, the difference in reported risk of bias may also account for the contradictory results. The current review had a better assessment of risk of bias. For instance, in the current review, more studies were randomized (4 in Lee and colleague's study [8] while 11 in this review). Concealment is another factor. In Lee and colleague's study [8], no studies satisfied the criteria of concealment but about 75% of the selected studies in this systematic review reported concealment.

4.2. New Phenomena Revealed. The findings in this systematic review reveal two phenomena. First, acupuncture has reduction effect on HRV. Acupuncture decreases the LF and LF/HF ratio among nonhealthy subjects. However, the effect of acupuncture on healthy subjects is inconclusive because there were no data of nonhealthy subjects for combined analysis.

Second, acupuncture does not have any effect on the HF component of HRV. Acupuncture also has reduction

Table 4: A summary of the heterogeneity for random effect.

Outcomes	No. of studies			Heterogeneity Chi-sq test			Higgins I^2 test		
	All studies	Healthy	Non-healthy	All Studies	Healthy	Non-healthy	All studies	Healthy	Non-healthy
HF	9	7	2	41.31 ($P = 0.0003$)	16.99 ($P = 0.11$)	24.31 ($P < 0.0001$)	64%	35%[#]	88%
LF	7	6	1	51.93 ($P < 0.0001$)	47.44 ($P < 0.0001$)	0.15 ($P = 0.70$)	77%	79%	0%
LF/HF ratio	11	9	2	977.50 ($P < 0.0001$)	913.49 ($P < 0.0001$)	0.75 ($P = 0.86$)	98%	99%	0%
HF norm	5	5	—	32.35 ($P < 0.0001$)	32.35 ($P < 0.0001$)	—	78%	78%	NA
LF norm	5	5	—	15.39 ($P = 0.03$)	15.39 ($P = 0.03$)	—	55%	55%	NA
LI4 on HF	2	—	—	0.00 ($P = 0.99$)	—	—	0%	—	—
LI4 on LF/HF ratio	4	—	—	73.89 ($P < 0.0001$)	—	—	93%	—	—
LI4 on HF norm	2	—	—	26.62 ($P < 0.0001$)	—	—	89%	—	—
LI4 on LF norm	2	—	—	13.43 ($P = 0.004$)	—	—	78%	—	—
PC6 on HF	3	—	2	24.71 ($P = 0.0002$)	—	24.31 ($P < 0.0001$)	80%	—	0%
PC6 on LF	2	—	—	6.33 ($P = 0.10$)	—	—	53%	—	—
PC6 on LF/HF ratio	6	—	2	85.78 ($P < 0.0001$)	—	0.75 ($P = 0.86$)	88%	—	0%
PC6 on HF norm	3	—	—	28.68 ($P < 0.0001$)	—	—	86%	—	—
PC6 on LF norm	3	—	—	15.22 ($P = 0.004$)	—	—	74%	—	—
ST36 on HF	3	2	—	33.34 ($P < 0.0001$)	0.49 ($P = 0.78$)	—	88%	0%	—
ST36 on LF	2	—	—	7.39 ($P = 0.02$)	—	—	88%	—	—
ST36 on LF/HF ratio	3	—	—	108.59 ($P < 0.0001$)	—	—	95%	—	—

[#]Higgins I^2 Test is ≤50%; fixed effect is used.

Table 5: A summary of the effect of acupuncture in HRV outcome measures in various subject groups and by different acupoints.

Outcomes	Overall effect, Z			Mean difference		
	All studies	Healthy	Non-healthy	All Studies	Healthy	Non-healthy
HF	4.69 ($P < 0.0001$)*	1.39 ($P = 0.17$)#	5.25 ($P < 0.0001$)*	0.08 [0.05, 0.10]*	0.07 [−0.03, 0.17]#	0.08 [0.05, 0.10]*
LF	0.03 ($P = 0.98$)	0.72 ($P = 0.47$)	2.04 ($P = 0.04$)*	0.01 [−0.38, 0.40]	0.15 [−0.26, 0.56]	−0.98 [−1.93, −0.04]*
LF/HF ratio	2.18 ($P = 0.03$)*	1.26 ($P = 0.21$)	15.82 ($P < 0.0001$)*	−0.33 [−0.63, −0.03]*	−0.26 [−0.66, 0.14]	−0.50 [−0.56, −0.44]*
HF norm	5.00 ($P < 0.0001$)*	5.00 ($P < 0.0001$)*	—	0.51 [0.31, 0.70]*	0.51 [0.31, 0.70]*	NA
LF norm	4.03 ($P < 0.0001$)*	4.03 ($P < 0.0001$)*	—	−0.30 [−0.44, −0.15]*	−0.30 [−0.44, −0.15]*	NA
LI4 on HF	15.82 ($P < 0.0001$)*	—	—	0.21 [−0.05, 0.47]	—	—
LI4 on LF/HF ratio	0.04 ($P = 0.97$)	—	—	−0.01 [−0.29, 0.27]	—	—
LI4 on HF norm	0.24 ($P = 0.81$)	—	—	1.20 [−8.64, 11.04]	—	—
LI4 on LF norm	0.36 ($P = 0.72$)	—	—	−1.45 [9.32, 6.41]	—	—
PC6 on HF	5.47 ($P < 0.0001$)*	—	5.25 ($P < 0.0001$)*	0.07 [0.05, 0.10]*	—	0.08 [0.05, 0.10]*
PC6 on LF	0.57 (0.57)	—	—	−0.05 [−0.24, 0.13]	—	—
PC6 on LF/HF ratio	2.23 ($P = 0.03$)*	—	15.82 ($P < 0.0001$)*	−0.27 [−0.51, −0.03]*	—	−0.50 [−0.56, −0.44]*
PC6 on HF norm	0.20 ($P = 0.84$)	—	—	0.80 [−6.99, 8.58]	—	—
PC6 on LF norm	0.15 ($P = 0.88$)	—	—	−0.45 [−6.42, 5.52]	—	—
ST36 on HF	4.47 ($P < 0.0001$)*	3.00 ($P = 0.003$)*	—	0.07 [0.04, 0.11]*	−99.51 [−164.56, −34.45]*	—
ST36 on LF	0.96 ($P = 0.34$)	—	—	−136.15 [−414.89, 142.59]	—	—
ST36 on LF/HF ratio	0.61 ($P = 0.54$)	—	—	−0.09 [−0.36, 0.19]	—	—

*Denoted "significant overall effect" and "significant mean difference", respectively.
#Higgins I^2 test is ≤50%; fixed effect is used.

effect on the HRV of sham/control. Sham/control decreases the HF for non-health subjects and HF norm for healthy subjects. Again, the effect of sham/control on healthy subjects is inconclusive because there were no data of nonhealthy subjects for combined analysis.

We find that changes in LF can change the values of LF/HF ratio or LF norm and HF norm. This is because LF or HF is a function of the LF/HF ratio or LF norm and HF norm. Therefore, it is worthwhile to look at the decreasing modulating effect of acupuncture on LF for nonhealthy subjects. LF refers to the parasympathetic influence on the balance between sympathetic and parasympathetic activities when one is in a relaxed state, like slowing heart rate, decreasing blood pressure, stimulating the gastrointestinal tract, eliminating waste, restoring energy, and building tissues. This modulating effect by acupuncture is important because, in general, parasympathetic activities represent conservative and restorative functions.

Effects of ST36 and PC6 acupoints on HRV were demonstrated that ST36 is an important acupoint of the Stomach Meridian which is documented to be related to general wellness and good *shen* (sound mind and good mood). ST36 is commonly used in acupuncture or acupressure for general health maintenance. This coincides with decrease of HF, that is, a decrease in parasympathetic activities. On the other hand, PC6 is an acupoint in the Pericardium Meridian. For nonhealthy subjects, we prefer to calm the meridian rather than exhausting it. The LF/HF is an indicator of sympathetic nerve activities; a lower number means decreased sympathetic activities or increased parasympathetic activities which may help clam the meridian. The interpretation of effect of acupoints warrants some concern because there is no information on the technique of needle insertion and no information on whether the subjects had excessive heat/cold at the time of receiving acupuncture.

4.3. HRV as an Indicator for the Therapeutic Effect of Acupuncture. Evidence from this systematic review partially supports the possible effect of acupuncture in modulating the low frequency component of heart rate variability. This may represent a mechanistic pathway for global physiological regulation, which is congruent with East Asian medical theory. It is important to highlight the significance of LF component of HRV. This modulation effect by acupuncture is important because, in general, parasympathetic activities represent conservative and restorative physiological activities. However, we do not have enough published work in this area to determine if HRV can be used as an indicator for the therapeutic effect of acupuncture because the dynamic change between LF and HF in maintaining the optimal status is yet to be answered in this systematic review.

4.4. Limitation and Further Work. We need more RCTs with high quality in this area so as to provide a direction for our evidence-based practice. We need larger sample sizes in the studies because this allows better randomization to work. The control for heterogeneity is an issue which can be overcome. We need to have studies in which both subjects and assessors can be blinded in order to minimize the psychological biases. Current studies were subject to psychological biases which would in turn affect the quality of the systematic review.

5. Conclusions

In this thesis, a systematic review with meta-analysis was conducted on 14 RCTs. All the studies on related HRV outcome measures showed moderate to high heterogeneity. Results showed a decreasing modulating effect of acupuncture on LF and LF/HF ratio for nonhealthy subjects and on LF norm for healthy subjects. However, the overall effect was in favour of the sham/control, not acupuncture group, for HF in nonhealthy subjects and for HF norm in healthy subjects. Evidence from this systematic review suggests that acupuncture modulates the low frequency component of HRV but not the HF component. However, we do not have enough published work in this area to determine if HRV can be an indicator for the therapeutic effect of acupuncture.

Conflict of Interests

The authors have no conflict of interests whatsoever to declare.

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Research Article

Impact of Colored Light on Cardiorespiratory Coordination

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Background. Light exposure to the eye can influence different physiological functions, for example, the suprachiasmatic nucleus (SCN). By affecting the autonomic nervous system, the SCN may influence the heart rate variability (HRV). Standardized colored light exposure alters HRV but the results are inconsistent. In this study we investigated the effects of nonstandardized red light (approx. 640 nm) and blue (approx. 480 nm) light (approx. 50 lx) on cardiorespiratory coordination and HRV. *Methods*. 17 healthy subjects (7 males, age: 26.5 ± 6.2 years) were exposed to the following sequence (10 minutes each): daylight-red light-daylight-blue light-daylight. Red and blue lights were created by daylight passing through colored glass panes. Spectral measures of HRV (LF: low frequency, HF: high frequency oscillations, and sympathovagal balance LF/HF) and measures of cardiorespiratory coordination (HRR: heart respiration ratio, PCR: phase coordination ratio) were analyzed. *Results*. The LF component increased and the HF component decreased after red light. Consequently, LF/HF increased after red light. Furthermore, during red light HRR and PCR confined to 4 : 1, that is, 4 heartbeats during one respiratory cycle. *Conclusion*. Nonstandardized red and blue lights are able to alter the autonomic control reflected by HRV as well as cardiorespiratory coordination.

1. Introduction

During normal vision, light is captured by the rods and cones. This way the circadian clock located in the suprachiasmatic nucleus (SCN) is entrained every day [1, 2]. Hence, light influences many other functions coordinated to the circadian clock. In addition to this impact of the so-called image forming visual system the existence of intrinsically photosensitive retinal ganglion cells (ipRGCs) has been discovered recently [3]. They form a nonimage forming visual system; that is, the light stimulus is not used to form images. Upon light stimulus the ipRGCs show a synaptically driven response (initiated by inputs from rods and cones) and an autonomous response (which is based on the photopigment melanopsin) [4]. The ipRGCs are more sensitive to short-wavelength (blue) light than to long-wavelength (red) light [5]. They also project onto the SCN [6, 7]. Hence, the image forming and the nonimage forming visual system influence the SCN in response to light.

As the SCN has an impact on the autonomic nervous system (ANS) [8, 9] different visual stimuli (e.g., different colors) may alter the ANS differently. Such alterations may be quantified utilizing the analysis of heart rate variability (HRV). In healthy subjects for example, respiration induces a modulation of heart rate mediated by parasympathetic activity (respiratory sinus arrhythmia, RSA) [10]. The extent of such modulations may be analyzed using spectral analysis [11]. In general, high frequency oscillations of heart rate are associated with modulations of the parasympathetic activity and the low frequency oscillations are due to modulations of sympathetic as well as parasympathetic activity. Additional information may be retrieved if cardiorespiratory interaction is analyzed. It has been shown that the oscillations of heartbeat and respiration may intermittently synchronize [12, 13]. During nighttime sleep heart rate and respiration show most likely a 4 : 1-synchronization; that is, 4 heartbeats occur at fixed times within each respiratory cycle [14–17].

Recent attempts to capture alterations of cardiovascular control caused by colored light were heterogeneous. Bright light (≥5000 lx) acutely increased the average heart rate in healthy subjects with blue light being most effective [18]. In neonates with physiologic jaundice the phototherapy increased heart rate and decreased respiratory rate during active sleep compared to active sleep without phototherapy [19]. In healthy subjects visual stimuli with different colors did not change the average heart rate but caused changes of HRV. During exposure to green or red light (700 lx) very low oscillations increased whereas during blue light these oscillations decreased compared to darkness [20]. Another study showed that after exposure to red or blue dim light (illuminance below 1 lux) the low frequency oscillations of HRV increased in healthy subjects with symptoms of anxiety and depression [21]. These studies indicate that each color stimuli may alter cardiovascular regulation differently.

The aim of this study was to investigate simultaneous effects during the exposure to red or blue lights on HRV, respiratory rate, and cardiorespiratory coordination in healthy subjects. We used a "naturalistic setting" which consisted of colored glass panes in front of a window illuminated by daylight. This way the level of illuminance is low (30 to 100 lx) and not standardized.

2. Methods

2.1. Subjects. 20 healthy subjects participated in the study (10 females, average age: 27 ± 6 years). None of the subjects had any history of cardiovascular diseases, especially, nor hypo- or hypertension or antiarrhythmic therapy. None of the subjects had any experience with any kind of light therapy. Three male subjects had to be excluded from the study because they deliberately changed their respiration. Hence, these subjects not only focused on the different visual stimuli but also on respiration. All subjects gave their written informed consent. The study was approved by the ethics committee of the University of Witten/Herdecke.

2.2. Protocol. The subjects sat comfortably in an armchair approx. 1.5 m in front of a window. The size of the window was matched to the size of the colored glass panes (approx. 0.6 m × 1.1 m) with black opaque curtains. A red and a blue glass pane were used to generate two different color stimuli. (The red colored pane was produced by addition of gold, the blue colored pane by addition of ferrous oxide. They were manufactured by Lucien Turci and Marianne Altmaier, Lichtblick e.V., Lörrach, Germany.) Each glass pane was mounted on a stand with reels which allowed the quick change of the visual stimuli. During color vision the black opaque curtains were placed such that color light was the only visual stimulus. Each subject was exposed to the following sequence of visual stimuli (level of illuminance: 30 to 100 lx):

$$\text{daylight-red ligh-daylight-blue light-daylight.} \tag{1}$$

The duration of each stimulus was 10 min (total duration: 50 min). In order to minimize a bias caused by circadian variations of HRV [22] the procedure was carried out between 10 am and 1 pm.

2.3. Data Acquisition. A 1-channel Holter electrocardiogram (ECG) and a respiratory trace (nasal/oral airflow captured by a thermocouple) were recorded during each session (Medikorder MK2, Schiller Engineering, Graz, Austria). The device's sampling rate of the ECG was 3000 Hz and, hence, the times of the device's internal R-peak detection had a precision <1 ms. To reduce memory consumption, the ECG was saved at a sampling rate of 250 Hz and the nasal airflow was saved at a sampling rate of 100 Hz. After transferring the ECG, the airflow trace and times of the R-peaks to a personal computer, the ECG, and the times of the R-peaks were inspected visually. In case of artefacts the times of associated R-peaks were corrected where necessary (e.g., by removing artifacts or by correcting the time of the R-peak). Less than 0.01% of R-peaks identified by the device had to be corrected.

The airflow trace was analyzed as follows. Inspiratory onsets were defined as local minima of the airflow because they were caused by the change from exhaling warm air (warmed be the respiratory tract) to inhaling air at the temperature of the environment. The times of the automatically identified inspiratory onsets were also inspected visually. Again, in case of artifacts the timings of inspiratory onsets were corrected.

The visual inspection of R-peaks, the subsequent HRV analysis (see Section 2.4) and the analysis of the airflow trace was carried out using custom programs written with Matlab (The Mathworks, Natick, MA, USA).

2.4. Heart Rate Variability. The normal-to-normal intervals between successive R-peaks (RR-interval series) served as the basis for the calculations. To avoid effects of transitions between different visual stimuli only the 5-minute epoch in the middle of each visual stimulus was analyzed. The mean normal-to-normal intervals and the accompanying standard deviation (SDNN) were calculated as basic time domain parameters. In the frequency domain, the extent of very low, low and high frequency oscillations of heart rate variations (VLF: ≤0.04 Hz, LF: 0.04–0.15 Hz, and HF: 0.15–0.4 Hz) and the ratio LF/HF were calculated using the fast Fourier transformation [11]. In addition to the spectral measures of HRV we also quantified self-similarity of the heart rate time series using the Detrended Fluctuation Analysis (DFA) [23, 24].

2.5. Cardiorespiratory Coordination. The mean respiratory rate and its standard deviation were calculated. Cardiorespiratory coordination was quantified by two different approaches. (a) The ratio of heart rate and respiratory rate (HRR, heart respiration ratio) served as a simple indicator of intermittent cardiorespiratory coordination [25]. (b) Furthermore, the "phase coordination ratio" (PCR) [14, 26] was calculated because it also takes into account the temporal coordination of heartbeat and respiration in more detail. This approach is advantageous compared to for example, analyzing cardiorespiratory synchronization using so-called synchrograms [12] because it is able to detect short and intermittent epochs of cardiorespiratory coordination (coordination can be detected after only two consecutive respiratory cycles) [14], whereas the analysis of synchrograms needs longer epochs to detect cardiorespiratory synchronization [13, 17].

The analysis was carried out as follows. First, the RR-tachogram RR_i ($i = 1, \ldots, N$) was transformed into a symbolic sequence symbolizing the acceleration and deceleration of heart rate (Figure 2):

$$S_i = \begin{cases} 0, & \text{if } RR_i - RR_{i-1} \geq 0, \\ 1, & \text{if } RR_i - RR_{i-1} < 0. \end{cases} \quad (2)$$

Next, $m:n$-coordination of heartbeat and respiration leads to specific sequences of 0 s and 1 s. For example, a 7 : 2-coordination (7 heartbeats during 2 respiratory cycles) is unambiguously characterized by the following binary pattern (Figure 1): 1001100. To minimize spurious detection of cardiorespiratory coordination the respective pattern must appear and also at least three subsequent symbols must belong to the same pattern. The following 7 $m:n$-ratios of cardiorespiratory coordination contain large parts of relevant information: 3 : 1, 7 : 2, 4 : 1, 9 : 2, 5 : 1, 11 : 2, and 6 : 1 [14]. The binary patterns reflecting these $m:n$-ratios are listed in Table 1. Subsequently, the PCR was calculated as the weighted mean of the detected $m:n$-ratios:

$$\text{PCR} = \frac{\sum_{i=1}^{7} m : n \cdot N(m : n)}{\sum_{i=1}^{7} N(m : n)}. \quad (3)$$

Here, $N(m:n)$ is the number of occurrences of the respective $m:n$-ratio. To quantify cardiorespiratory coordination HRR and PCR were calculated for the middle 5-minute epochs of each visual stimulus.

2.6. Statistics. The objective of this exploratory pilot study was to assess the effects of red and blue visual stimuli on HRV and cardiorespiratory coordination. Each 5-minute epoch was quantified by the following parameters: mean RR-interval, SDNN, VLF, LF, HF, LF/HF, mean and standard deviation of respiratory rate, HRR, and PCR. To minimize effects of transitions between successive visual stimuli the 5-minute epoch in the middle of each visual stimulus was analysed. A nonparametric 1-way analysis of variance with repeated measurements (Friedman test) was used to quantify differences within the succession of different visual stimuli. Post hoc differences between different visual stimuli were calculated using mean ranks and a correction for multiple comparisons according to Bonferroni. A $P < 0.05$ was considered statistically significant.

A specific feature of HRR is the centering towards a ratio of 4 : 1 during nighttime sleep [17, 27]. We analyzed whether cardiorespiratory coordination centers more strict to this 4 : 1-ratio during the exposure to colored light. Centering of HRR towards 4 : 1 during color light is defined as

$$\begin{aligned} \text{HRR}_{\text{dur\_col}} - \text{HRR}_{\text{bef\_col}} > 0, \quad & \text{if } \text{HRR}_{\text{bef\_col}} < 4 : 1, \\ \text{HRR}_{\text{dur\_col}} - \text{HRR}_{\text{bef\_col}} < 0, \quad & \text{if } \text{HRR}_{\text{bef\_col}} > 4 : 1. \end{aligned} \quad (4)$$

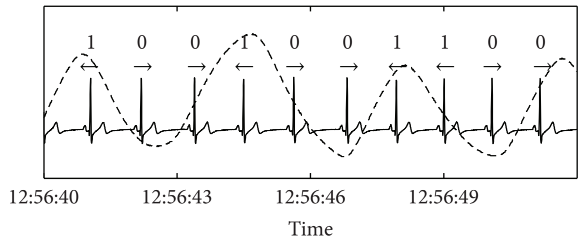

Figure 1: A short example of an ECG recording and the respiratory trace (dashed line). The symbols denote the encoding of acceleration (1) and deceleration (0) of the instantaneous heart rate as a consequence of respiratory sinus arrhythmia, that is, the modulation of heart rate by respiration. The sequence of binary symbols can be used to detect cardiorespiratory coordination. In this example, a cardiorespiratory coordination with a ratio of 7 : 2 (7 heartbeats in 2 respiratory cycles) would be detected.

Table 1: Binary patterns used for the analysis of the different $m:n$-ratios of cardiorespiratory coordination.

$m:n$-ratio	Binary pattern	Complementary binary pattern
3 : 1	001001	110110
7 : 2	0010011	1101100
4 : 1	00110011	11001100
9 : 2	000110011	111001100
5 : 1	0001100011	1110011100
11 : 2	00011000111	11100111000
6 : 1	000111000111	111000111000

Furthermore, the larger the deviation of HRR before the color stimulus ($\text{HRR}_{\text{bef\_col}}$) from the 4 : 1-ratio the larger the corresponding difference to achieve a centring (or confinement) towards 4 : 1 during the color stimulus ($\text{HRR}_{\text{dur\_col}}$). To quantify this centring, we plotted $\text{HRR}_{\text{bef\_col}}$ versus ($\text{HRR}_{\text{dur\_col}} - \text{HRR}_{\text{bef\_col}}$). According to the definition, centring is indicated by a negative correlation in this diagram. Hence, we calculated Pearson's correlation coefficient r and its accompanying P value. Spurious correlations caused by outliers were detected as follows: the correlation coefficient was also calculated omitting the points of minimal and maximal difference $\text{HRR}_{\text{dur\_col}} - \text{HRR}_{\text{bef\_col}}$. The correlation was considered significant only if the P-value of both correlations (original and without outliers) was $P < 0.05$. The centering of PCR towards the 4 : 1-ratio was analyzed analogously.

3. Results

An example of a complete RR-interval series, II-interval series, and heart respiration ratio (HRR) is shown in Figure 2. Note that the 5-minute epoch in the middle of each visual stimulus was analyzed. The level of illuminance was approx. 50 lx during colored light. The average RR-interval was 1002 ms (i.e., approx. 60 beats per minute) at the beginning and did not change during the succession of the different visual stimuli (see Table 2). SDNN as a measure in the time

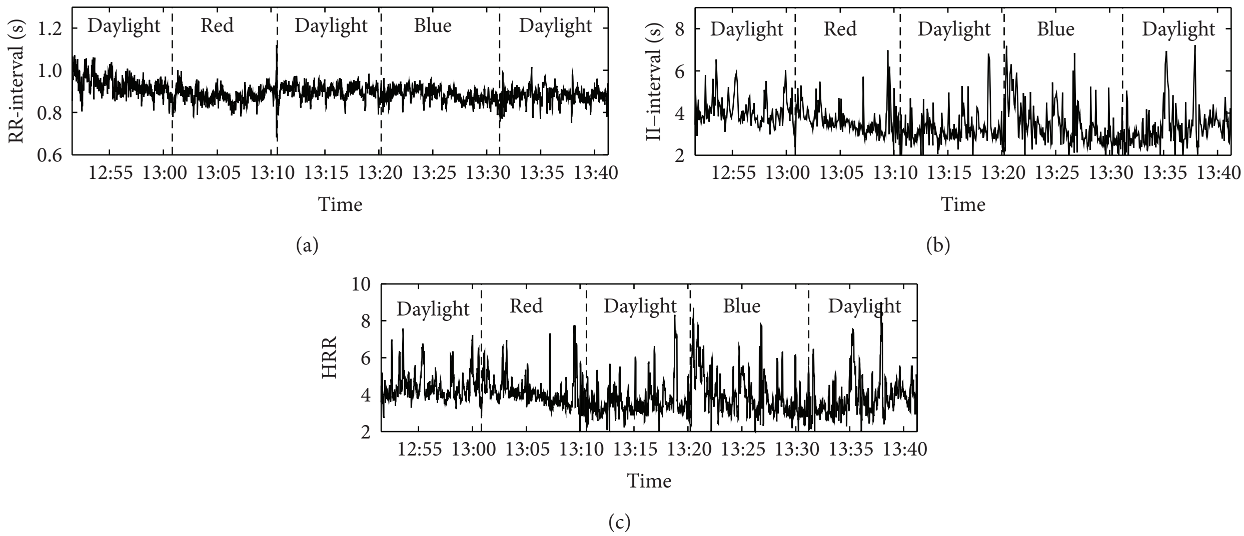

Figure 2: Example of the RR-interval series, II-interval series, and the heart respiration ratio (HRR) of one subject. The sequence of the visual stimuli is shown in each diagram.

Table 2: Results of HRV and cardiorespiratory coordination. All values are mean ± SD.

	Daylight	Red light	Daylight	Blue light	Daylight
RR-interval (ms)	1002 ± 149	999 ± 132	992 ± 123	995 ± 1113	986 ± 108
SDNN (ms)	73 ± 31	74 ± 33	79 ± 35	78 ± 33	83 ± 28
VLF ($\ln ms^2$)	6.61 ± 0.75	6.88 ± 0.75	6.99 ± 0.83	7.31 ± 0.75	7.51 ± 0.75
LF ($\ln ms^2$)§§	$6.84^{*,\#}$ ± 0.88	6.94^{*} ± 0.98	7.31 ± 1.07	7.18 ± 0.99	7.39 ± 0.77
HF ($\ln ms^2$)§	$7.33^{\#}$ ± 1.00	7.10 ± 1.00	7.02 ± 1.03	6.91 ± 1.23	6.86 ± 1.06
ln (LF/HF)§§§	$-0.24^{**,\#\#}$ ± 0.93	0.12 ± 0.87	0.47 ± 0.91	0.39 ± 0.74	0.64 ± 0.81
$\alpha^{\S\S}$	$0.80^{**,\#\#,\$}$ ± 0.19	0.94 ± 0.22	1.03 ± 0.23	1.06 ± 0.24	1.08 ± 0.19
Resp. rate (min^{-1})	15.5 ± 2.0	15.6 ± 2.0	15.8 ± 2.0	16.4 ± 2.1	16.2 ± 2.8
SD (resp. rate) (min^{-1})	2.3 ± 0.9	2.4 ± 1.0	3.0 ± 0.9	3.0 ± 1.2	2.9 ± 1.8
HRR	4.1 ± 1.2	4.2 ± 1.1	4.2 ± 1.2	4.0 ± 1.2	4.2 ± 1.3
PCR	4.1 ± 1.1	4.2 ± 1.1	4.1 ± 1.2	4.0 ± 1.1	4.0 ± 1.1

$^{\S}P < 0.05$, $^{\S\S}P < 0.01$, and $^{\S\S\S}P < 0.001$; $^{*}P < 0.05$ versus daylight (end); $^{**}P < 0.01$ versus daylight (end); $^{\#}P < 0.05$ versus daylight (middle); $^{\#\#}P < 0.01$ versus daylight (middle); $^{\$}P < 0.05$ versus blue light.

domain was constant throughout the procedure (73 ms). In the frequency domain LF, HF, and LF/HF showed variations with respect to the sequence of the visual stimuli, whereas the changes of VLF were slightly above the level of significance ($P = 0.0526$). LF was lower during daylight at the beginning compared to daylight in the middle ($6.84 \ln ms^2$ versus $7.31 \ln ms^2$, $P < 0.05$) and daylight at the end ($6.84 \ln ms^2$ versus $7.39 \ln ms^2$, $P < 0.05$). Furthermore, LF was also lower during red light compared to daylight at the end ($6.94 \ln ms^2$ versus $7.39 \ln ms^2$, $P < 0.05$). On the contrary, HF was only higher during daylight at the beginning compared to daylight in the middle ($7.33 \ln ms^2$ versus $7.02 \ln ms^2$, $P < 0.05$). LF/HF was lower during daylight at the beginning compared to daylight in the middle (−0.24 versus 0.47; $P < 0.01$) and compared to daylight at the end (−0.23 versus 0.64, $P < 0.01$). With respect to fractal properties of heart rate variations the self-similarity parameter α was also lower during daylight at the beginning compared to daylight in the middle, blue light and daylight at the end (0.80 versus 1.03, $P < 0.01$; 0.80 versus 1.06, $P < 0.05$ and 0.80 versus 1.08, $P < 0.01$).

The average respiratory rate was 5.5 to 16.4 cycles per minute (cpm). The standard deviation of respiratory rate ranged from 2.3 to 3.0 cpm. Both parameters did not change during the succession of the different visual stimuli. The average $m : n$-ratio of cardiorespiratory coordination as quantified by HRR and PCR did not also change. They were both close to 4 during the different visual stimuli.

The centring (or confinement) of cardiorespiratory coordination with respect to the 4 : 1-ratio was as follows. During red light a clear linear relation was found in the centering diagram as indicated by the correlation coefficient r (see Figures 3(a) and 3(c); HRR: $r = -0.72$, $P < 0.01$; PCR: $r = -0.68$, $P < 0.01$). Hence, the larger the deviation from 4 during daylight before the color stimulus the larger the difference $HRR_{dur\_col} - HRR_{bef\_col}$ to achieve the centring (confinement) during the color light stimulus. As centring leads to

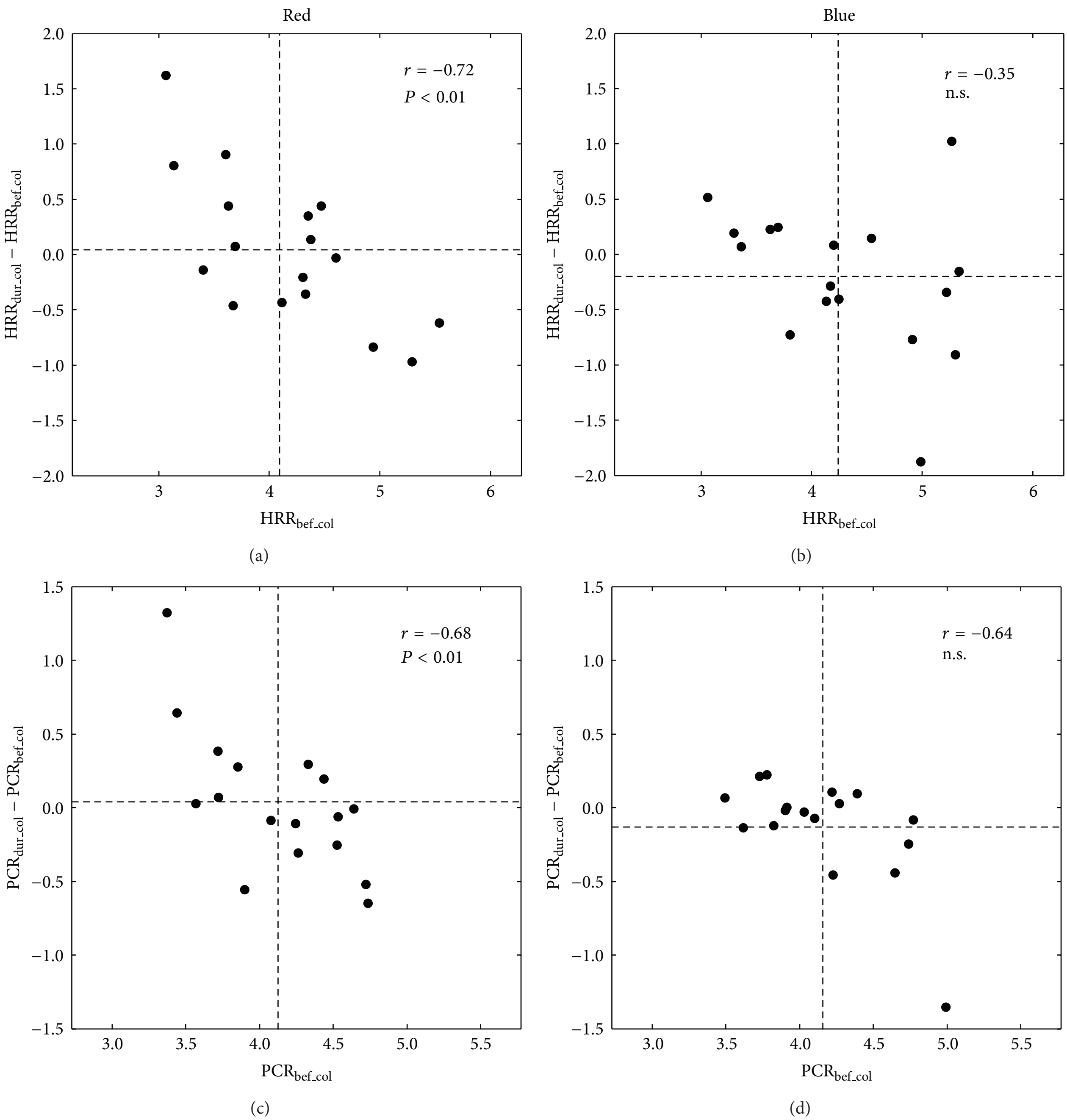

Figure 3: Analysis of centering of cardiorespiratory coordination towards 4 : 1 during exposure to red and blue color lights. (a), (c) During red light the significant negative correlation coefficient r for HRR and PCR indicates a centering towards 4 : 1. (b), (d) During blue light a centring does not occur; that is, the correlation is not significant. Note that in diagram (d) the high correlation r is spuriously caused by the outlier in the lower right part of the diagram. Dashed lines indicate average HRR_{bef_col} (and PCR_{bef_col}) and average $HRR_{dur\_col} - HRR_{bef\_col}$ (and $PCR_{dur\_col} - PCR_{bef\_col}$), respectively.

a narrower distribution of HRR (and PCR) the standard deviation of HRR (and PCR) was also lower during red light (see Table 1). Note that centring does not affect the average HRR (and PCR). During blue light HRR and PCR did not centre (Figures 3(b) and 3(d); HRR: $r = -0.35$, n.s., PCR: $r = -0.64$, n.s.). Note that the relatively large correlation coefficient in Figure 3(d) is caused by the outlier (correlation coefficient without outlier: $r = -0.44$, $P = 0.10$). We also checked centring diagrams for all other combinations of visual stimuli. None of these centring diagrams showed a significant negative correlation, that is, centring towards the 4 : 1-ratio.

4. Discussion

Visual stimuli of red and blue light without standardization of illuminance did not change average heart rate and respiratory rate throughout the procedure. In brief, LF oscillations increased during the procedure whereas HF oscillations decreased during the first part of the procedure. LF/HF showed more detailed results: this parameter only increased during the exposure to daylight. That is, red and blue lights did not alter LF/HF, whereas daylight in the middle and daylight at the end did. The self-similarity parameter α increased during daylight at the end compared to daylight at the beginning. Furthermore, during red light cardiorespiratory coordination as expressed by HRR and PCR centred (confined) towards 4 : 1 (4 heartbeats in one respiratory cycle). That is, HRR and PCR were closer to 4 : 1 during red light than during daylight at the beginning. Blue light did not show any effect on cardiorespiratory coordination.

Recent studies showed clear effects of visual stimuli on heart rate and HRV. Healthy subjects exposed to bright light (>5000 lx) showed an increase of the average heart rate [18]. The authors state that the increase of heart rate may be primarily dependent on the light-induced increase of sympathetic activity of the ANS. In another study, HRV was altered during the exposure to colored light for 10 minutes, whereas the average heart rate did not change [20]. VLF oscillations increased during red and green lights, whereas they decreased during blue light. Straight after the color stimuli HRV returned to the values that were observed before the color stimulus. The origins of the VLF component are still a matter of debate [11, 28]. Some authors suggest that both, the very low frequency variations of vagal baroreflex sensitivity and the very low frequency variations of HRV, are caused by vagal modulations [29]. Hence, the changes of the VLF component could be cautiously interpreted in terms of changes of vagal activity. LF oscillations did not change and HF oscillations decreased during green light only. Hence, vagal activity decreased during green light. These results were obtained only during the visual stimulation with a standardized level of illuminance (700 lx). Visual stimuli with dim light (1 lx) also led to alterations of the ANS [21]. However, these results were obtained after the visual stimulation. Heart rate decreased after visual stimulation with dim red, blue, and green lights compared to baseline. LF oscillations increased after red and blue lights, whereas HF decreased after red light. As a consequence, the sympathovagal balance LF/HF decreased after red light.

In the present study we found an increase of LF oscillations and a decrease of HF oscillations after the visual stimulation with red light. That is, sympathetic oscillations increased, whereas parasympathetic oscillations decreased after red light. Consequently, LF/HF increased. These findings are in accordance with the above mentioned findings of Choi et al. [21]. Furthermore, we found an increase of the self-similarity parameter α after the red light stimulus. This increase is associated with changes in the overall spectral density as α reflects the $1/f^{\beta}$ decay of the spectrum. In this particular case, more power is shifted to lower frequencies after the red light stimulus. In contrast to previous findings, we did not find any alteration of the average RR-interval, that is, average heart rate, caused by a color light stimulus. The differences during the color light stimulus as described by Schäfer and Kratky [20] could also not be confirmed.

The differences of the results with respect to the study by Schäfer and Kratky could be caused by the different levels of illuminance (700 lx versus 30 to 100 lx). Even low levels of illuminance may have an impact on the ANS because the ipRCGs of the nonimage forming visual system even respond to few photons [30] and, hence, may also affect the SCN and the ANS. The synaptically mediated response of the ipRCGs caused by low light stimulus [4] supports this notion.

The only simultaneous effect during colored light exposure was the centring of cardiorespiratory coordination towards 4 : 1 during red light as indicated by HRR and PCR. Note that HRR is a simple measure of cardiorespiratory coordination [25], whereas PCR is more strict with respect to the temporal coordination of heart beat and respiration [14]. Hence, during red light the coordination was adjusted such that the 4 : 1-ratio emerged. Such a coordination has been observed in healthy subjects during quiet rest [12, 13] as well as during nighttime sleep [17, 27, 31]. The emergence of this coordination indicates a process of relaxation and recovery of the organism. We note that this kind of relaxation is different from, for example, an increase of the high frequency oscillations of HRV as an indicator of increased vagal activity [32].

With respect to the limitations we note that especially the effects of blue light may be confounded by the previous red light stimulus. The response of the ipRGCs upon visual stimuli is more sustained than that of conventional RGC [4]. Hence, carry over effects could be responsible for the results of the blue light stimulus. A randomized balanced design with respect to the succession of colored light stimuli may also reveal specific effects of blue light on HRV or cardiorespiratory coordination. Ideally, each color stimulus should be investigated separately to avoid carry over effects. The increase of LF oscillations and decrease of HF oscillations during the procedure could be avoided if more time would be allowed to acclimatize before starting the procedure. However, a longer adaptation period could also lead to an increased tiredness caused by low levels of alertness during the adaptation period.

In conclusion, the exposure to red light showed a centering of cardiorespiratory coordination towards 4 : 1. Furthermore, red and blue lights altered autonomic nervous functions as expressed by HRV. These results may lead to a rationale for the application of colored light as a therapeutic means as used in Anthroposophic Arts Therapies. The physiological mechanisms leading to these alterations need to be explored.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Research Article

Auricular Acupressure to Improve Menstrual Pain and Menstrual Distress and Heart Rate Variability for Primary Dysmenorrhea in Youth with Stress

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Background. Dysmenorrhea and accompanying symptoms can have a negative impact on academic achievement, physical activity and functioning, and quality of life. Unfortunately, stress increases the sensitivity and severity of pain, activating sympathetic responses while inhibiting parasympathetic responses. *Objective*. This study used objective, physiological measurements to evaluate the effects of auricular acupressure on menstrual pain and menstrual distress in young college students with primary dysmenorrhea across two menstrual cycles. The aim was to determine if significant differences could be detected between the intervention and follow-up phases after controlling life stress. *Design*. A one-group experimental research design was used, and repeated measurements and followups were done. Thirty-two women completed questionnaires and physiological parameters were measured. *Results*. Significant differences between the intervention and follow-up phases were found for high frequency (HF) and blood pressure on day 1 and no significant differences in menstrual pain and menstrual distress, heart rate variability, low frequency (LF), LF/HF ratio, or heart rate. *Conclusion*. Auricular acupressure effectively increases parasympathetic activity to maintain autonomic function homeostasis in young women with primary dysmenorrhea and may have a value in alleviating menstrual pain and menstrual distress in a high-stress life. Future studies should consider stress, stimulus dose of auricular acupressure, severity of menstrual pain, and a longitudinal research design.

1. Introduction

The prevalence of dysmenorrhea in adolescents and young women ranges between 40% and 90% and varies with age, country of residence, and population density [1–5]. Dysmenorrhea refers to cramp-like, dull, and throbbing pain that emanates from the lower abdomen [6, 7], often accompanied by nausea, vomiting, headaches, backaches, weakness, diarrhea, sleeplessness, or nervousness [8]. Such debilitating symptoms limit daily activity [9] leading to short-term school absenteeism and have negative consequences on health-related quality of life [9, 10]. In addition, dysmenorrhea is associated with decreased exercise performance [11], increased pain sensitivity [12], and changes in gray-matter volume [13] and brain metabolism [14]. Alleviating menstrual pain and menstrual distress is a critical women's health concern. Dysmenorrhea is significantly and positively associated to perceived levels of stress, occurring with an odds ratio of 2.4 in high-stress female populations compared to low-stress populations [15]. Stress refers to a state of threatened homeostasis and ubiquitous presence [16]. It tends to increase a person's sensitivity to pain although there is wide variability among individuals [17]. Dysmenorrhea has been found by many to be associated with psychological stress [18, 19], as well as stress from daily life [20] and work [21, 22].

Stress and pain both activate the sympathetic nervous system to release epinephrine and norepinephrine, which increase heart rate (HR), cardiac contractility, vascular smooth muscle contraction, and blood pressure (BP) [16, 23]. At the same time, stress and pain reduce activation of the parasympathetic nervous system (PNS) responses [24, 25]. Heart rate variability (HRV) is a convenient noninvasive method for measuring overall autonomic nervous system activity, based on interactions between the sympathetic and parasympathetic nervous systems [26]. HRV measurements incorporate a low-frequency (LF) component that represents sympathetic nerve activity, and a high-frequency (HF) component reflecting vagal activity. The LF/HF ratio mirrors the sympathovagal balance and reflects modulations in sympathetic activity [27]. HR and BP must also be considered as elements of the sympathetic reaction [16]. Autonomic fluctuations in response to pain usually lead to changes in cardiovascular parameters, such as increases in BP [28, 29] and HR [24, 30] and a decrease in HRV [24, 30]. Women experiencing primary dysmenorrhea possess low parasympathetic nerve activity throughout their menstrual cycle [31], but such activity increases after acupuncture stimulation [32]. In healthy women, however, there is no association between pain perception and HR [33]. Investigating the relationship between pain and autonomic regulation in women with dysmenorrhea is the focus of this study.

Approximately 67% of young females with primary dysmenorrhea take analgesic drugs [34], which can only alleviate pain temporarily [35]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and oral contraceptives pills (OCPs) are prescribed for the relief of menstrual pain, and a systematic review study concluded that such treatments are effective [7]; however, it was noted that NSAIDs and OCPs can induce or exacerbate preexisting hypertension [36]. NSAIDs are associated with a variety of adverse effects including gastrointestinal disorders, nephrotoxic, and hepatotoxic effects, hematologic abnormalities, and fluid retention [37] and fail to alleviate menstrual pain in 20% to 25% of women [38]. OCPs also have a host of side effects including nausea, vomiting, headaches, breast tenderness, acne, weight gain, and depression [39]. Therefore, the search for an alternative yet effective nonpharmacological interventions to relieve pain in dysmenorrhea is necessary. The result of a recent systematic review and meta-analysis indicates that acupoint stimulation is an effective intervention for primary dysmenorrhea [40]. Auricular acupressure is a noninvasive acupoint stimulation that transmits signals to the brain and to specific organs to modulate and harmonize physiological function [41]. The positive effects of auricular acupressure to alleviate menstrual pain and menstrual distress have been studied and reported [42–46], although some of these studies lacked methodological rigor [40] or failed to measure physiological indicators [45].

2. Purpose Statements

In this study, physiological parameters were measured across two menstrual cycles in young college students with primary dysmenorrhea in order to objectively evaluate the effects of auricular acupressure. We hypothesized that after controlling for life stress, significant differences would be identified for menstrual pain, menstrual distress, and HRV physiological parameters between the intervention and follow-up periods.

3. Materials and Methods

3.1. Research Design and Participants. A single-group experimental research design was used, and repeated measures were done in the intervention and follow-up phases. A convenient sample of young college students with primary dysmenorrhea was recruited from a college in northern Taiwan. All participants were given auricular acupressure to relieve their menstrual pain and menstrual distress for a certain period. The inclusion criteria were (1) 18 to 25 years of age, (2) menstrual-cycle duration between 25 and 40 days, (3) body mass index between 18.5 and 24.9 kg/m^2, and (4) pain score 3. Women meeting the following criteria were excluded: (1) diagnosis of pelvic disease, gynecological disease or surgery, or secondary dysmenorrhea, (2) chronic disease, such as diabetes, renal disease, or cardiovascular disease, (3) serious arrhythmia or pacemaker user, (4) habitual smoking or consumption of stimulant beverages such as tea, coffee, or alcohol, and (5) swelling, infections, and ulcers in both ears. Sample size was estimated using G Power software and was based on the study of Yeh et al. [45], which indicated that auricular acupressure was effective in relieving menstrual pain, providing an average (±standard deviation) improvement of 5.14 ± 2.32 points in the visual analog pain score. An estimated sample size of 32 would be required to demonstrate significant at the 5% probability level with 80% power. Figure 1 shows the flowchart of research design and participants of this study. Menstrual pain, menstrual distress, and HRV physiological parameters were measured.

3.2. Intervention. Based on a literature review of auricular acupressure for treating dysmenorrhea, six common auricular acupoints were used: *internal genitals*, *endocrine*, *shenmen*, *sympathesis*, *liver*, and *kidney*. The *internal genitals* acupoint was selected to dredge the meridian and normalize circulation, eliminate stasis, and alleviate pain. The *endocrine* acupoint was targeted to harmonize physical function, regulate menstruation, and improve menstrual distress. The *shenmen* acupoint was used to reduce pain and provide tranquility [47], while the *sympathesis* acupoint was to normalize autonomic nervous system and vasomotor functions, relieve muscle spasm, and enhance the analgesic effect [47, 48]. The *liver* acupoint was stimulated to disperse stagnated liver qi for relieving stagnation and to regulate the flow of qi for alleviating pain, and the *kidney* acupoint was stimulated to coordinate Chong and conception vessels, invigorate kidney qi, and active qi and blood for the relief of pain [48]. Cowherb seeds with adhesive patches were embedded on the specific acupoints two to three days before menstruation, and the application of pressure was initiated at the onset of menstrual pain. All participants were instructed to press each acupoint for 1 minute, 4 times per day until they achieved relief of menstrual pain. They were also informed

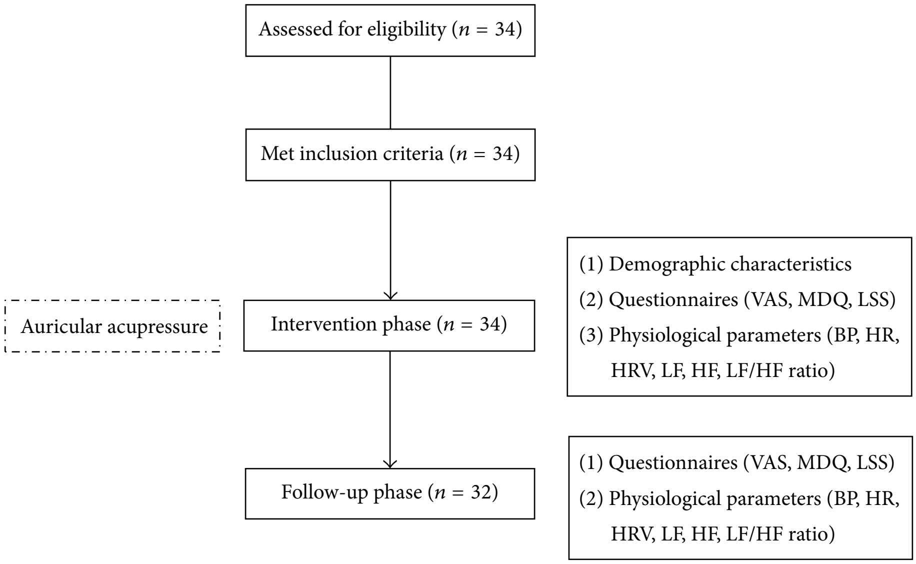

Figure 1: Study flowchart.

that they may experience various sensations while applying pressure: numbness, swelling, mild pain, or warmth. The adhesive patch and Cowherb seed were removed accordingly only if pain had been relieved for 48 hours.

3.3. Measures. Menstrual pain was evaluated using a 100 mm horizontal visual analogue scale (VAS) where 0 represented no pain and 100 indicated unbearable pain. Participants were instructed to indicate a point on the scale corresponding to the pain intensity. The distance from the left end to the selected point was measured to calculate the pain score in millimeters. Higher scores represent higher intensity of menstrual pain. Menstrual distress was measured using the modified 16-item Menstrual Distress Questionnaire (MDQ) that assesses menstruation related symptoms (pain, water retention, and autonomic reactions) during the premenstrual and menstrual periods [49]. Each item was scored from 1 (no symptoms) to 4 (severe symptoms), with higher scores reflecting higher severity of distress. Cronbach's alpha for the internal consistency reliability was 0.83 in the earlier study [45] and 0.80 in this study.

HRV was measured using an ANSWatch wrist monitor (Taiwan Scientific Co., Taipei, Taiwan). This monitor uses multiple piezoelectrical sensors in the cuff to measure blood pressure waveforms in the radial artery. HRV, LF, HF, and LF/HF ratio were analyzed based on the international standard [27]. The accuracy of ANS monitor was represented by the correlation between HRV parameters and EKG [50]. HRV measurements were taken between the hours of 8 pm and 10 pm, and participants were instructed to refrain from eating, drinking stimulant beverages (such as tea, coffee, and alcohol), smoking cigarettes, and exercising 2 hours prior to the measurements. Participants were first subjected to rest quietly for 10 minutes in a sitting posture; and were then assisted to wear the ANS monitor on the left wrist, instructed to close eyes, to relax and remain quiet, and to not move for 7 minutes while waveforms were being recorded. HR and BP were measured at the same time. Data were downloaded to a notebook using the ANS Watch Manager Pro software.

The Chinese version of the Life Stress Scale (LSS) [51] was used to measure life stress over the preceding month. The LSS consisted of 29 items categorized into six subscales: including academic stress, family stress, interpersonal stress, emotional stress, employment stress, and self-cognition stress. Each item was scored from 0 (no stress) to 4 (extremely stressful). Higher scores indicated a higher level of life stress. Cronbach's alpha was 0.92 from a previous study [51] and 0.92 in this study.

3.4. Procedures and Data Analysis. The study protocol and design were reviewed and approved by the Chang Gung Medical Foundation Institutional Review Board (reference number: 100-2728A3). Verbal and written informed consent were obtained from all participants after informing them of the study design, intervention, data collection, and the rights of the participants. They were made aware that all data remained confidential at all times and that they were free to withdraw at any time during the study without affecting their academic grades. Interventions and data collection were performed by the researcher and trained research assistants. Menstrual pain and HRV parameters were measured repeatedly during the intervention phase and the follow-up phase; while menstrual distress and life stress levels were measured once at the end of menstruation during the intervention phase and follow-up phase. Day 1 indicated the day of greatest menstrual pain during the menstrual cycle. Adverse effects of the intervention were also recorded. Data were analyzed using IBM SPSS 20.0 for Windows. Descriptive statistics was used to analyze demographics. A paired t-test was used to test for differences between the two phases in VAS, MDQ, physiological parameters, and LSS. $P < 0.05$ was considered statistically significant.

Table 1: Participants' demographic characteristics.

Characteristics	Mean ± SD	n (%)
Age (years)	20.78 ± 1.53	
Age at menarche (years)	11.94 ± 0.91	
Menstrual cycle (days)	30.97 ± 3.28	
Menses duration (days)	6.28 ± 1.37	
Past pain intensity	7.75 ± 1.53	
Menstrual regularity		
Yes		25 (78.1%)
No		7 (21.9%)
Initial onset of menstrual pain		
Menarche		3 (9.4%)
<1 year after menarche		14 (43.8%)
1-2 years after menarche		9 (28.1%)
Others		6 (18.8%)
Time of dysmenorrhea		
Day before menses		8 (25%)
First 2 days in menses		23 (71.9%)
Others		1 (3.1%)

4. Results

Thirty-four women were recruited for the study, two of whom later withdrew due to personal reasons. Thus, 32 women completed the study, and the attrition rate was 5.88%. Table 1 shows the demographic characteristics of the participants. The mean age for women in the study was 20.78 ± 1.53 years, and the mean age at menarche was 11.94 ± 0.91 years. The average menstrual cycle length was 30.97 ± 3.28 days, and the mean menses duration was 6.28 ± 1.37 days. Most participants had regular menstruation and first experienced menstrual pain less than two years after menarche. Menstrual pain occurred in the first two days of menses. Past pain intensity was 7.75 ± 1.53.

Table 2 shows the comparison of MDQ and LSS between the intervention phase and follow-up phase. The MDQ was slightly higher in the intervention phase, but the difference was not statistically significant ($P = 0.26$). LSS was found to be significantly higher during the intervention phase compared to the follow-up phase ($P = 0.001$). Figure 2 shows the comparison of VAS and physiological parameters on days 1–3 of the two phases. Significant differences between the two phases were found on day 1 for HF ($P = 0.01$), systolic BP ($P = 0.005$), and diastolic BP ($P = 0.001$), but not for menstrual pain ($P = 0.75$), HRV ($P = 0.70$), LF ($P = 0.40$), LF/HF ratio ($P = 0.12$), and HR ($P = 0.89$). No significant differences were found for VAS or other parameters on days 2 and 3 ($P > 0.05$).

5. Discussion

The average age of menarche for the participants in this study was 12 years. Most participants first experienced menstrual pain within two years of menarche, and the menstrual pain continued for nearly 7 to 8 years. Menstrual pain

Table 2: Menstrual distress and life stress in the intervention and follow-up phases.

Variables	Intervention Mean ± SD	Follow-up Mean ± SD	Paired t-test
Menstrual distress	29.47 ± 5.95	28.31 ± 5.95	1.15
Life stress	31.97 ± 16.32	28.53 ± 16.38	3.75^{**}

$^{**}P < 0.01$.

of the participants in this study persisted for 2 to 3 days during the menstrual cycle, which is consistent with other studies [37, 52]. Menstrual pain was similar during the first three days of the cycle in both the intervention phase and follow-up phase, whether auricular acupressure was used or not. For both the intervention and follow-up phases, menstrual pain level was at 5.74, 4.07, and 2.22 on days 1, 2, and 3, respectively. Without considering stress, this finding is not consistent with the effects of auricular acupressure for improving dysmenorrhea reported in other studies [42, 45, 46]. Indeed, life stress impacts menstrual pain in the intervention phase. With life stress influences [17], menstrual pain would be more serious if auricular acupressure was not given. Therefore, the effect of auricular acupressure on reducing menstrual pain was insignificant. Comparing to the results from follow-up phase with low life stress, this study supports that the effect of auricular acupressure is reduction of menstrual pain.

In addition, participants have an obviously lowered pain perception in the intervention (5.66) phase and follow-up (5.81) phase compared to the average pain intensity (7.75) from the previous month prior to intervention. This indicates that auricular acupressure may improve menstrual pain during the intervention phase and that the effect may persist through the follow-up phase. One the other hand, the effects of auricular stimulation for acute pain were immediate rather than long term while comparing standard medical care alone or in combination with auricular acupuncture [53]. It should be noted that no obvious reduction of menstrual pain was found between the intervention and follow-up phases in this study, and it seems that the stimulant dose of auricular acupressure is insufficient to alleviate menstrual pain. This study suggests that the protocol for acupressure application can be considered multiple times per day that may improve the overall duration of the effect. Further studies should also increase stimulus dose, including acupressure frequency, duration, and intensity and examine a prolonged course of intervention for achieving a long-term effect.

This study found that menstrual distress from menstrual-related symptoms was due primarily to pain, water retention, and autonomic reactions. Although auricular acupressure was provided, menstrual distress during the intervention phase was similar to the follow-up phase; making the result inconsistent with previous studies [42, 45, 46]. As mentioned previously, the participants would have had more pain and autonomic responses during the high life stress of the intervention phase, which should also be observed as a factor affecting the results of menstrual distress. Thus, there were no obvious effects of auricular acupressure in reducing

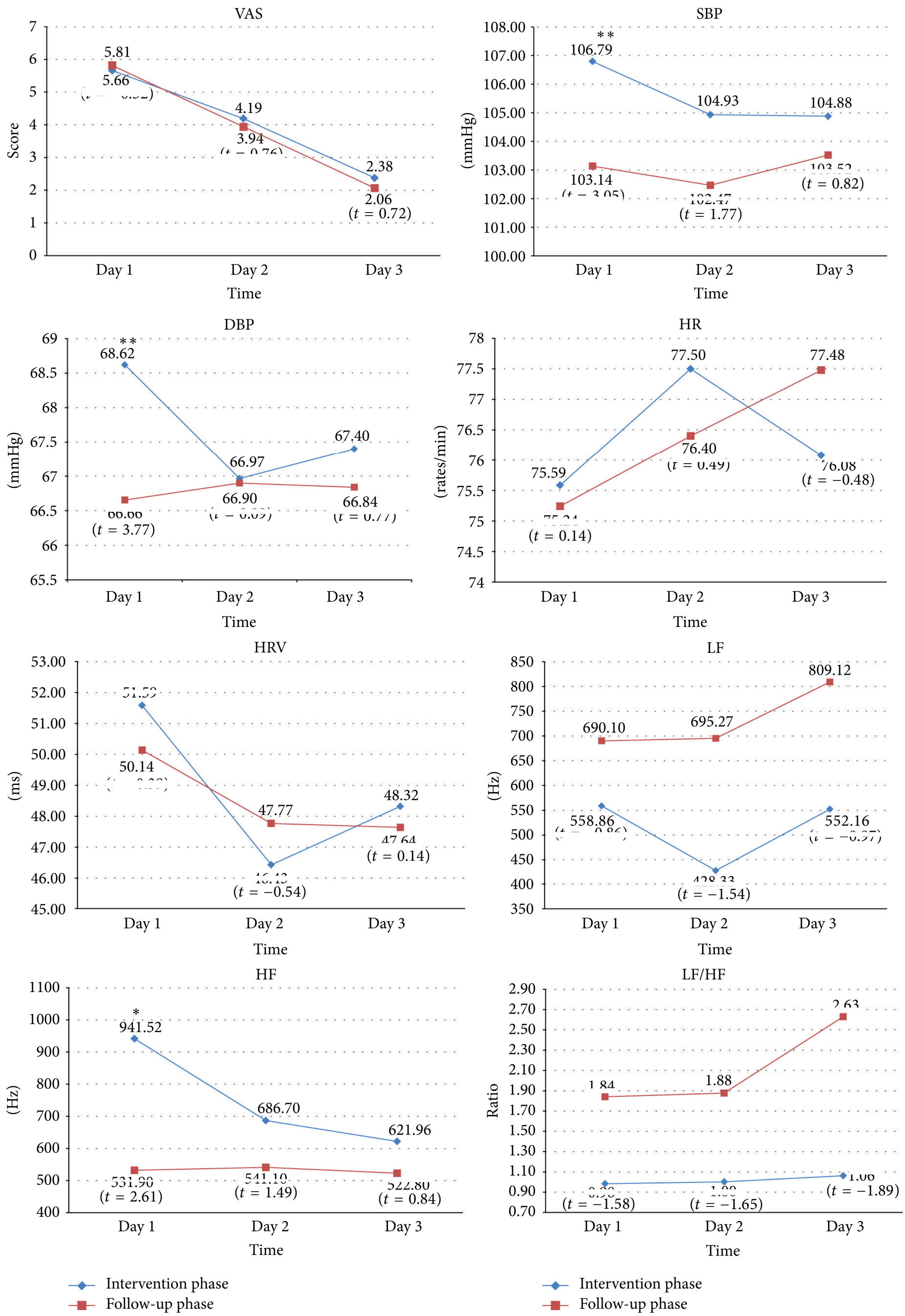

Figure 2: The comparison of VAS and physiological parameters on days 1–3, $^{*}P < 0.05$, $^{**}P < 0.01$.

menstrual distress. It is not surprising this is inconsistent with other studies that found that acupoint stimulation decreased water retention and the autonomic reactions of menstrual distress [53, 54].

This study supports the effect of auricular acupressure to effectively maintain the autonomic homeostasis in young women with primary dysmenorrhea, in terms of increasing the HF activity that regulates the menstrual cycle, but not LH activity or the LF/HF ratio. This is in agreement with other studies in postmenopausal women with insomnia [55] and in healthy adults [56, 57]. Auricular acupressure may stimulate the auricular branch of the vagus nerve, leading to an increase in parasympathetic activity and modifying both autonomic and central nervous system activity [58]. HF reflects vagal activity that contributes to the maintenance of homeostasis during menstrual pain. This finding is similar to that of other studies in which HRV was increased and LF/HF ratio was unchanged in healthy adults [57] and individuals with chronic insomnia [59], while sympathetic activity remained unchanged in healthy adults [56]. In contrast, LF signals reflect sympathetic activity. Other studies have found an increase in LF due to an increase in the intensity of the BP regulatory mechanism [60] or a decrease in LF due to response for improving insomnia [55]. In comparison to the reference LF/HF ratio of 0.8 to 1.5, we measured ratios of 0.98 to 1.06 in the intervention phase and 1.84 to 2.63 in the follow-up phase, which indicate a balance of sympathetic and vagal activity during the intervention phase. Therefore, this finding indicates that auricular acupressure increases parasympathetic activity and regulates the homeostasis of autonomic function in young women with primary dysmenorrhea.

Systolic and diastolic BP, but not HR, was significantly elevated on day 1 between two phases. This is inconsistent with previous studies that reported either a decrease [57] or no change in BP [61, 62]. Many studies have shown a decrease in HR for healthy adults [57, 60], individuals with chronic insomnia [59], and those experiencing anxiety associated with dental extractions [63]. Others have reported no change in HR for healthy adults [56, 61] and those experiencing anxiety before surgery [62]. Activating the vagus nerve typically causes a reduction in HR and BP [58]. However, in the presence of pain and stress, HR and BP are increased in response to vagus nerve stimulation [16, 23]. Accordingly, participants with high stress and pain in the intervention phase could activate sympathetic reactions, leading to an elevation of BP, while HR would remain unchanged due to acupoint-stimulation related simultaneous activation of the parasympathetic nervous system. Various ages, physical conditions, auricular stimulus doses, and measure time points may lead to different results. In addition, previous studies have shown no relationship between HR and pain perception in women [33]. Thus, HR may not be an appropriate indicator of pain outcome for women [62].

5.1. Limitations. This study has some limitations. The absence of a control group and baseline measurements makes it difficult to directly relate the outcome measures to the interventions. In addition, a small sample population was from a single college, which limited our ability to perform subgroup analysis (such as mild, moderate, and severe pain), and the extent to which the results can be generalized. Because the intervention was given during one menstrual period and the outcomes were measured over two cycles only, the long-term effects are unknown.

6. Conclusion

Auricular acupressure is an effective noninvasive intervention that increases HF to maintain autonomic function homeostasis in young women with primary dysmenorrhea. It may be valuable in alleviating menstrual pain and menstrual distress in high-life stress conditions. Life stress can increase menstrual pain and impact the effect of auricular acupressure. Increasing the simulation requirement of auricular acupressure in high life stress conditions should be considered. Further studies considering stress, using a longitudinal randomized-controlled design, expanding recruiting sites, enlarging sample sizes, involving individuals with differences in the severity of dysmenorrhea, modifying intervention doses, and increasing additional endpoints of timing should be considered.

Authors' Contribution

Mei-Ling Yeh and Jang-Geng Lin contributed equally to this work as cocorrespondence authors.

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Research Article

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

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

1. Introduction

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

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

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

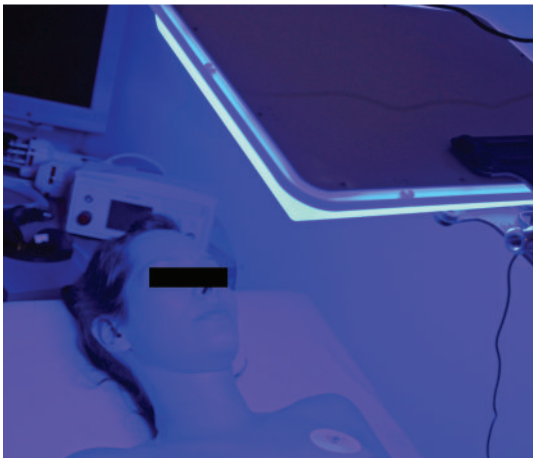
2. Materials and Methods

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

2.2. New Colored Light Stimulation Methods. Two color light panels (collaxx, mse elektronik, Frankenburg, Austria) were used in this study (see Figures 1(a) and 1(b)).

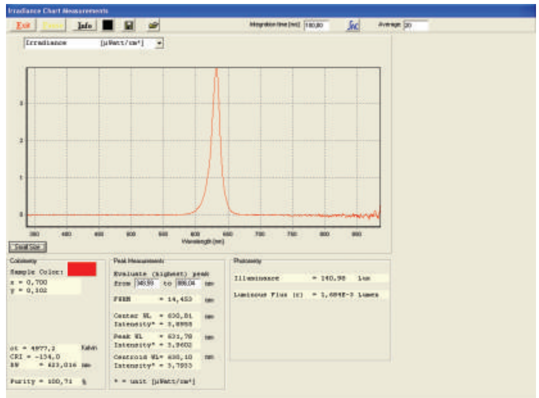
Both colors had almost the same illuminance (red: 140.98 lux, and blue: 140.27 lux, measured at a distance of 40 cm). Figure 2 shows the spectra of the two colors. In addition, the dominant wavelengths (DWred = 623.0 nm and

(a)

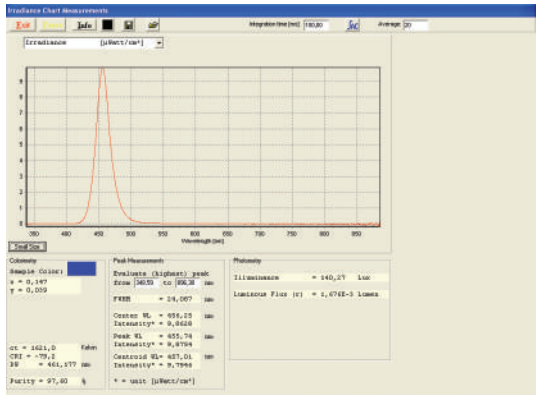


(b)

Figure 1: Stimulation with red (approx. 631 nm; (a)) and blue (approx. 456 nm; (b)) color panels (approx. 140 lux).



(a)



(b)

Figure 2: Colorimetry and photometry of the two light panels used in the study. (a) 631 nm, red; (b) 456 nm, blue.

DWblue = 461.2 nm) are indicated. DW is the wavelength which is important for the sensitivity of the human eye. The peak wavelength differs from the DW because both colors, red and blue, are located at the (opposite) margins of the visible light spectrum, and the human eye's sensitivity for brightness is drastically reduced in these regions.

2.3. Temperature Measurements. The temperature measurements were performed using a Flir i7 (Flir Systems, Wilsonville, USA) infrared camera which operates at a wavelength range from 7.5–13 μm. The focal distance of the infrared lens is $f = 6.8$ mm. The temperature measurement range is between −20°C and +250°C. Its accuracy lies at ±2% of the reading. Sensitivity is <0.1°C at 30°C, and the infrared resolution is 140 × 140 pixels. The system is ready for use in 15–20 seconds. We chose the forehead and the tip of the nose as locations for the thermographic measurements. Both areas were measured during illumination and also during the control phases before and after illumination.

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

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

2.5. Procedure. The experiment used a repeated-measures design with two different light conditions (see Figure 3) and took place during daytime (between 09:00 and 11:00) in July 2013 (room temperature: 28–30°C). Every volunteer completed the investigation. The persons were lying on a bed with closed eyes in a special lab of the Medical University of Graz, and the measurements started after a resting period of 5 minutes. The volunteers were exposed to the two differently colored stimulations for 10 minutes in randomized order.

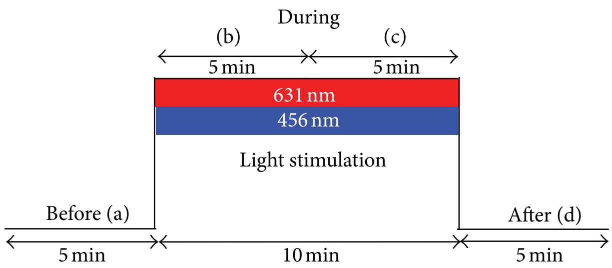


Figure 3: Experimental protocol.

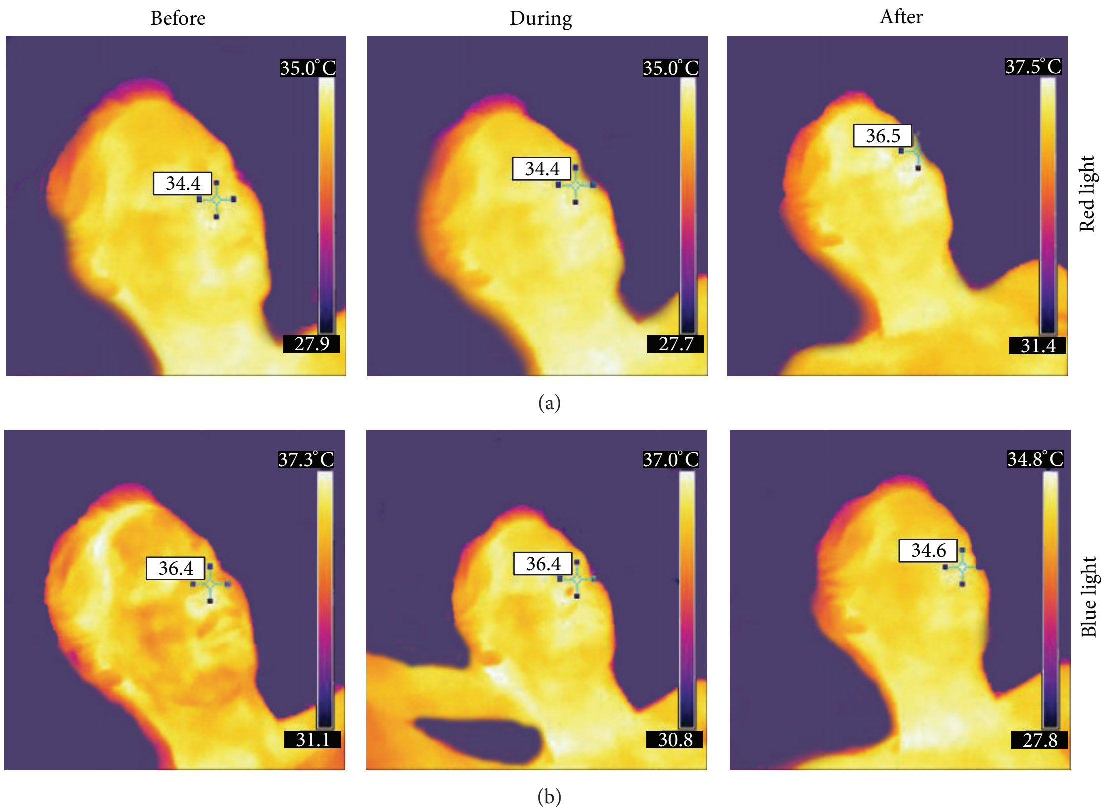


Figure 4: Six thermal images from a 23-year-old healthy female volunteer before, during and after red (a) and blue (b) light stimulation with closed eyes. Note the increase of the temperature of the nose after red and also the decrease after blue light irradiation.

Between the two different stimulation modalities there was a resting period of 10 min. This duration was chosen for practical reasons (e.g., HR should return to baseline values in this time). HRV was measured during stimulation ((b) and (c) in Figure 3), and also for 5 min before (a) and after (d) illumination with red or blue light. After exposure to the red and blue light, respectively, the volunteers reported their state of mind for each modality. These reports were categorized on a scale from 0 to 10 (0: positive, comfortable, and relaxed; 10: negative, uncomfortable, and anxious).

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

3. Results

A typical example of the results of thermal imaging is shown in Figure 4. The example demonstrates the face of a 23-year-old female. The room temperature was 30°C. It is interesting that after red light stimulation the temperature of the nose (marker in Figure 4) increases from 34.4°C to 36.5°C. In contrast, after blue light stimulation the temperature of the nose of the same person decreases from 36.4°C to 34.6°C.

Figure 5 summarizes data extracted from the thermal images. Blue light decreases temperature in most of the

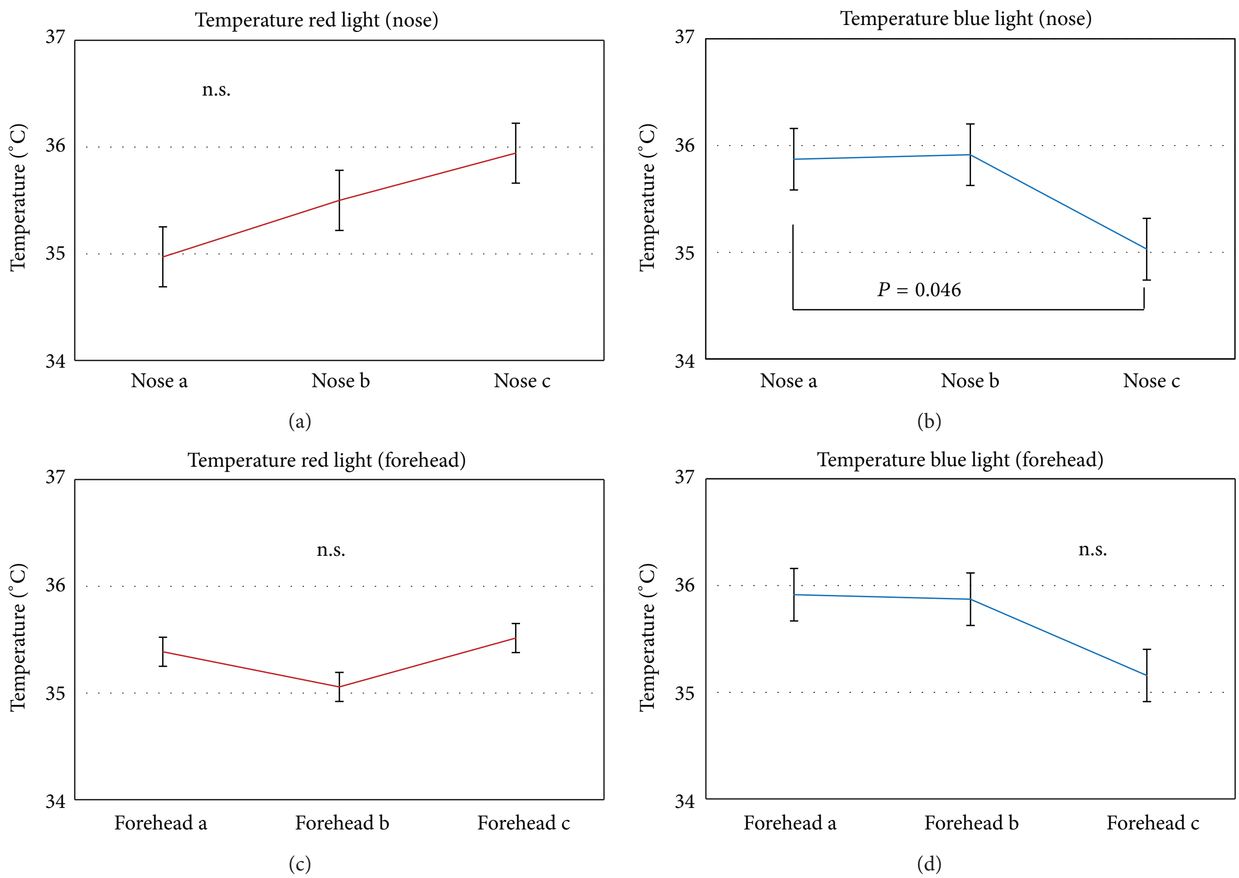


Figure 5: Temperature values at the nose and forehead before (a), during (b), and after (c) irradiation with red and blue light. Note the significant decrease of the temperature at the nose after blue light stimulation.

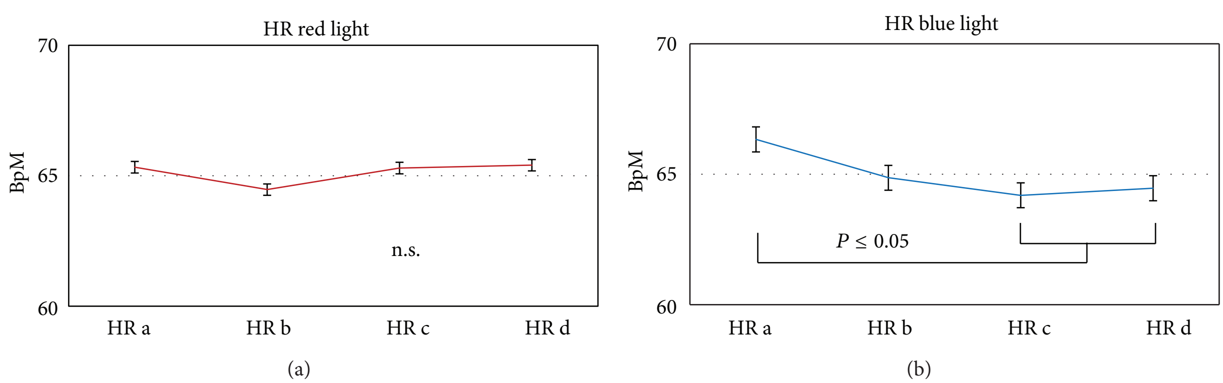
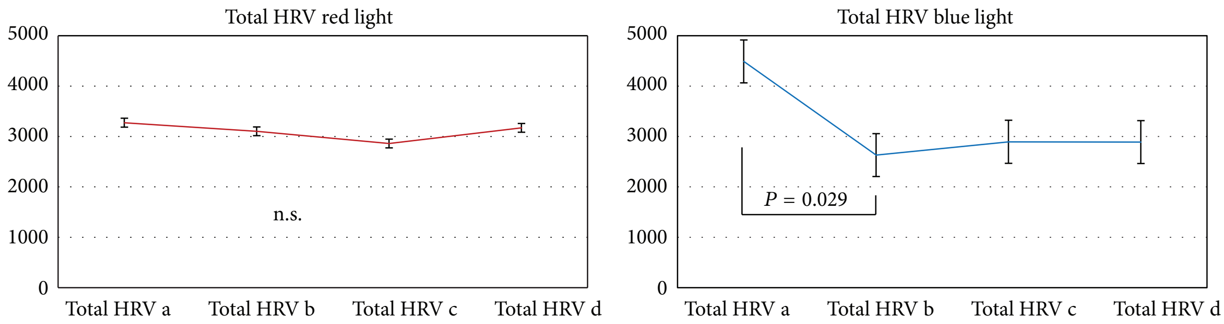


Figure 6: Graphics displaying the changes in mean heart rate (HR) of the 7 subjects receiving red light stimulation (a) and the same persons receiving blue light stimulation (b). HR decreased significantly only following stimulation with a wavelength of 456 nm.

volunteers (measured at the nose and forehead), whereas red light leads to a slight increase.

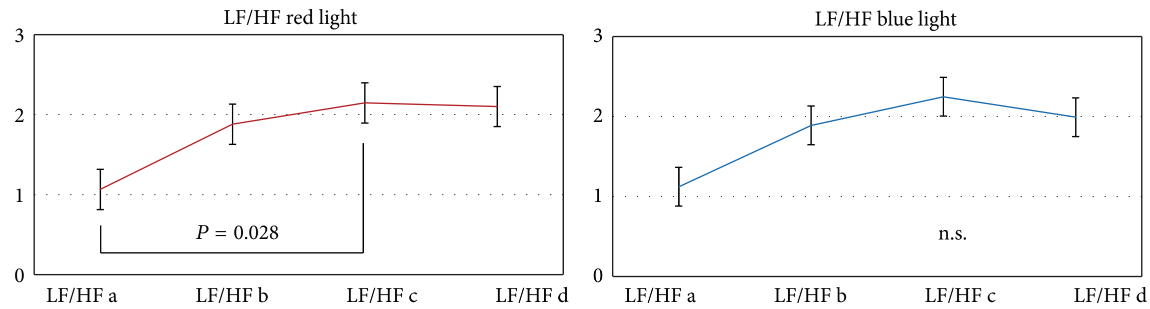
Figures 6 and 7 show the mean HR and total HRV from the ECG recordings of altogether seven healthy volunteers during the four measurement phases (a–d). There was a slight but not significant decrease in HR after stimulation onset with red light. However, in the blue light session HR decreased significantly ($P < 0.05$) during the second half of the stimulation phase and also in the 5 min period afterwards (Figure 6).

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



(a) (b)

FIGURE 7: Changes in total heart rate variability (HRV). Blue light (right) stimulation induced significant stimulation-related changes in total HRV in the seven subjects investigated in this study. No significant changes were found for red light stimulation.



(a) (b)

FIGURE 8: The low frequency (LF)/high frequency (HF) ratio changed significantly during red light stimulation ($P = 0.028$).

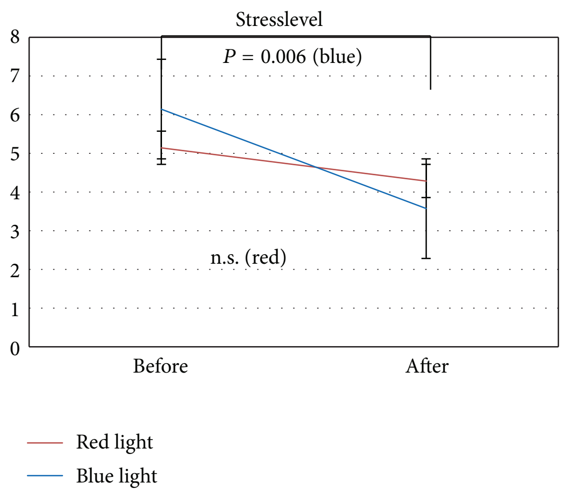


FIGURE 9: Stress level score evaluated in the 7 subjects.

Continuous HRV monitoring also showed significant alterations in the LF/HF ratio during red light stimulation (see Figure 8).

The results of the state of mind questionnaires are summarized in Figure 9. A significant ($P = 0.006$) decrease in the sense of a positive effect of well-being was found after blue light stimulation.

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

Conflict of Interests

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

Acknowledgments

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Research Article

Pilot Study of Acupuncture Point Laterality: Evidence from Heart Rate Variability

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The specificity of acupuncture points (acupoints) is one of the key concepts in traditional acupuncture theory, but the question of whether there is adequate scientific evidence to prove or disprove specificity has been vigorously debated in recent years. Laterality, or the tendency for acupoints on the right or left side of the body to produce different physiological effects, is an important aspect of acupoint specificity. Data is particularly scarce regarding the laterality of the same channel, same-named acupoint located on opposite sides of the body. The aim of this study was to investigate whether Neiguan (PC6) has laterality. A total of eighteen healthy female volunteers were recruited for this study. Electrocardiograms were recorded and heart rate variability was analyzed before, during, and after PC6 was stimulated on either the left or the right side. The results show that during acupuncture, there were significant differences in the standard deviation of RR intervals (STDRR), root mean square of successive differences between RR intervals (RMSSD), and total power between the left PC6 stimulation group and the right PC6 stimulation group, which indicates that PC6 may have laterality.

1. Introduction

Acupuncture has been widely used as a healing modality for at least 2500 years [1]. Over the past 30 years, researchers have demonstrated the neurobiological basis for the analgesic effects of acupuncture, which has led to greater acceptance of acupuncture in the scientific community [2, 3]. However, a number of well-designed clinical trials have reported that although "true" acupuncture is superior to usual care, it does not significantly outperform sham acupuncture [1, 4]. These findings are apparently at odds with traditional theories regarding acupuncture point specificity because in traditional Chinese medicine (TCM) theory, acupoint specificity is an essential principle for therapeutic efficacy. On the other hand, some clinical trials [5, 6] and studies using fMRI technology [7, 8] have demonstrated acupoint specificity.

For example, in a previous study performed by our group, we found that ipsilateral stimulation of Hegu (LI4) corresponded to increased blood perfusion in the contralateral Hegu (LI4) [9]. Moreover, the increased degree of blood perfusion was asymmetrical [10], which suggests the laterality, or specificity, of this acupuncture point [11].

In clinical practice, Neiguan (PC6) is one of the most commonly used acupoints and is indicated for treating cardiovascular related disorders in classical texts [12–14]. However, the differences between the bilateral PC6 acupoints have never been scientifically investigated. The aim of this study is to investigate the specificity of bilateral PC6 points based on heart rate variability.

2. Materials and 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. 18 healthy female volunteers were recruited in this study. All subjects were students from the China Academy of Chinese Medical Sciences and Beijing University of TCM. None of the subjects had a history of prior disease

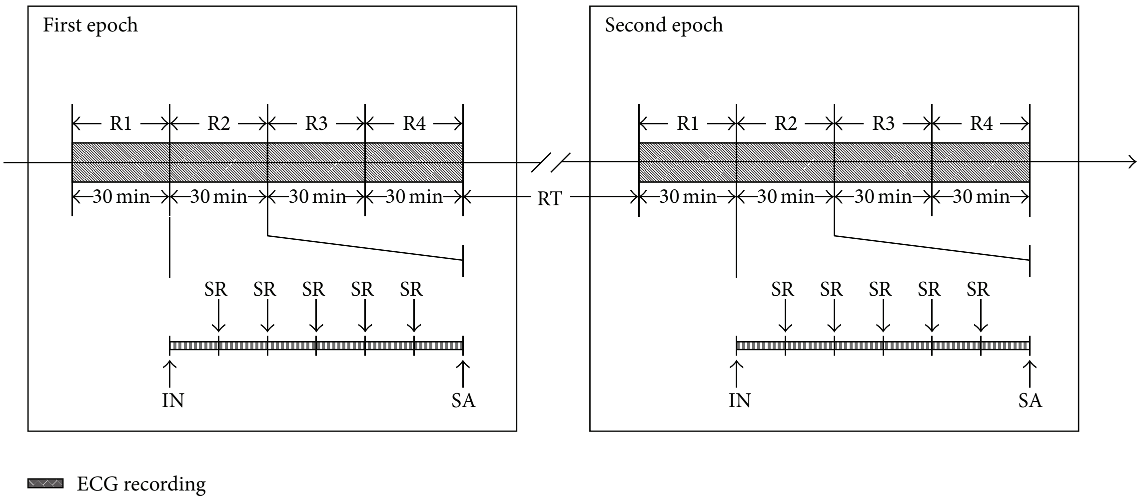

Figure 1: Procedure of acupuncture and measurement. R_i ($i = 1, 2, 3, 4$): ECG recording time point; IN: insert needle; SA: stop acupuncture; SR: slowly rotate the needle every 5 min (the needle was slowly rotated every 5 min for a total of 30 min during the R2 acupuncture session); RT: rest time between two epochs, the range is 8–22 days.

Table 1: Basic characteristics of the study participants ($n = 18$).

Characters	Mean ± SD	Range
Age (years)	25.61 ± 2.38	33–21
High (cm)	161.78 ± 4.60	172–150
Body weight (kg)	50.50 ± 4.89	60–42
Body mass index (BMI)	19.28 ± 1.58	22.04–15.79
Interval of two measures (days)	13.28 ± 3.99	22–8

nor had they taken any medication in the six months prior to the study. Each subject was provided with informed consent and had an adequate understanding of the procedure and purpose of this study. Basic characteristics of the participants are shown in Table 1.

2.3. Electrocardiogram Measurement Protocol. Before the laboratory procedure began, subjects were placed in a temperature-controlled room (24–26°C) to rest for 10 minutes. The ECG recordings were processed with standard II electrocardiographic lead on NeurOne system (NeurOne, MEGA Electronics Ltd, Finland). The data were digitized with a sampling rate of 500 Hz. In every epoch, the 4 segments of successive ECG were recorded and symbolized as R1 to R4. In each segment, a 30 min ECG recording was obtained using the NeurOne system (shown in Figure 1).

2.4. Acupuncture Protocol. For every participant, either the right or left PC6 was stimulated during the first epoch of the study and the opposite side PC6 was stimulated during the second epoch. The stimulus order was determined randomly and the interval time between the two epochs was at least 8 days. For the acupuncture procedure, a small acupuncture needle, 0.25 × 25 mm (100112, Zhen Huan), was gently inserted to a depth of 15 mm in Neiguan (PC6). The needle was slowly rotated every 5 min for a total of 30 min during the acupuncture session in order to maintain the soreness and numbness sensation of De-Qic [9, 15]. The acupuncture process is illustrated in Figure 1.

2.5. Data Analysis. The raw data recorded by NeurOne system was exported with ASC format and then imported into Kubio HRV software and analyzed [16]. The analysis parameter was default. In the time domain, the mean heart rate (HR), the standard deviation of RR intervals (STDRR), and the root mean square of successive differences (RMSSD) were analyzed. In the frequency domain, the power spectrum density was analyzed with AR spectrum method in normalized units. The low frequency (LF) and high frequency (HF) were defined as 0.04–0.15 Hz and 0.15–0.4 Hz, respectively. Data are expressed as Mean ± SD. For every recording point, the paired t-test was performed between RS group and LS group. The level of significance was defined as $P < 0.05$. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 and Figure 2(a) present the mean value of heart rate during the two epochs of the experiment. HR did not change significantly between the right side (RS) group and left side (LS) group.

3.1. Time Domain Results. Table 2 and Figures 2(b) and 2(c) present the results of time domain analysis during the preacupuncture, acupuncture, and postacupuncture periods. The results show that before and after acupuncture (R1, R3, and R4), there were no significant differences in STDRR and RMSSD between the LR-group and RS group. However,

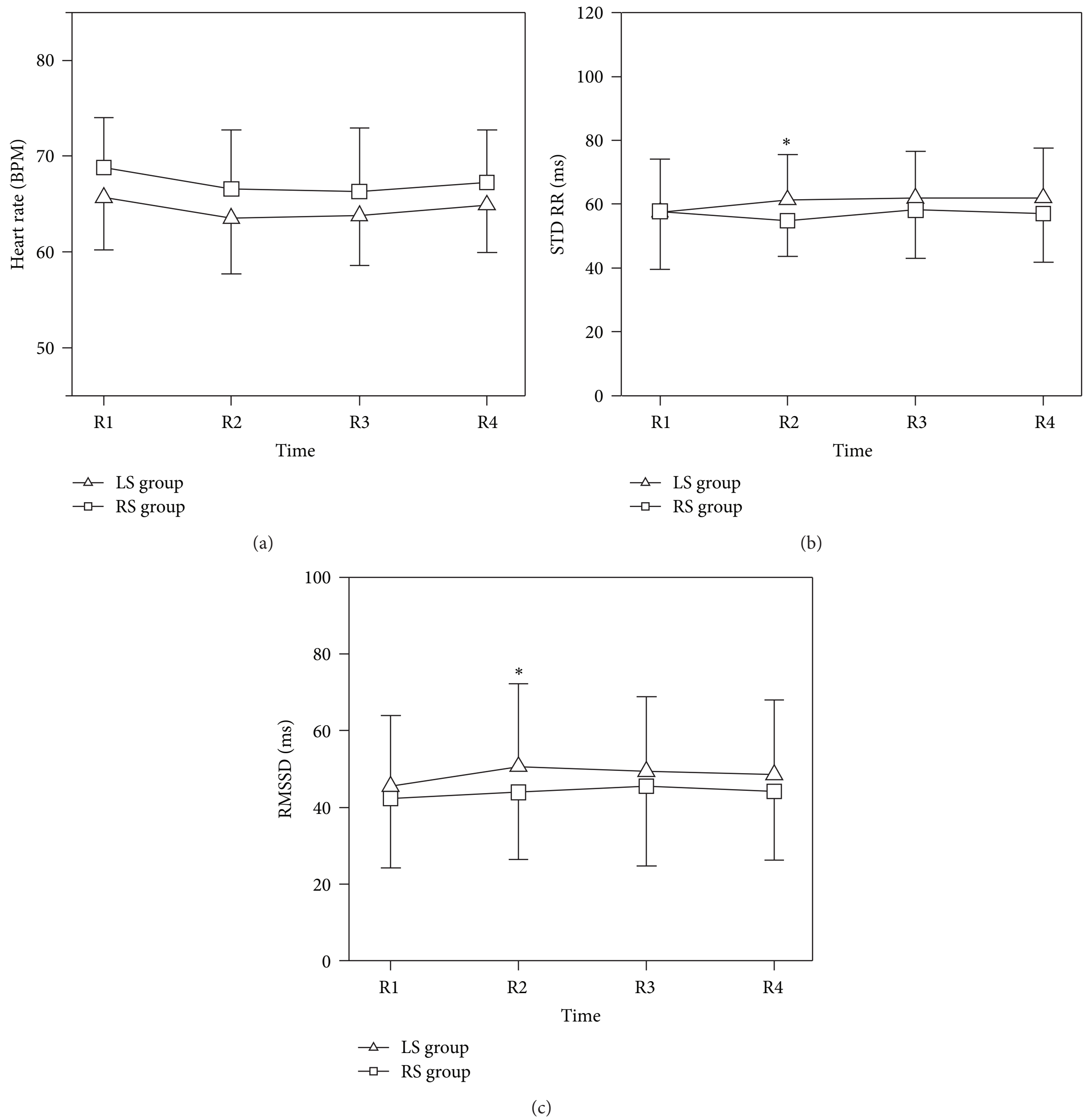

Figure 2: Time domain results. LS: left Neiguan acupoint was stimulated; RS: right Neiguan acupoint was stimulated; STDRR: standard deviation of RR intervals; RMSSD: root mean square of successive differences between RR intervals. Data are expressed as Mean ± SD; $^*P < 0.05$; LS group versus RS group; one-sample paired t-test.

during the acupuncture, there were significant differences between the two groups.

3.2. Frequency Domain Results. Table 3 and Figure 3 present the results of frequency domain analysis during the preacupuncture, acupuncture, and postacupuncture periods. The results show that before, during, and after acupuncture (R1, R2, R3, and R4), there was no significant difference in LF, HF, or LF/HF ratio between the LR-group and RS group. However, during the acupuncture, there were significant differences in total power between the two groups.

4. Discussion

When a needle is inserted into a point on the body, various neural and neuroactive components are activated [17, 18]. Acupuncture has been shown to have clear central nervous

Table 2: Time domain result of heart rate variability.

	Time	LS group	RS group	T value	P value
Mean HR (BPM)	R1	65.669 ± 5.439	68.801 ± 5.237	−1.914	0.073
	R2	63.505 ± 5.771	66.561 ± 6.131	−1.886	0.077
	R3	63.752 ± 5.145	66.314 ± 6.581	−1.581	0.132
	R4	64.897 ± 4.970	67.240 ± 5.457	−1.641	0.119
STDRR (ms)	R1	57.495 ± 16.635	57.737 ± 18.111	−0.046	0.964
	R2	61.386 ± 14.168	54.763 ± 11.235	2.238	0.039
	R3	61.886 ± 14.773	58.182 ± 15.163	0.891	0.386
	R4	62.010 ± 15.453	57.088 ± 15.399	1.748	0.099
RMSSD (ms)	R1	45.418 ± 18.573	42.256 ± 18.084	0.993	0.335
	R2	50.629 ± 21.593	43.886 ± 17.490	2.492	0.023
	R3	49.330 ± 19.469	45.540 ± 20.906	1.122	0.277
	R4	48.488 ± 19.496	44.151 ± 17.963	1.714	0.105

LS: left Neiguan acupoint was stimulated; RS: right Neiguan acupoint was stimulated; HR: heart rate; STDRR: standard deviation of RR intervals; RMSSD: root mean square of successive differences between RR intervals; BPM: beat per minute. Data are expressed as Mean ± SD.

Table 3: Frequency domain result of heart rate variability.

	Time	LS group	RS group	T value	P value
LF	R1	44.308 ± 13.443	47.128 ± 16.006	−1.325	0.203
	R2	44.94 ± 14.216	48.373 ± 16.697	−1.429	0.171
	R3	45.693 ± 14.727	48.755 ± 19.521	−0.927	0.367
	R4	46.747 ± 15.164	50.605 ± 15.892	−1.605	0.127
HF	R1	55.618 ± 13.433	52.806 ± 15.981	1.322	0.204
	R2	54.988 ± 14.217	51.548 ± 16.68	1.432	0.170
	R3	54.245 ± 14.728	51.182 ± 19.511	0.927	0.367
	R4	53.184 ± 15.169	49.332 ± 15.889	1.604	0.127
Total Power	R1	3258.025 ± 1758.083	3397.377 ± 2253.850	−0.218	0.830
	R2	3613.889 ± 1537.027	2892.476 ± 1143.268	2.375	0.030
	R3	3738.289 ± 1736.069	3374.885 ± 1663.170	0.781	0.445
	R4	3761.157 ± 1960.836	3241.175 ± 1700.276	1.408	0.177
LF/HF	R1	0.912 ± 0.517	1.087 ± 0.713	−1.634	1.121
	R2	0.928 ± 0.463	1.142 ± 0.693	−2.055	0.056
	R3	0.979 ± 0.547	1.271 ± 0.928	−1.855	0.081
	R4	1.052 ± 0.672	1.247 ± 0.743	−1.627	0.122

LF: low frequency; HF: high frequency; n.u.: normalized unit; LF/HF: low frequency high frequency ratio. Data are expressed as Mean ± SD.

system and autonomic nervous system effects both in humans [19, 20] and in animals [21]. Previous studies showed that manual stimulation of Hegu (LI4) resulted in specific changes in alpha EEG frequency and in HRV parameters. The relationship between the HRV parameters and the special EEG band might point to a specific modulation of cerebral function by acupuncture [22]. On the other hand, power spectral analysis of heart rate variability (HRV) has recently been used as a sensitive index of autonomic nervous system activity. The analysis of HRV provides quantitative information regarding autonomic control mechanisms in the body [23]. For these reasons, HRV has recently been adopted as an index used to evaluate the effects of acupuncture [24].

A previous study indicated that the cardiac modulatory balance differs between genders and is characterized by a greater influence of the autonomic vagal component in women and by the sympathetic component in men [25]. Another study investigated the influence of age and gender on the short-term HRV indices and revealed significant modifications of the indices especially by age but partly also by gender, especially in the younger groups [26]. To exclude gender bias, we only recruited the healthy adult females in this study.

According to the traditional acupuncture theory, acupoints are distributed along meridians and will often have different effects in treatment. PC6 is a classic acupuncture point, and it is considered to be effective in treating cardiovascular disorders. Evidence has recently shown that violet laser stimulation at the PC6 induces a significant increase of total heart rate variability [27].

Previous studies demonstrated that acupuncture manipulation significantly decreased the LF spectral component of HRV and significantly reduced LF/HF, which is an index of sympathetic activity [28]. In the present study, acupuncture

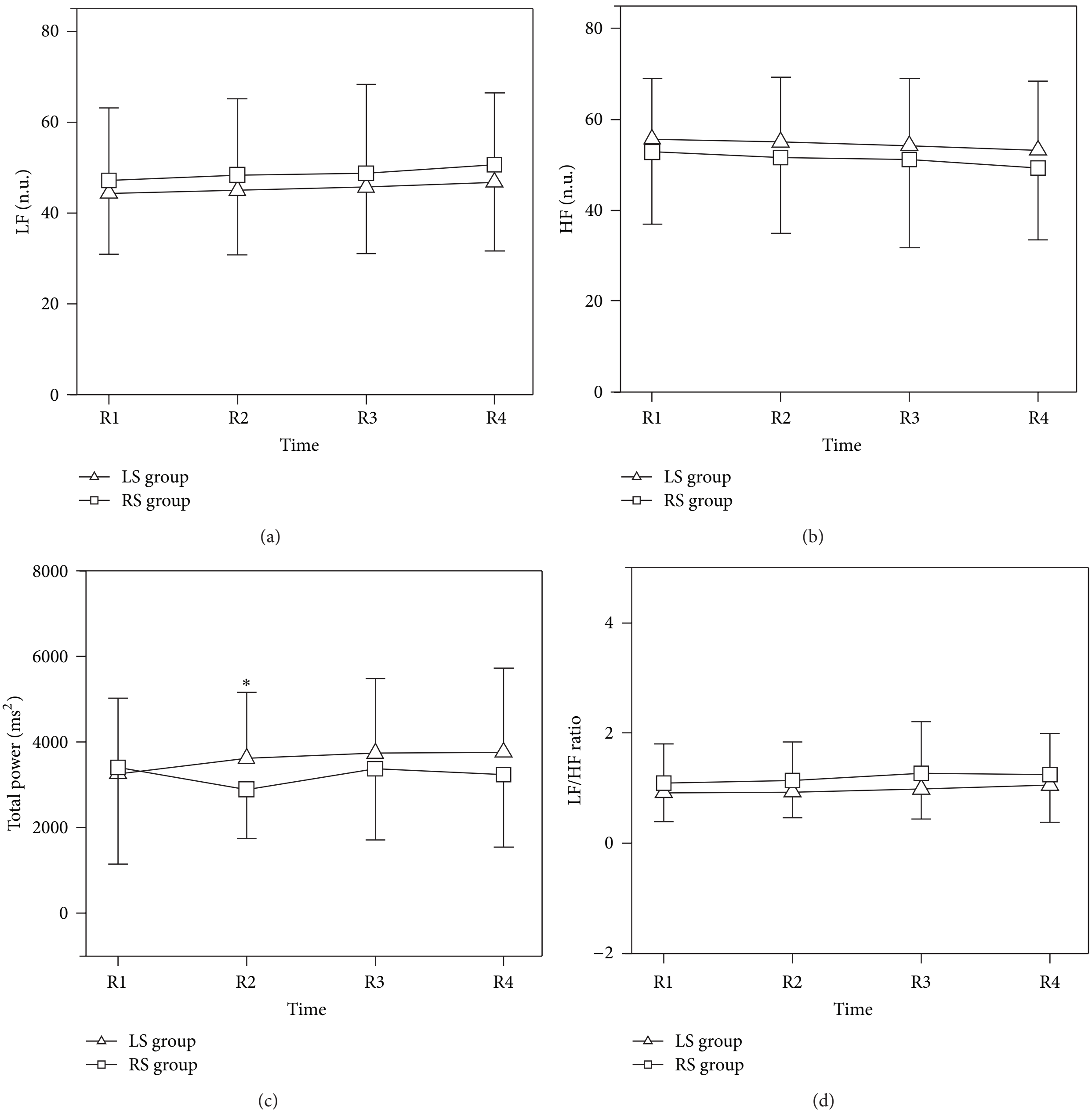

FIGURE 3: Frequency domain results. LS: left Neiguan acupoint was stimulated; RS: right Neiguan acupoint was stimulated; LF: low frequency; HF: high frequency; n.u.: normalized unit; LF/HF: low frequency high frequency ratio; PSD: Power spectrum density. Data are expressed as Mean ± SD; $^*P < 0.05$; LS group versus RS group; one-sample paired t-test.

effects on heart rate variability mainly occurred in the acupuncture period. After the acupuncture was discontinued, this effect disappeared. Since the effects of acupuncture are the result of central nervous system regulation, we can expect laterality due to the fact that information is processed in the brain, which has hemispheric dominance [29]. In this study, differences in LF, HF, and LF/HF between the two groups were not observed; however, the difference in total power was significant.

Obviously, this is a plot study in acupoint laterality, the conclusions just resulted from the female healthy subjects aged from 21 to 33, and just the PC6 was investigated in the study. So, we cannot be sure whether all acupoints have the laterality, and we also cannot be sure whether this laterality will be changed under different conditions such as disorder, handedness, or aging. In particular, we note that the laterality of PC6 was just based on the HRV analysis. The mechanism and factors analysis needed further research.

Acknowledgments

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Research Article

Manual Acupuncture and Laser Acupuncture for Autonomic Regulations in Rats: Observation on Heart Rate Variability and Gastric Motility

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This study focused on the effects of laser acupuncture (LA) and manual acupuncture (MA) at different acupoints on gastric motility and heart rate variability (HRV) simultaneously to elucidate the site specific effects of acupoints and the correlation between changes of gastric motility and low frequency/high frequency (LF/HF) ratio. Gastric motility and HRV were recorded before and during MA or LA. Stimulating PC-6 or ST-36 significantly enhanced gastric motility, while BL-21 caused no changes. In contrast, MA or LA at CV-12 significantly suppressed gastric motility. Stimulating PC-6 or ST-36 significantly increased heart rate (HR), while CV-12 or BL-21 induced no significant changes of HR. Stimulating PC-6 significantly increased LF/HF, while ST-36, CV-12, or BL-21 induced no significant effects. These results indicated that there was acupoint specificity in the effects of acupuncture on gastric motility and HRV. The stimulatory effect of MA and LA at PC-6 and ST-36 on HR was associated with sympathetic activity. The stimulatory effect of MA or LA at PC-6 or ST-36 on gastric motility was associated with vagal activity. Laser needle can be used as an alternative stimulation therapy.

1. Introduction

Acupuncture has been used for regulating autonomic dysfunction in gastrointestinal and cardiovascular diseases. Manual acupuncture (MA) is a traditional method used widely in the world. With the development of technology, different kinds of acupuncture methods have been used in clinic. Laser acupuncture (LA) is a new technology stimulation with low energy and no pain, and it can also be used noninvasively instead of acupuncture needles; thus it becomes easily accepted by more and more patients especially in Europe and America [1].

MA or LA has been used for autonomic regulation [2]. Heart rate variability (HRV) has been used to evaluate the balance between the parasympathetic and sympathetic activity. Low frequency (LF) represents sympathetic activity, while high frequency (HF) represents parasympathetic or vagal activity. Therefore, LF/HF can be an indicator of the sympathovagal balance. In a previous study, different frequencies of violet LA on HRV were investigated. The result showed that there was a significant difference in mean HR and LF/HF ratio before, during, and after 2 or 100s violet LA [3]. It was also demonstrated that HR changed and HRV increased significantly during interstitial LA stimulation of PC-6 [4], but whether the effect of LA is the same as that of MA still remains unclear. In the present study, the effect of MA or LA at different acupoints such as PC-6 (Neiguan), ST-36 (Zusanli), CV-12 (Zhongwan), and BL-21 (Weishu), respectively, on HRV and gastric motility was investigated simultaneously in rats, to determine the specificity of acupoints related to the internal organs and if the effects were affected by the stimulating methods.

2. Animals and Methods

2.1. Animal Preparation. Ten male healthy Sprague-Dawley rats (weight: 190–250 g, purchased from the Institute of Animals, China Academy of Chinese Medical Sciences) were kept in an animal house maintained at 24 ± 1°C, with a 12-hour light-dark cycle and free access to food and water for one day before the experiment. The animals were anesthetized with an intraperitoneal injection of 10% urethane (1.2 g/kg, Sigma-Aldrich, St. Louis, USA). Animals were sacrificed by an overdose of anesthetics after the study. The study was approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences and was in accordance with National Institutes of Health guidelines.

2.2. Gastric Motility Recording. After intraperitoneal injection of urethane, the animals were under deep anesthesia. A small longitudinal incision was made in the duodenum about 1 cm from the pylorus. A small balloon made of flexible condom rubber was inserted via incision of the duodenum into the pyloric area of rat and kept in position by tying the connecting catheter to the duodenum. Another catheter (inner diameter 1 mm) was also inserted into the same hole by incision in order to drain digestive juices secreted from the stomach. The balloon was filled with about 0.2-0.3 mL warm water to keep pressures at about 100 mmH_2O. Pressure in the balloon was measured by a transducer through a thin polyethylene tube (1.5 mm in outer diameter) and then input into a polygraph amplifier (NeuroLog, NL900D; AutoMate Scientific, Berkeley, CA, USA). The signal was captured online and analyzed offline using a data acquisition system (Power-Lab/4s; AD Instruments, Colorado Springs, CO, USA) and Chart 5.2 software. Demifasting gastric motor activity was recorded as a control for at least 30 min before any stimulation. The gastric motility induced by stimulation was compared with the background activity in terms of integral (returns the integral of the selection, calculated as the sum of the data points multiplied by the sample interval). Rectal temperature was kept constant at around 37°C by a feedback-controlled heating blanket.

Gastric motility during stimulation was compared with background activity. If the change rates of gastric motility during or after stimulation were 15–20% of the basal activity, the response was considered to have an excitatory or inhibitory effect. The first stimulation was applied when gastric motility wave maintained stability, usually about one hour after the surgical procedure [5].

2.3. Electrocardiographic Monitoring. To collect electrocardiographic (ECG) data in rats, three needle electrodes were placed separately in subcutaneous muscles, in the left and right forelimbs and the left hindlimb separately [6]. The data for HRV analysis was derived from ECG recording using a special software (HRV module for Chart5, AD Instruments, Colorado Springs, CO, USA). The mean HR and LF/HF ratio of HRV were evaluated.

2.4. Stimulation

2.4.1. Laser Acupuncture. LA was employed to stimulate PC-6, CV-12, ST-36, or BL-21 separately. PC-6 is located proximal to the accessory carpal pad of the forelimb, between the flexor carpi radialis and palmaris longus ligaments. CV-12 is located in the medioventral line, 3 mm above the umbilicus. ST-36 is located on the anterolateral side of the hindlimb near the anterior crest of the tibia below the knee under the tibialis anterior muscle. BL-21 is located at 5 mm lateral to the spinal process of the 12th thoracic vertebrae in rats [7]. The laser needle (length 35 mm, diameter 0.55 mm) was a Modulas needle (type: IN-Light, Schwa-Medico, Ehringshausen, Germany). It emits red laser light in continuous wave mode with a wavelength of 658 nm and an output power of 50 mW [4]. In this study, laser acupuncture was performed interstitially (needle penetration depth was 1–3 mm).

2.4.2. Manual Acupuncture. MA was also performed at the four acupoints, respectively. For MA stimulation, sterile single-use needles (length: 15 mm, diameter: 0.3 mm; Huan Qiu, Suzhou, China) were inserted perpendicularly to the skin with a depth of approximately 3–5 mm at the acupoint. The needles were stimulated clockwise and counterclockwise at a frequency of 2 Hz. The stimulation was performed immediately after inserting the needle, continued for 3 minutes, and then withdrawn [8].

Any stimulation was only applied when the gastric motility and HRV had recovered to control state. The two signals before and during stimulations were recorded continuously, 180 s for each session. Two stimulation methods were performed randomly, with an interval of no less than 10 min.

2.5. Statistical Analysis. Quantification of gastric motility was studied by calculating the motility index (MI). The MI is equivalent to the area under the curve of motility recording. MI was calculated every 3 min before and during acupuncture using Power Lab software (AD instruments, Colorado Springs, CO, USA).

MI after EA or LA was compared with the background activity and expressed as % change of MI in each rat. The data obtained before and during treatment in the same group were compared by a paired t-test; different groups were compared by independent-sample t-test. $P < 0.05$ was considered as of a statistical significance. All data are expressed as mean ± standard error (SE).

3. Results

3.1. Gastric Motility under Resting Condition. The gastric motility of the rats was detected by recording the intragastric pressure. When the intrapyloric balloon pressure was increased to about 80–200 mmH_2O, the rhythmic waves of contractions in the pyloric area were observed. With regard to gastric motor characteristics, both the changes of intragastric pressure and rhythmic contraction were noteworthy. Generally, the intragastric pressure represents the index of gastric tone motility and rhythmic contraction represents gastric peristalsis induced by circular muscle contractions, similar to slow wave of gastric motor activity. The pressure was maintained at about 100 mmH_2O as baseline by expanding the volume of the balloon with warm water; rhythmic contractions occurred at a rate of four to six per minute.

3.2. Effects of MA and LA on Gastric Motility Induced by Different Acupoints. The gastric motility was enhanced during LA or MA at PC-6. Figure 1 shows that the MI increased from 41.86 ± 7.52 cmH_2O to 51.67 ± 8.44 cmH_2O· ($P < 0.05$) induced by LA; and it also increased from 27.72 ± 5.02 cmH_2O· to 46.59 ± 9.73 cmH_2O· by MA ($P < 0.05$). The gastric motility was also facilitated by LA or MA at ST-36 (LA: MI from 35.56 ± 8.56 cmH_2O· to 56.98 ± 10.59 cmH_2O· ($P < 0.05$) and MA: MI from 19.99 ± 3.17 cmH_2O· to 30.32 ± 5.18 cmH_2O· ($P < 0.05$)). In contrast, the gastric motility was inhibited by LA or MA at CV-12. The MI was inhibited from 42.19 ± 9.37 cmH_2O· to 30.85 ± 6.99 cmH_2O· by LA ($P < 0.05$), and for MA it decreased from 43.40±9.74 cmH_2O· to 24.81 ± 5.20 cmH_2O· ($P < 0.05$). However, LA or MA at BL-21 did not induce significant changes in gastric motility (see Figure 1). The effect of MA at PC-6 on gastric motility was stronger than that of LA (LA: 15.12 ± 1.59% and MA: 37.62 ± 5.43%, $P < 0.05$) (Figure 2).

3.3. Effects of MA and LA at Different Acupoints on HRV. Both LA and MA stimulation at PC-6 induced an increasing effect on HR which was from 362.3 ± 8.6/min to 373.8 ± 8.8/min (LA, $P < 0.05$) and from 373.3 ± 9.8/min to 390.1 ± 10.2/min (MA, $P < 0.05$). LA or MA at ST-36 also increased HR from 355.0 ± 8.3/min to 368.9 ± 9.7/min (LA, $P < 0.05$) and from 363.9 ± 8.2/min to 387.7 ± 9.5/min (MA, $P < 0.05$). However, LA or MA at CV-12 or BL-21 produced no significant difference in HR (Figure 3). MA at PC-6 or ST-36 had a stronger influence on the change of HR than LA ($P < 0.05$) (Figure 4).

The HF power provides an index of parasympathetic (vagal) activity, while LF/HF ratio is an indicator of sympathovagal balance. Both LA and MA stimulation at PC-6 induced an increase of LF/HF. The LF/HF ratio increased from 0.51 ± 0.048 to 0.63 ± 0.058 (LA, $P < 0.05$) and from 0.40 ± 0.03 to 0.48 ± 0.042 (MA, $P < 0.05$). However there was no significant difference in LF/HF induced by LA or MA at ST-36, CV-12 or BL-21, respectively, (Figure 5). The change rate of LF/HF induced by MA at PC-6 was stronger than that by LA ($P < 0.05$) (Figure 6).

4. Discussion

We previously showed that electroacupuncture (EA) at ST-36 caused gastric contractions, while EA at CV-12 caused gastric relaxations in rats, and there existed an "intensity-response" relationship between stimulation and effects on gastric motility. TRPV1 receptor was involved in the regulation process of EAS [5]. Previous studies also suggested that acupuncture at the lower limbs stimulated gastric motility via vagal cholinergic pathways, while acupuncture at the abdomen inhibited gastric motility via sympathetic pathways in rats [9]. Now more and more studies demonstrated that the effects of acupuncture at ST-36 and CV-12 on gastric motility were related to the autonomic nervous system [10].

The spectral analysis of HRV of ECG has been used to evaluate the balance between the parasympathetic and sympathetic activity in humans. Although many previous articles studied the effect of acupuncture on gastric motility [11] or HRV [12], only a few studies have assessed both parameters simultaneously especially in laser acupuncture. In the present study, the effects of MA or interstitial LA on gastric motility and HRV induced by stimulation of different acupoints were compared. The results showed that both MA and LA at PC-6 and ST-36 enhanced gastric motility and increased HR simultaneously, while at CV-12 suppressed gastric motility. LA or MA at PC-6 increased LF/HF, while MA or LA at the other stimulated acupoints had no significant difference on LF/HF. These results indicated that stimulations at different acupoints evoked different effects on gastric motor function and cardiac autonomic nerve function in anesthetized rats, which confirmed that there is acupoints specificity in regulating autonomic functions. Therefore, acupoint selecting is important in treating diseases.

Acupuncture as a somatic stimulation induced two kinds of reaction forms of alterations in visceral function including segmental and systemic regulation [13]. The segmental somatosympathetic reflex occurs between the surface and visceral organs of the same nerve segment. The central part of the spinal cord was involved in the sympathetic regulation. In the present study, either LA or MA at PC-6 increased HR which is perhaps mediated by the segmental somatosympathetic reflex and enhanced gastric motility which is perhaps mediated by sympathetic vagal regulation. It has also been found that either LA or MA at ST-36 enhanced gastric motility which may be mediated by vagal regulation, whereas LA or MA at CV-12 suppressed gastric motility via segmental somatosympathetic reflex in the spinal level.

Part of our results was not in accordance with previous studies [11]. Li and Wang showed that stimulation at CV-12 increased the vagal HRV component without affecting the dominant ECG frequency, while stimulation at BL-32 decreased the dominant ECG frequency without affecting the vagal HRV component [14]. It was also suggested that the stimulatory effect of electroacupuncture at ST-36 on gastric motility is associated with its stimulatory effect on vagal activity [15]. Further studies will focus on the question which acupoints are more important on autonomic function in terms of sympathetic and parasympathetic influence.

As a modern technique, laser stimulation has been used in clinic and research of acupuncture. LA showed a clinically and statistically significant benefit with reducing symptoms of depression on objective measures including different clinical scores [16]. LA at ST-36 elicited significant antinociceptive effects against acetic-acid- and formalin-induced behavior in rats, and this effect is mediated by activation

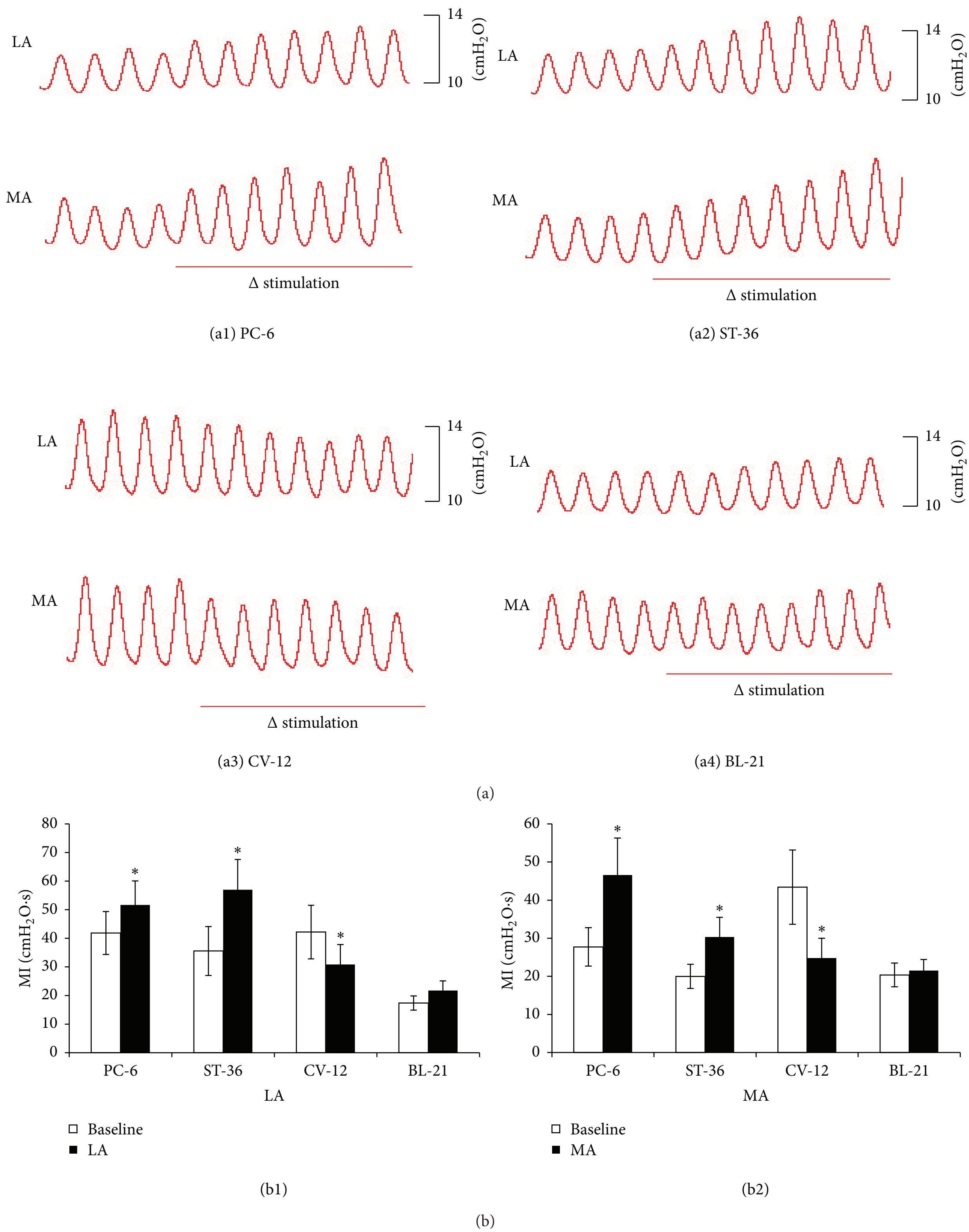


Figure 1: Effects of MA and LA on gastric motility induced by different acupoints. (a) Representative examples of the alteration of gastric contraction wave. (a1), (a2), (a3), and (a4) represent the stimulation of acupoints PC-6, CV-12, ST-36, and BL-21, respectively. (b1) Gastric motility index (MI) at baseline and during LA ($n = 10$ per group). (b2) MI at baseline and during MA ($n = 10$ per group). $^*P < 0.05$, compared with baseline. Stimulation for 180 seconds is indicated by horizontal bars.

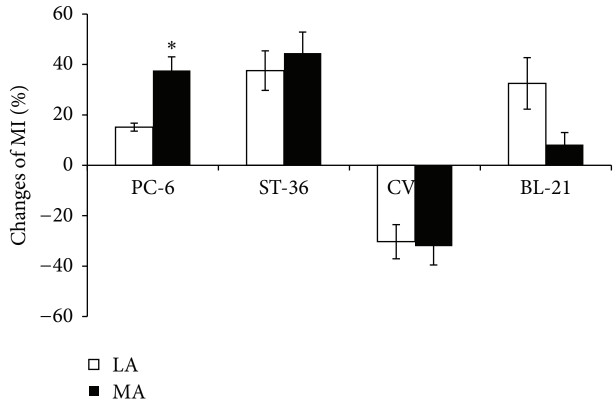


Figure 2: Comparison of changes of the MI induced by LA or MA at four acupoints, respectively ($n = 10$ per group). $^*P < 0.05$, compared with LA.

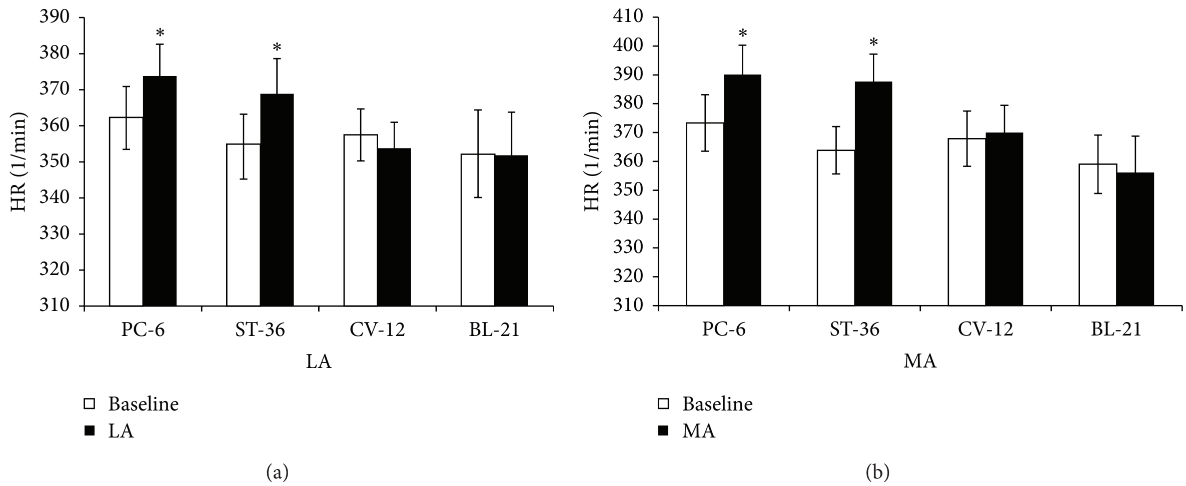


Figure 3: HR changes induced by LA or MA at four acupoints, respectively. (a) HR by LA at PC-6, ST-36, CV-12, or BL-21, respectively. (b) HR by MA at PC-6, ST-36, CV-12, or BL-21, respectively ($n = 10$ per group). $^*P < 0.05$, compared with baseline.

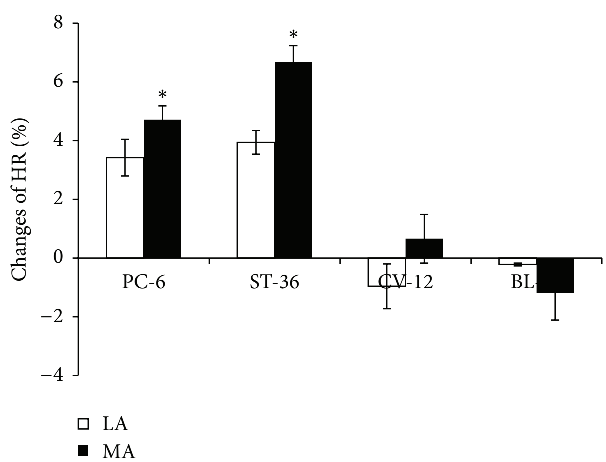
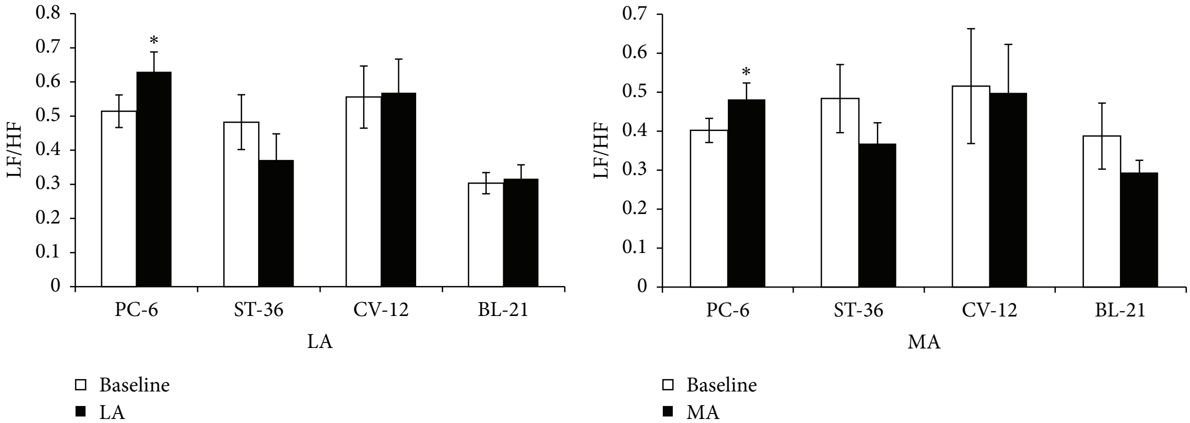


Figure 4: Changes of HR induced by LA or MA at four acupoints, respectively ($n = 10$ per group). $^*P < 0.05$, compared with LA.



(a) (b)

Figure 5: Change rate of LF/HF induced by LA or MA at four acupoints, respectively. (a) shows LF/HF by LA and (b) shows LF/HF by MA at PC-6, ST-36, CV-12, or BL-21, respectively ($n = 10$). $^*P < 0.05$, compared with baseline.

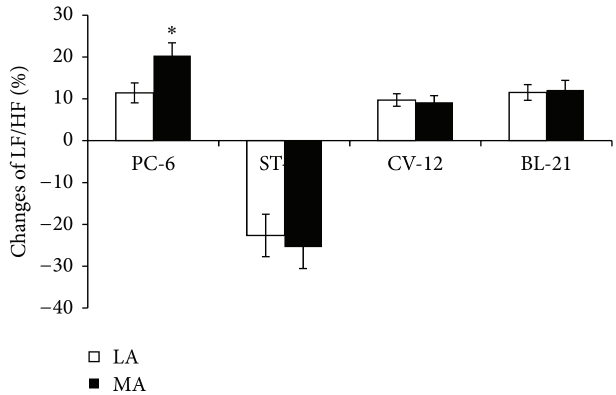


Figure 6: Changes of LF/HF induced by LA or MA at four acupoints, respectively ($n = 10$ per group). $^*P < 0.05$, compared with LA.

of the opioidergic and serotonergic (5-HT1 and 5-HT2A receptors) systems [17]. According to our results, both MA and interstitial LA induced similar effects on autonomic functions, while acupuncture at different acupoints induced different effects on autonomic functions, which indicates that acupoint position is more important than acupuncture stimulation method in treating diseases. Compared with MA, LA has a potential role in clinic because of its painlessness, noninvasiveness, and safety, making it an alternative method of MA for those who are afraid of manual acupuncture.

5. Conclusion

There is acupoint specificity in regulating autonomic functions. The stimulatory effect of MA and LA at PC-6 and ST-36 on HR was associated with sympathetic nerve activity. The stimulatory effect of MA and LA at PC-6 and ST-36 on gastric motility was associated with vagal activity. The choice of the acupoint is more important than the stimulation method in treating diseases by acupuncture. Laser needle can be used as a noninvasive alternative stimulation therapy.

Conflict of Interests

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

Acknowledgments

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Research Article

Heart Rate Variability and Hemodynamic Change in the Superior Mesenteric Artery by Acupuncture Stimulation of Lower Limb Points: A Randomized Crossover Trial

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Objective. We investigated the relationship between superior mesenteric artery blood flow volume (SMA BFV) and autonomic nerve activity in acupuncture stimulation of lower limb points through heart rate variability (HRV) evaluations. *Methods*. Twenty-six healthy volunteers underwent crossover applications of bilateral manual acupuncture stimulation at ST36 or LR3 or no stimulation. Heart rate, blood pressure, cardiac index, systemic vascular resistance index, SMA BFV, and HRV at rest and 30 min after the intervention were analyzed. *Results*. SMA BFV showed a significant increase after ST36 stimulation (0% to 14.1% ± 23.4%, $P = 0.007$); very low frequency (VLF), high frequency (HF), low frequency (LF), and LF/HF were significantly greater than those at rest (0% to 479.4% ± 1185.6%, $P = 0.045$; 0% to 78.9% ± 197.6%, $P = 0.048$; 0% to 123.9% ± 217.1%, $P = 0.006$; 0% to 71.5% ± 171.1%, $P = 0.039$). Changes in HF and LF also differed significantly from those resulting from LR3 stimulation (HF: 78.9% ± 197.6% versus −18.2% ± 35.8%, $P = 0.015$; LF: 123.9% ± 217.1% versus 10.6% ± 70.6%, $P = 0.013$). *Conclusion*. Increased vagus nerve activity after ST36 stimulation resulted in increased SMA BFV. This partly explains the mechanism of acupuncture-induced BFV changes.

1. Introduction

In traditional medicine in East Asia, acupuncture therapy is achieved through acupoints, which are reactive points on the surface of the body. Each acupoint is thought to achieve specific and sometimes unique effects on human organ systems [1]. However, unique organ-specific effects associated with acupoints have been difficult to evaluate quantitatively, because of the lack of a quantitative method of evaluation of the effects of acupuncture. With this in mind, we have evaluated the effects on blood flow in the superior mesenteric artery, radial artery, brachial artery, and retrobulbar artery as a result of acupuncture stimulation at specific acupoints [2–6].

Acupuncture stimulation of ST36 is considered to have beneficial effects on gastrointestinal symptoms and has been used since ancient times [1]. We have reported that acupuncture stimulation of ST36 significantly increased superior mesenteric artery blood flow volume (SMA BFV) using ultrasonographic diagnostic equipment [5]. The mechanism of the increase in blood flow volume has not yet been fully elucidated but is speculated to result from vasodilation caused by the suppression of abdominal sympathetic nerve activity, promotion of abdominal vagus nerve activity, and increases in secondary blood flow due to promoted intestinal movement, as well as other mechanisms.

Noninvasive methods are preferable to elucidate mechanisms by which the autonomic nervous system controls physiological phenomena such as increases in blood flow volume. In research and clinical practice, the spectral analysis of heart rate variability (HRV) has gained popularity and has gained wide popularity as a noninvasive monitor of the autonomic response [7]. Spectral analysis of HRV allows a quantitative evaluation of autonomic nerve activity by calculating the power of each frequency domain through a fast Fourier analysis of HRV obtained from the R-R interval on an electrocardiogram [7].

In the present study, we evaluated the involvement of autonomic nerve activity and changes in SMA BFV resulting from acupuncture stimulation of acupoints using a spectral analysis of HRV.

2. Method

Crossover trials of stimulation at ST36 and LR3 and nonstimulation (CTL) were conducted (Figure 1). The subjects were 26 healthy volunteers (12 men and 14 women with a mean age of 28.9 ± 7.0 years). The subjects were randomly divided into 3 groups in which acupuncture stimulation of ST36, the same stimulation of LR3, and nonstimulation were conducted in a crossover fashion with at least 7 days between trials.

Before each trial, resting measurements were obtained for each subject after 10 min of rest in the supine position at a room temperature of 25-26°C. Thereafter, acupuncture stimulation was performed by bilateral insertion of a number 1 needle (diameter: 0.16 mm, length: 40 mm; Seirin Co. Ltd., Shizuoka, Japan) at ST36 or LR3 to a depth of 1 cm over 18 sec, twirling the needle for 18 sec, and leaving the needle for 15 min. In the CTL group, the subject was observed for the same duration without acupuncture stimulation. An acupuncturist with over 5 years of acupuncture experience administered the acupuncture stimulation. ST36 is located on the lower leg, 3 units below the lateral "eye" of the knee and approximately 1 finger width lateral to the tibia [8]. LR3 is located on the foot, 1.5–2 units above the web between the first and second toes [8].

In each trial, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac index (CI), systemic vascular resistance index (SVRI), SMA BFV, and power of each frequency domain calculated from HRV (very low frequency (VLF), low frequency (LF), high frequency (HF), and LF/HF) at rest (before) and 30 min after stimulation were extracted for analysis. The details of the extracted items were as follows.

2.1. HR and Spectral Analysis of HRV. In each trial, an electrocardiogram recorded values from rest until 30 min after acupoint stimulation. HR and HRV were recorded from electrocardiographic data obtained from a standard limb lead using the AD conversion system PowerLab (AD Instruments Pty Ltd., Australia). The data were extracted from the record for 3 min at rest and at 30 min after the intervention to avoid the period of the SMA BFV measurement using ultrasonographic diagnostic equipment. The data were subjected to a spectral analysis using LabChart analysis software (AD Instruments Pty Ltd., Australia) (Figure 2). The Task Force of the European Society of Cardiology and the North American

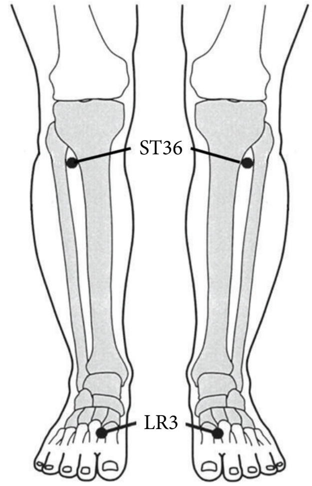

Figure 1: Locations of ST36 and LR3 where acupuncture stimulations were performed.

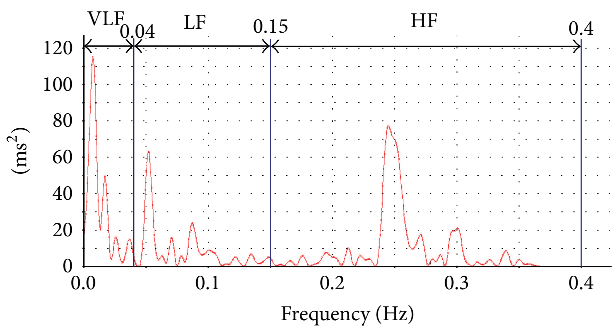

Figure 2: A spectrum screen in LabChart in which the power of each frequency domain (ms^2) is calculated. The very low frequency (VLF), low frequency (LF), and high frequency (HF) domains are indicated in the figure. The solid red line in the figure represents the power calculated in each frequency band by spectral analysis.

Society of Pacing and Electrophysiology define 2–5 min as the extraction time required for a spectral analysis of HRV [7]. In the present experiment, to avoid the period of SMA BFV measurement required for the control of respiration, the extraction time for each period was set to 3 min. The sampling rate was 2 k/s. The baseline fluctuations were removed by a differential calculus wave pattern. The frequency domain analyses were performed using a nonparametric fast Fourier transform (FFT). The FFT spectra were then calculated using a Hann periodogram method and were as follows [7].

(i) VLF: very low frequency domain (≤0.04 Hz) power (ms^2) generally reflects functions such as thermoregulation and renin-angiotensin system activity.

(ii) LF: low frequency domain (0.04–0.15 Hz) power (ms^2) generally reflects both the sympathetic and parasympathetic nervous systems.

(iii) HF: high frequency domain (0.15–0.40 Hz) power (ms^2) generally reflects the parasympathetic nervous system.

(iv) LF/HF: the power ratio of LF to HF generally reflects the balance between the sympathetic and parasympathetic nervous systems.

2.2. SMA BFV. SMA BFV was measured within 2-3 cm from the origin of the artery using ultrasonographic diagnostic equipment (Prosoundα10; Hitachi Aloka Co. Ltd., Tokyo, Japan).

2.3. Blood Pressure Measurements (SBP and DBP). SBP and DBP were measured using an oscillometer, BP-608 Evolution II (Colin Healthcare Co. Ltd., Tokyo, Japan).

2.4. CI. CI was calculated from intrathoracic impedance measured using 4 dual sensors of a BioZ ICG Module, Dash 3000 (GE Healthcare, USA).

For the statistical analysis, values of HR, SBP, CI, SVRI, SMA BFV, and the power of each HRV frequency domain (VLF, LF, HF, and LF/HF) at rest and 30 min after stimulation in each group were represented by the percentage change (%) relative to the resting value. The values before and after the intervention were compared by a paired t-test. A 2-sample t-test was used for intergroup comparisons. The difference was considered significant when the P value was less than 0.05.

This study was conducted after approval by the Ethics Committee of Tohoku University School of Medicine. All subjects provided written consent regarding the contents of the experiments.

3. Results

Tables 1–3 show the changes in the values 30 min after acupuncture stimulation at ST36 and LR3 and nonstimulation (CTL) compared to values before the intervention. Table 4 shows a summary of changes in parameters.

In stimulation at ST36, VLF, LF, HF, and LF/HF significantly increased ($P = 0.045$, $P = 0.006$, $P = 0.048$, and $P = 0.039$, resp.), SBP significantly decreased ($P = 0.037$), and SMA BFV significantly increased ($P = 0.007$) (Table 1).

In LR3 stimulation, HF significantly decreased ($P = 0.014$) and LF/HF significantly increased ($P = 0.013$), while SMA BFV showed no significant change (Table 2).

In the CTL group, VLF and SVRI significantly increased ($P = 0.047$, $P = 0.002$) and CI significantly decreased ($P < 0.001$), while SMA BFV showed no significant change (Table 3).

In the comparison between ST36 and LR3 stimulations, a significant difference ($P = 0.009$) was observed in the change in SMA BFV as well as in LF and HF ($P = 0.013$, $P = 0.015$) (Table 5).

4. Discussion

In the present study, acupuncture stimulation at ST36 resulted in an increased SMA BFV together with significantly increased VLF, LF, HF, LF/HF, and significantly decreased SBP values. The intergroup comparison between the 2 acupoint stimulations showed significant differences in LF and HF.

Table 1: Changes in parameters after acupuncture stimulation at ST36.

	Before	30 minutes after ST36 stimulation percentage change (%)	P value
VLF	0	479.4 ± 1185.6	0.045*
LF		123.9 ± 217.2	0.006*
HF		78.9 ± 197.6	0.048*
LF/HF		71.5 ± 171.1	0.039*
SBP		−2.6 ± 5.8	0.037*
DBP		−2.2 ± 9.0	0.252
HR		−0.4 ± 6.8	0.774
CI		−2.4 ± 5.7	0.053
SVRI		0.5 ± 9.6	0.791
SMA BFV		14.1 ± 23.4	0.007*

The values indicate the percentage change (%) calculated by setting the values at rest to 0 (%) and are represented by the mean ± standard deviation. $^*P < 0.05$.

Table 2: Changes in parameters after acupuncture stimulation at LR3.

	Before	30 minutes after LR3 stimulation percentage change (%)	P value
VLF	0	90.0 ± 307.1	0.140
LF		10.6 ± 70.6	0.444
HF		−18.2 ± 35.8	0.014*
LF/HF		58.6 ± 113.5	0.013*
SBP		−0.3 ± 6.2	0.839
DBP		−0.8 ± 8.1	0.623
HR		−1.3 ± 7.8	0.432
CI		−1.4 ± 7.3	0.345
SVRI		1.2 ± 8.3	0.492
SMA BFV		−7.6 ± 31.4	0.250

The values indicate the percentage change (%) calculated by setting the values at rest to 0 (%) and are represented by the mean ± standard deviation. $^*P < 0.05$.

4.1. Change in Parameters after ST36 Stimulation. The SMA supplies blood widely to the small and large intestines, and its blood flow is regulated by the peritoneal vagus and abdominal sympathetic nerves [9]. HF in HRV is considered to reflect parasympathetic nerve (cardiac vagus nerve) activity [7]. LF is considered to reflect both cardiac sympathetic nerve and cardiac vagus nerve activity [10]. LF/HF is considered to reflect the sympathovagal balance (balance between the sympathetic and parasympathetic nervous systems) [11, 12]. In the present experiment, ST36 stimulation significantly increased VLF, LF, HF, and LF/HF. Because CI, HR, and SVRI did not change while SBP decreased, we considered that the increase in SMA BFV induced by ST36 stimulation might have been caused by the augmentation of parasympathetic nerve (vagus nerve) activity rather than the suppression of sympathetic nerve activity.

4.2. Changes in Parameters after LR3 Stimulation. LR3 stimulation caused no significant change in SMA BFV. In addition,

Table 3: Changes in parameters after nonstimulation (CTL).

	Before	30 min after precondition percentage change (%)	P value
VLF	0	37.6 ± 93.8	0.047*
LF		60.2 ± 233.1	0.191
HF		69.6 ± 283.3	0.213
LF/HF		24.0 ± 93.3	0.194
SBP		-0.5 ± 5.9	0.713
DBP		-1.2 ± 7.9	0.453
HR		-1.3 ± 9.0	0.471
CI		-6.9 ± 5.8	<0.001*
SVRI		6.2 ± 8.5	0.002*
SMA BFV		-2.8 ± 17.0	0.432

The values indicate the percentage change (%) calculated by setting the values at rest to 0 (%) and are represented by the mean ± standard deviation. $^*P < 0.05$.

Table 4: Summary of changes in parameters after ST36 stimulation, LR3 stimulation, and nonstimulation (CTL).

	30 minutes after stimulation or precondition		
	ST36	LR3	CTL
VLF	↑↑	→	↑↑
LF	↑↑	→	→
HF	↑↑	↓↓	→
LF/HF	↑↑	↑↑	→
HR	→	→	→
SBP	↓↓	→	→
DBP	→	→	→
CI	↓	→	↓↓
SVRI	→	→	↑↑
SMA BFV	↑↑	→	→

→: no change, ↑ or ↓: a trend ($0.05 < P \leq 0.1$), and ↑↑ or ↓↓: a significant difference.

Table 5: Intergroup comparisons between ST36 and LR3 stimulations and between ST36 or LR3 stimulation and nonstimulation (CTL).

	ST36 versus LR3 (P value)	ST36 versus CTL (P value)	LR3 versus CTL (P value)
VLF	0.104	0.059	0.401
LF	0.013*	0.303	0.294
HF	0.015*	0.886	0.116
LF/HF	0.746	0.211	0.226
SBP	0.180	0.207	0.913
DBP	0.589	0.706	0.859
HR	0.683	0.686	0.978
CI	0.605	0.012*	0.007*
SVRI	0.806	0.041*	0.047*
SMA BFV	0.009*	0.006*	0.513

A 2-sample t-test was used for comparisons. $^*P < 0.05$.

when HR, SBP, CI, and SVRI showed no significant change, HF significantly decreased, LF/HF significantly increased, and LF showed no significant change. LR3 stimulation might have suppressed parasympathetic nerve (cardiac vagus nerve) activity while exerting no influence on sympathetic nerve activity.

4.3. Changes in Parameters in CTL. In CTL, while no significant change was seen in SMA BFV, CI significantly decreased, SVRI significantly increased, and HR and SBP showed no significant changes. While VLF significantly increased, HF, LF, and LF/HF showed no significant changes. Although there is a report stating that an increase of VLF indicates an enhancement of parasympathetic nerve activity [13], the increase of VLF was unlikely solely the result of an increase of parasympathetic nerve activity, because no change in HF, an indicator of parasympathetic nerve activity was seen. Therefore, it is likely that no changes in autonomic nerve activity related to HRV occur while resting in the supine position.

4.4. Difference in Reaction Depending on the Acupoint Stimulated. In the comparison between ST36 and LR3 stimulations in the present experimental system, SMA BFV as well as HF and LF in HRV showed significant differences. These 2 acupoints are present in the same segment, that is, on the same afferent fiber. Mori et al. performed acupuncture stimulation at 2 acupoints in the same segment (afferent fiber) and indicated that the reaction of the autonomic nervous system may vary because of differences in the muscle (muscular segment), even though the input is made on the same afferent fiber [14]. Zhao et al. also mentioned the presence of acupoint specificity [15]. The presence of differences in SMA BFV and cardiac vagus nerve activity depending on the site (acupoint) in the present experimental system indicates that the reaction of the autonomic nervous system varies because of the difference in the site (acupoint), even though the acupuncture stimulation is applied to the same limb; this difference may be demonstrated by a spectral analysis of HRV.

4.5. Interpretation of the Hypothesis in a Previous Study. Watanabe et al. speculated that increased SMA BFV after ST36 stimulation resulted from the suppression of the sympathetic nervous system through stimulation of the parasympathetic nervous system and spinal reflex [5]. In the present experiment, when ST36 stimulation caused an increase of the SMA BFV, we obtained a result indicating that activation of the parasympathetic nervous system increases in VLF, HF, LF, and LF/HF in HRV. This result supported the promotion of the parasympathetic nervous system, which was part of the hypothesis proposed by Watanabe et al.

5. Limitations

5.1. Effect of Respiratory Rate. HR effects due to sympathetic nerve activity have distinctly different characteristics from those due to vagus nerve activity. HRV does not exceed 0.15 Hz with sympathetic nerve transmission, whereas HRV up to around 1 Hz occurs with cardiac vagus nerve transmission because of differences in intracellular communication mechanisms downstream of β and Ach receptors [16]. To evaluate the HR effect of the cardiac vagus nerve separately from that of the sympathetic nerve, the frequency of the HF component needs to be maintained above 0.15 Hz (a respiratory rate of over 9 times/min), which is the limit of the frequencies that the sympathetic nerve can transmit [17, 18]. In the present study, the measurement items did not include respiratory rate. However, we speculate that the participants were breathing more than 9 times per min because we instructed them to stay awake during the trial, and healthy adults breathe 12–15 times per min at rest [19]. Further, a peak of more than 0.15 Hz is shown in the spectrum analysis in Figure 2. This peak is regarded to represent respiratory sinus arrhythmia, which confirms that the respiratory rate was more than 9 times/min.

5.2. Extraction Time in HRV Analysis. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology define 2–5 min as the extraction time required for a spectral analysis of HRV [7]. Notably, VLF assessed from short-term recordings (i.e., <5 min) is considered unreliable and should be avoided. In the present experiment, to avoid the period of SMA BFV measurement required for the control of respiration, the extraction time was set to 3 min. To verify whether an accurate analysis could be conducted with extraction time of 3 min, we preliminarily compared VLF data from 5 min extractions with those from 3 min extractions. As a result, no significant difference was observed between the values of HRV calculated from 5 min and 3 min of data. The range of VLF components is less than 0.04 Hz. In other words, there are approximately 7 waves in 3 min and approximately 12 waves in 5 min in the VLF components. With the above analysis, we confirmed that there was no difference in data obtained from 7 and 12 waves. Therefore, we considered that the analytical results in the present experiment were valid, even though the extraction time was 3 min.

5.3. Respiratory Control in the Measurement of SMA BFV. Because ultrasonographic diagnostic equipment is used to measure SMA BFV, respiration needs to be controlled for a short time during the measurement. We preliminarily investigated the presence or absence of the influence of respiratory control on HRV. We investigated the change in HRV after ST36 stimulation in a protocol without SMA BFV measurement (respiratory control) and compared it with that with SMA BFV measurement. As a result, no influence of respiratory control on HRV was seen. Thus, when examining multiple organs, we need to preliminarily check possible interference among examination procedures because multiple examinations need to be conducted simultaneously.

6. Conclusion

When SMA BFV increased after ST36 stimulation, HF and LF in HRV showed a significant increase and differed from those observed after LR3 stimulation. This indicated that an increase of vagus nerve activity most likely is involved in an increase of SMA BFV induced by ST36 stimulation. This result also demonstrated acupoint specificity.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Soichiro Kaneko took part in planning the study, performed the data analysis, and wrote the paper. Masashi Watanabe took part in planning the study and performed the measurements. Shin Takayama and Takehiro Numata took part in planning the study, provided advice for statistical analysis, and wrote the paper. Junichi Tanaka and Seiki Kanemura provided advice for writing the paper. Yoshitaka Kimura, Tadashi Ishii, and Nobuo Yaegashi were in charge of the study design and execution and assisted in writing the paper. Takashi Seki is the original proposer of the study. All authors read and approved the final paper.

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Research Article

Effectiveness of Interstitial Laser Acupuncture Depends upon Dosage: Experimental Results from Electrocardiographic and Electrocorticographic Recordings

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The purpose of this study was to evaluate the influence of the duration of interstitial laser acupuncture therapy effects on neurovegetative and neurobioelectrical parameters like heart rate (HR), heart rate variability (HRV), and electroencephalogram (EEG). We investigated 6 male Sprague-Dawley rats. They underwent 10 min, 20 min, and 30 min interstitial laser acupuncture (in randomized order, with a break of at least 30 min between the different measurement conditions) at the acupoint Neiguan. HR changed significantly only during 20 min red laser stimulation, whereas 10 and 30 min stimulation did not induce significant changes. HRV did not change significantly during any of the different durations; however, an increase was found during 20 min irradiation. Neither the LF/HF ratio of HRV nor the integrated EEG showed significant changes. In this study, it could be experimentally proved that some effects of laser acupuncture are time dependent, and therefore the dosage, as well known from theory, also depends on the time factor. We could especially demonstrate that different treatment times lead to different effects on neurovegetative and neurobioelectrical parameters. Further studies are needed to verify or refute these results.

1. Introduction

In a previous study, our research team from China and Austria investigated interstitial (i.st.) laser acupuncture in anesthetized Sprague-Dawley rats for the first time [1]. In that preliminary study, we explored i.st. laser acupuncture, intravenous laser blood irradiation, and electroacupuncture under stable conditions and analyzed the effects on physiological neurovegetative parameters and bioelectrical brain activity. We found changes in the rat model; however, the question of the adequate dosage of laser is still an open one in scientific literature [2, 3].

Tunér and Hode, both are very renowned researchers on laser therapy, stated in 2010 [4]: "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?" [4].

The goal of the present study was to change the dosage of the laser acupuncture treatment via the duration of the i.st. irradiation at the acupoint Neiguan in order to find an optimal treatment time [2]. The data were recorded in Beijing, China, and the analysis was performed in Graz, Austria.

Figure 1: Interstitial laser acupuncture in a rat model.

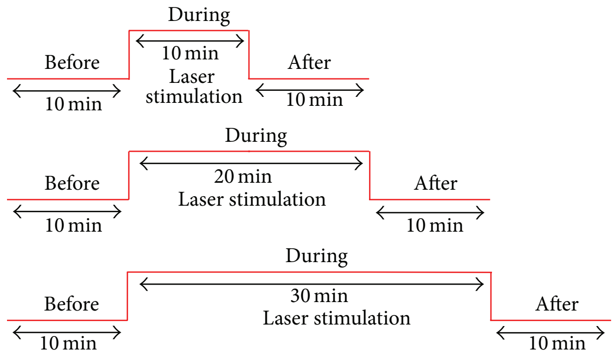

Figure 2: Experimental procedure for the different durations of i.st. laser acupuncture stimulation.

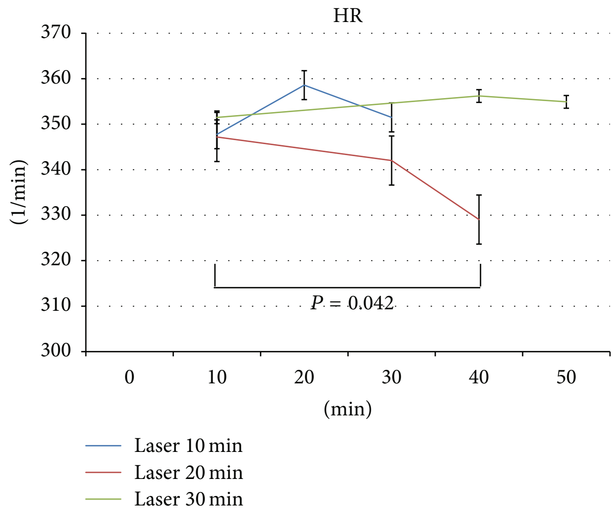

Figure 3: Mean heart rate (HR) of the 6 rats. Note the different stimulation durations (10, 20, and 30 min). Significant changes were only found for a duration of 20 min (red line). The error bars indicate the standard error (SE).

2. Animals and Methods

2.1. Sprague-Dawley Rats. Six male healthy Sprague-Dawley rats (weight: 190–300 g) were kept in an animal house maintained at 24 ± 1°C, with a 12-hour light-dark cycle and free access to food and water for seven days before the experiment. The procedure was the same as in our previous work published recently [1]. The animals were initially anesthetized with an intraperitoneal injection of 10% urethane (1.2 g/kg, Sigma-Aldrich, St. Louis, MO, USA). Additional sodium pentobarbital was administered if necessary to prolong the anesthetic state. Animals were sacrificed by an overdose of anesthetics after the study. The study was approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences and was in accordance with the National Institutes of Health guidelines.

2.2. Interstitial Laser Acupuncture. The laser needle for i.st. laser irradiation (length: 35 mm; diameter: 0.55 mm) was a Modulas needle (type: IN-Light, Schwa-Medico, Ehringshausen, Germany). It emits red laser light in continuous wave mode with a wavelength of 658 nm and an output power of 50 mW (Figure 1). We stimulated the acupoint Neiguan (PC6) on the left side using i.st. laser acupuncture. The laser needle was inserted about 3 mm in the acupoint Neiguan. This acupoint is located proximal to the accessory carpal pad of the forelimb, between the flexor carpi radialis and palmaris longus ligaments [1].

2.3. Procedure. Figure 2 shows the measurement profile. Three measurement periods were compared: one before, one during, and one after stimulation. This scheme was used for all three conditions (10, 20, and 30 min i.st. laser acupuncture) in the same rat. The order of the stimulation methods was randomized, and the time between the separate measurement conditions was at least 30 min.

2.4. Measurement Parameters. As in the previous study [1], we registered electrocardiographic (ECG) and electroencephalographic (EEG) parameters using a biophysical amplifier AVB-10 (Nihon Kohden, Japan). For the ECG, we evaluated heart rate (HR), heart rate variability (HRV), and the LF (low frequency)/HF (high frequency) ratio of HRV. Again, EEG was registered directly on the brain; high cutoff frequency was 100 Hz, and the low cutoff frequency was 0.5 Hz.

2.5. Statistical Analysis. The data were analyzed using Friedman repeated measures analysis of variance (ANOVA) on ranks (SigmaPlot 12.0, Systat Software Inc., Chicago, IL, USA). Post hoc analysis was performed using Holm-Sidak test. The level of significance was defined as $P < 0.05$.

3. Results

The analysis of the HR of all 6 rats is shown in Figure 3. Note the significant ($P = 0.042$) decrease of HR after 20 min i.st. laser acupuncture stimulation at the left Neiguan acupoint. It is also interesting that stimulation durations of 10 and 30 min, respectively, did not lead to the same effects in the rat model.

In contrast to HR, total HRV increased (insignificantly), also during 20 min laser stimulation. No increases in total

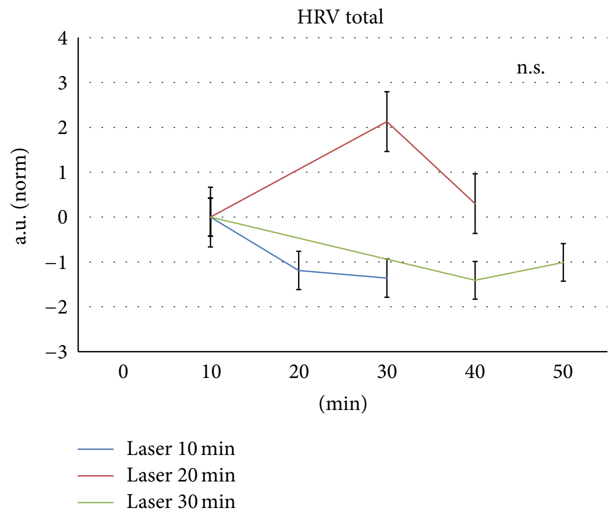

Figure 4: Changes in total heart rate variability (HRV total) before, during, and after the three stimulation procedures. a.u. (norm): normative arbitrary units. For further explanation, compare with Figure 3.

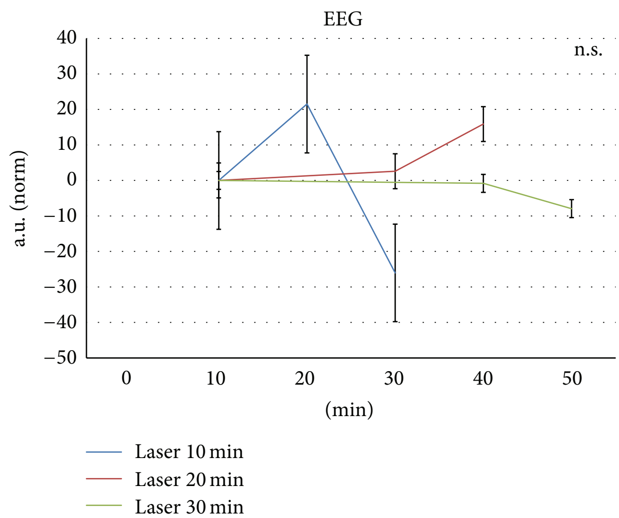

Figure 6: Integrated electrical rat brain activity. Note the (insignificant) increase of the integrated EEG after 20 min laser stimulation. For further explanation, see Figures 3 and 4.

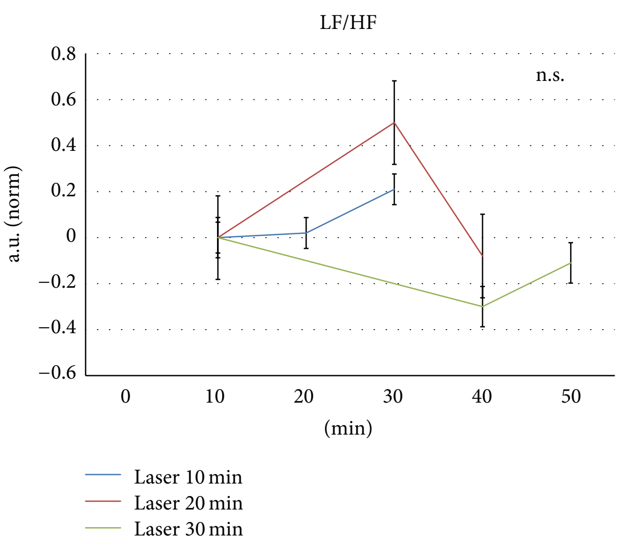

Figure 5: LF/HF of the 6 investigated rats. For further explanation, see Figures 3 and 4.

HRV were seen during or after 10 min or 30 min laser stimulation (Figure 4).

Figure 5 shows the changes of LF/HF HRV ratio. No significant changes were found during any of the stimulation procedures.

Analysis of the bioelectrical brain activity (EEG, Figure 6) did not reach the level of statistical significance.

4. Discussion

Interstitial laser acupuncture is a new acupuncture modality that also allows treatment of different body areas like spinal nerves or joints. The application of laser energy can be performed directly in the region of interest.

In our first animal experimental study, we could demonstrate that there are significant changes in neurovegetative parameters like HR and HRV after i.st. laser stimulation of the left Neiguan acupoint in rats [1]. In a human pilot study, it could also be shown that the pain intensity of patients with shoulder pain and spine-associated pain could be significantly reduced [5]. Interstitial laser therapy was also already used as a therapy for liver metastases in Western medicine. First results of a clinical phase I study were published already 10 years ago by a German research team [6]. The authors of that study stated that i.st. laser therapy of liver malignancies is a minimally invasive procedure with little side effects which produces sharply defined, yet small volumes of necrosis [6].

In the last years, there was a continuous increase in publications concerning laser acupuncture (see http://www.pubmed.gov/). However, there are still some important questions concerning dosage and in this context especially treatment duration.

The term treatment dose is identical to energy density, which is measured in watt seconds per cm^2 (= joule (J) per cm^2). Dosage refers to the amount of energy per unit area brought to bear on tissue or cell culture [4].

As we have shown in many previous studies, some people can feel the laser, others not [7]. Maybe it is appropriate to begin with a low dose for a new patient in the first treatment session to be sure that one does not enter a biosuppressive dose range [4]. In laser acupuncture, the dose is often given in joules per point. It is assumed that a "point" is something small. Tunér and Hode [4] have defined an "acupuncture point" as an area that is 5 mm in diameter (~0.2 cm^2) or less. They stated that "this means if we hit the skin with the light concentrated to this small area and administer 1 J "per point," we have given 1 J "per point," and in this "point" (~0.2 cm^2)

the dose value is 5 J/cm^2" [4]. The authors of the book [4] further stated that the most common situation in laser therapy is the wish to administer a certain dose (D) to a specified area (A) with a laser, having an (average) output power (P), and therefore it is necessary to calculate the treatment time (t) for the laser probe at hand. If the problem to treat is situated at a depth (d) (where $d = 0$ to 4 cm; this is the maximum penetration depth of the red laser [2, 8]), the following approximate formula can be used to find the treatment time:

$$t = \frac{D \times A}{P} \times (1 + d) \ [\text{sec}] . \tag{1}$$

For this formula to work, the correct units to be used are as follows: P must be given in watts (not milliwatts); D must be given in J/cm^2; A must be expressed in cm^2; and d in cm. The treatment time will then come out in seconds.

If we use this formula for our laser acupuncture experiment in rats, the calculated time for effects seen in laser acupuncture is too small ($t = \{(5 \times 0.2)/0.04\} \times (1 + 1) =$ 25 sec). Even if we insert $d = 4$ cm (humans), the estimated treatment time ($t \approx 2$ min) seems to be too short. Our measurements within this study only showed significant effects on neurovegetative parameters in rats during or after the 20 min stimulation duration.

Although the therapeutic use of laser acupuncture in general is gaining popularity, objective evaluation of the dosage-dependent effects is very difficult [9]. Only few studies describe the important parameters like wavelength, irradiance, and the beam profile in detail. For a complete description of the dosage-dependent effects, energy transmission factors have also to be taken into account. These factors are, for example, skin properties such as thickness or pigmentation [9]. The thickness of the skin starts decreasing at the age of 45 years, and the difference in pigmentation between the Caucasian and, for example, African population, which is caused by different concentrations of melanin [10], leads to different penetration depths of the laser beam.

Our present study is the first one comparing different i.st. laser stimulation treatment times (durations) in rats. It is well known that the effectiveness of laser acupuncture depends upon dosage [3, 4]. We used a red laser (658 nm) with an output power of about 40 to 50 mW, which results in a very high dosage. This dosage is also time dependent. In our study with the experimental rat model, we could demonstrate that different treatment times lead to different effects on neurovegetative and neurobioelectrical parameters. Further studies are needed to verify or refute these results.

Conflict of Interests

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

Authors' Contribution

Wei He and Gerhard Litscher contributed equally to this study.

Acknowledgments

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Research Article

Continuous Auricular Electroacupuncture Can Significantly Improve Heart Rate Variability and Clinical Scores in Patients with Depression: First Results from a Transcontinental Study

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The goal of this study was to investigate the impact and acceptability of providing continuous auricular electroacupuncture as an adjunct to conventional medications for patients with depression. Ten patients with a mean age ± SD of 43.3 ± 10.4 years were able to provide informed consent. The quantitative and qualitative outcome measures were heart rate, heart rate variability (HRV), and different clinical scores. The study documented that a special kind of auricular electro acupuncture, applied over a period of three days, can improve various aspects of quality of life significantly but also highlighted the significant increase of HRV whilst having acupuncture treatment. In conclusion, our study shows stimulation-related and quantifiable clinical and physiological alterations in parameters after continuous auricular acupoint stimulation in patients with depression. Improved access to electro acupuncture treatment would be of major benefit for these patients. Further studies are necessary in order to verify the gained results.

1. Introduction

A review article from 2011 summarizes 30 randomized controlled trials which evaluated manual acupuncture, electroacupuncture, or laser acupuncture in nearly 3000 patients with major depressive disorder [1]. No consistent benefit was noted with any form of acupuncture [1]. However, our research group recently found acute stimulation effects on neurovegetative parameters like heart rate (HR) and heart rate variability (HRV) in patients with depression [2, 3] and insomnia [4].

In previous studies it has also been shown that both the autonomic and the central nervous system could be modified by auricular vagal stimulation via projections from the auricular branch of the vagus nerve to the nucleus of the solitary tract [5]. Auricular acupuncture is proposed to prevent neurodegenerative diseases via vagal regulation [5]. However, there is a controversy on the specificity and the efficacy of auricular acupoints for treating diseases [5].

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 [2, 6–8]. The present clinical study was performed at the Military Acupuncture Centre at the People's Liberation Army General Hospital in Beijing, in cooperation with the Austrian center mentioned above.

This research describes results from continuous auricular electroacupuncture measurements in patients with depression using computer-based HRV recordings before, during, and after long-lasting electroacupuncture under standardized clinical conditions in Beijing, China. All data analyses were performed in Graz, Austria.

(a) (b) (c)

Figure 1: Ear acupuncture using P-Stim in Beijing (a)–(c).

2. Subjects and Methods

2.1. Patients. Ten patients (7 females, 3 males; mean age ± SD 43.3 ± 10.4 years; range 29–59 years) suffering from depression (Chinese diagnosis "Yu Zheng") and therefore receiving acupuncture treatment were investigated at the Chinese People's Liberation Army Hospital in Beijing. The experiment started in all patients at the morning. The clinical evaluation of the patients, performed immediately before the first HRV data recording, used three main scales: the Hamilton anxiety rating scale (HAM-A) [9], the Athens insomnia scale (AIS) [10], and the Hamilton rating scale for depression (HRSD) [11]. No patient was under the influence of centrally active medication and had a history of heart or cerebrovascular disease, respiratory or neurological problems, or hypertension. The study was approved by the ethic committee of the Chinese People's Liberation Army Hospital and carried out in compliance with the Declaration of Helsinki. All patients with depression gave oral informed consent.

2.2. Biosignal Recording in China 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.

For the joint investigations, the researchers in China used a medilog AR12 HRV (Huntleigh Healthcare, Cardiff, United Kingdom) system from the TCM Research Center at the Medical University in Graz. The system has a sampling rate of 4096 Hz [12], the raw data are stored digitally on a CompactFlash memory card, and 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. The biosignals were analyzed and HRV was displayed in a way to help to judge the function of the autonomic nervous system [6–8].

Similar to previous studies [2–4, 12–14], 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 [15].

2.3. Auricular Electroacupuncture (Punctual Stimulation) and Procedure. A method for ear acupuncture is the electrical point stimulation system (P-Stim; Biegler, Austria). Ultrathin permanent needles are applied at the ear. A generator, located behind the acupunctured ear, produces electrical stimulation impulses (using a constant AC current of 1 mA; impulse duration: 1 ms; stimulus frequency: 1 Hz; alternating 15 min active stimulation and 15 min break (no active stimulation) over a period of three days), which are transferred to the acupuncture areas via the needles. The ear is chosen because a concentration of free nerve ends/acupuncture points are located here [16]. P-Stim allows continuous, intermitting stimulation up to several days combined with absolute mobility of the patient.

Selected acupuncture points at the ear were chosen by an experienced Chinese acupuncturist. A position tape previously prepared with the P-Stim application pointer was applied. This procedure was repeated until all acupuncture points were marked. Then, the needles could be taken up by the application pointer and applied.

A Chinese physician adhered the actual device behind the acupunctured ear with the integrated adhesive electrode. Then, the wires were connected to the permanent needles by snapping over the plastic rings, and everything was fastened with adhesive tape. Finally, P-Stim was activated by removing the adhesive foils from the batteries and by opening the lid. Further methodological details are described in previous publications [17–19].

The following ear acupoints were used in this study: Shenmen, Small Intestine, and Heart (see also Figure 1(c)).

Patients had to come to the clinic on three consecutive days. On day 1, they first answered the score questionnaires;

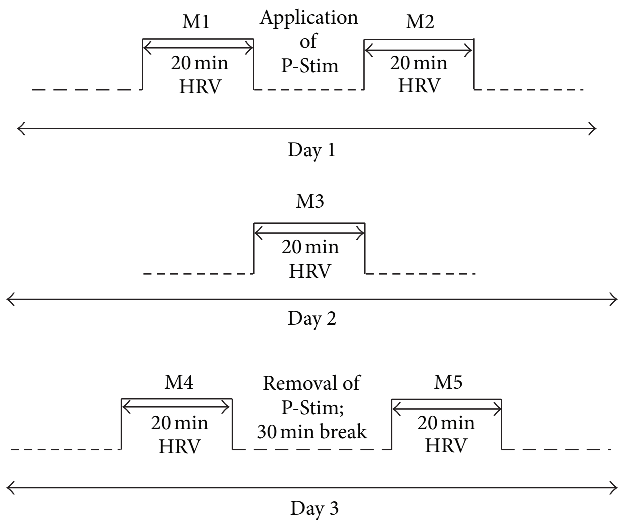

Figure 2: Measurement procedure.

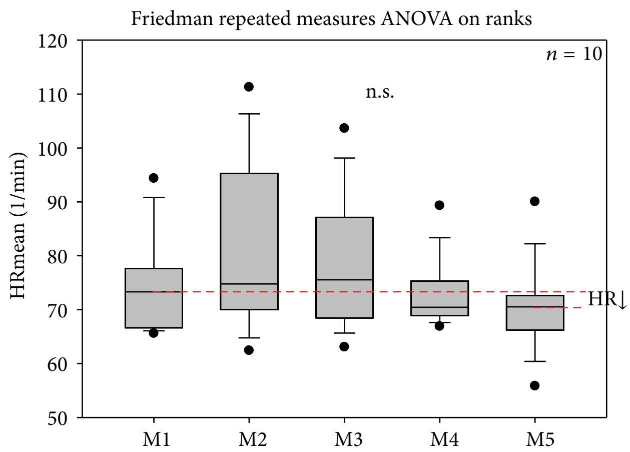

Figure 3: Box plots displaying the changes in mean heart rate (HR) of the 10 patients. After the fifth measurement, on the third day, HR had decreased; however, the changes were not significant. The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles.

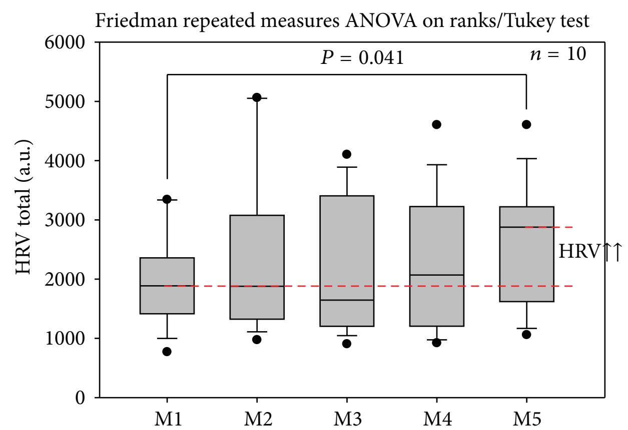

Figure 4: Changes in total heart rate variability (HRV). Electrical auricular stimulation induced a significant increase in total HRV in the ten patients investigated in this study. For further explanations, see Figure 3.

then a first HRV measurement (M1 in Figure 2) was performed for 20 min. After that, the P-Stim auricular stimulation equipment was applied and activated, and immediately after that, a second HRV measurement (M2 in Figure 2) took place. On the second day, the patient came to the clinic again, still wearing the P-Stim device; the questionnaire was answered again and a third HRV measurement (M3 in Figure 2) was recorded. And finally, on day 3, the patient came again, still wearing the auricular punctual stimulation. A fourth HRV measurement (M4 in Figure 2) was performed before the P-Stim device was removed. After a break of 30 min, a last HRV measurement (M5 in Figure 2) followed, and the questionnaire was answered one last time.

2.4. Statistical Analysis. Data 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 or Holm-Sidak test. The criterion for significance was $P < 0.05$.

3. Results

Figures 3 and 4 show the mean HR and total HRV from the ECG recordings of 10 patients with depression during the five measurement phases (M1–M5). There was a slight decrease in HR, but no significant change before, during, or after the stimulation sessions.

In contrast to HR, HRV increased significantly ($P = 0.041$) after continuous auricular electroacupuncture over a period of three days (see Figure 4, M5).

Furthermore, continuous HRV monitoring showed no significant alterations in the LF/HF ratio during or after acupuncture stimulation at the ear (see Figure 5).

The analysis of the three clinical scores revealed interesting results. In all scores there was a significant reduction already after the first day of continuous electroacupuncture with P-Stim (see Figures 6(a)–6(c)).

The data of the blood pressure values of all 10 patients showed insignificant results (systolic blood pressure (mean ± SD): M1: 107.5±10.1 mmHg, M5: 105.6±9.9 mmHg; diastolic blood pressure (mean ± SD): M1: 73.8 ± 7.4 mmHg, M5: 72.1 ± 5.5 mmHg).

4. Discussion

Depression is one of the most disabling diseases in Europe and Asia, causing a significant burden both to the individual and to society [20, 21]. The World Health Organization (WHO) data suggests that depression causes 6% of the burden of all diseases in Europe. Already in 2006, at least 21 million people were affected by depression in 28 European countries (with an overall population of 466 million) [20]. The total annual cost of depression in Europe in 2004 was

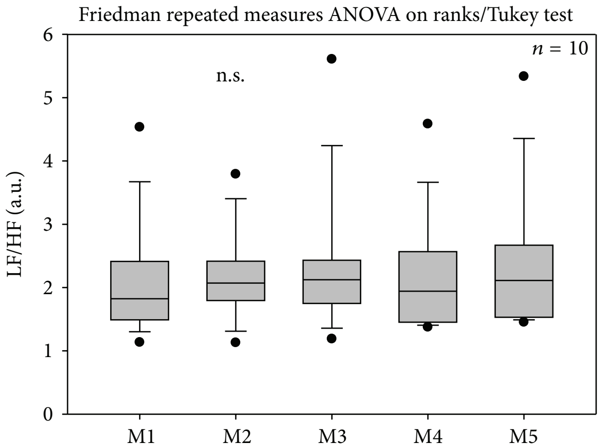

Figure 5: The low frequency (LF)/high frequency (HF) ratio did not change significantly during the three days of the investigation. For further explanations, see Figure 3.

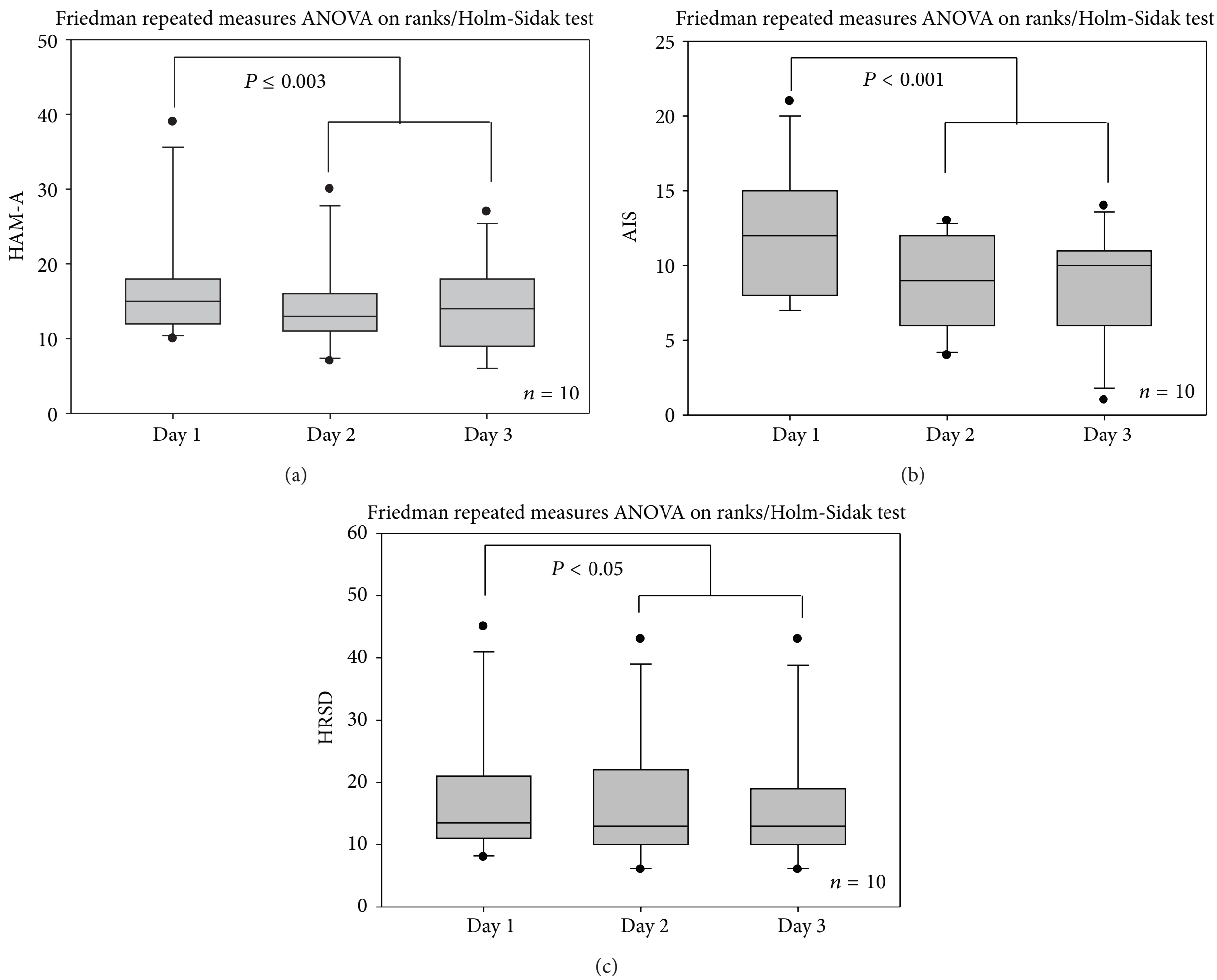

Figure 6: Significant decreases of the three scores investigated within this study. (a) Hamilton anxiety rating scale (HAM-A); (b) Athens insomnia scale (AIS); (c) Hamilton rating scale for depression (HRSD). For further explanations, see Figure 3.

estimated to be 118 billion Euro [20], which corresponds to costs of 253 Euro per inhabitant [20].

A recent survey in China [21] indicated that the 12-month prevalence rate of depressive disorders was 2.5% in Beijing and 1.7% in Shanghai. These disorders may result in disability, premature death, and severe suffering of those affected and their families. The total estimated cost of depression in China is about 6,264 million US dollars (USD, at 2002 prices). Direct costs were 986 million USD, which corresponds to about 16% of the total cost of depression. Indirect costs were, accordingly, 5,278 million USD, or 84% of the total cost of depression [21].

One important way to stop this cost explosion in Europe and China is through increased research efforts in this field. Better detection, prevention, treatment, and patient management are imperative to reduce the burden of depression and its cost [20].

In order to achieve better outcome for the patients, it is mandatory that scientists develop new strategies and that physicians have an up-to-date knowledge of recent advances in evidence-based complementary medicine.

Auricular acupuncture is a special kind of stimulation which is slowly becoming accepted as an evidence-based complementary medical treatment method [16, 17]. Electroacupuncture at auricular points is also used in patients with depression [22, 23]. Especially continuous electrical stimulation of acupoints over several days can increase the effects of acupuncture [17–21, 24–29].

This study introduces first measurements in patients with depression using a miniaturised system for continuous electric acupoint stimulation at the ear. In previous studies, the influence on cerebral functions could be observed [17, 28]. One of the main advantages of the P-Stim system is the complete mobility for the patients, which is not the case when using other systems. In addition to treating patients with neurological diseases like depression, this concept seems to be useful for treating addiction, allergies and in special areas of anaesthesiology [24–29].

Results of a clinical point stimulation study show that a marked decrease in VAS (Visual Analogue Score) of pain occurred in 31 persons who underwent electric stimulation [17, 30]. The average stimulation duration was 36.6 hours (treatment time range: 18–72 hours). A significant decrease in VAS during stimulation was observed in 67% of the patients, and 29% of the patients reported a moderate reduction in pain. A relevant reduction in pain medication was observed in 70% of the patients. No accompanying medication was necessary in half of the patients treated. Almost all patients reported an improvement in their general health situation [17, 30]. This is in accordance with the results of our present study. All clinical scores (HAM-A, AIS, HRSD) showed a significant improvement already after 24 hours of continuous electrical stimulation. In addition, HRV, which is a reliable indicator of the state of health [2–4, 6, 7], also improved significantly.

In conclusion, our study shows stimulation-related and quantifiable clinical and physiological alterations in parameters after continuous auricular acupoint stimulation in patients with depression. Further studies are necessary in order to verify the gained results.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

X. Shi and G. Litscher contributed equally to this study.

Acknowledgments

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Research Article

Improvement of the Dynamic Responses of Heart Rate Variability Patterns after Needle and Laser Acupuncture Treatment in Patients with Burnout Syndrome: A Transcontinental Comparative Study

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We investigated manual needle and laser needle acupuncture as a complementary therapy for patients with burnout syndrome. Twenty patients with a mean age ± SD of 38.7 ± 8.4 years were assigned to two groups, each consisting of ten patients. One group was treated with manual needle acupuncture and the other with laser needle acupuncture. Heart rate, heart rate variability (HRV), and a new score called dynamic acupuncture treatment score (DATS) served as evaluation parameters. The study documented significant effects on heart rate after needle acupuncture treatment and significant effects on HRV caused by both needle and laser needle acupuncture. Based on new neurovegetative acupuncture treatment evaluation scores, it can be stated that both noninvasive laser needle acupuncture and manual needle acupuncture have the potential to be a powerful approach for evidence-based complementary treatment of patients with burnout syndrome. Further transcontinental studies to verify or refute the preliminary findings are in progress.

1. Introduction

In the year 1974, Freudenberger defined the "Burnout syndrome" as a state of physical and mental exhaustion which develops slowly from continuous stress and use of energy to exhaustion because of excessive demands [1]. Since Freudenberger's "Staff Burnout," published in 1974, burnout has become a synonym for psychosomatic and psychological symptoms and social consequences of a long-lasting workload exceeding an individual's capacity [1–3].

Due to unclear diagnosis, the plurality of symptoms, and diverse reasons for burnout, there are uncertainties in the literature regarding its therapy [2].

Fatigue is a common complaint mainly in the working population, with a reported prevalence varying from 7 to 45% [4–6]. It can be understood as a continuum, ranging from mild to severe, disabling fatigue, as in the chronic fatigue syndrome [4]. The phenomenon of burnout is conceptually linked with fatigue [7]. However, it is important to realize that persistently fatigued workers are not burnt out by definition and that burnt out workers might not experience fatigue as one of their major complaints [7].

Both persistent fatigue and burnout are reported to be serious conditions, but little research is available on their clinical features. In this respect, chronic fatigue syndrome has received more attention. It is characterized by persistent, medically unexplained fatigue for at least six months [7].

At the moment, not only psychotherapy, especially cognitive behavioral therapy, phytotherapy, and physiotherapy, but also complementary treatments such as acupuncture are used to treat fatigue, burnout, and chronic fatigue syndrome (see Table 1).

For the present study, patients with burnout syndrome were treated with manual needle acupuncture or laser needle acupuncture at the Beijing Hospital of Traditional Chinese Medicine (TCM) affiliated to Capital Medical University. The dynamic responses of heart rate (HR) and its variability (HRV) patterns were analyzed with respect to the different modalities of the acupuncture therapy applied. Analysis was performed at the TCM Research Center at the Medical University of Graz. This is a further clinical transcontinental teleacupuncture study between the two centers in Europe and China [8].

2. Patients and Methods

2.1. Patients. Twenty patients (10 females, 10 males; mean ages ± SD 38.7 ± 8.4 years; ranges 25–57 years) suffering from burnout syndrome and therefore receiving manual needle (group A) or laser needle (group B) acupuncture treatment were investigated at the Beijing Hospital of TCM affiliated to Capital Medical University. The clinical evaluation of the patients was performed immediately before the first HRV data recording by Chinese experts. The patients were randomly assigned to group A or B. In group A ($n = 10$; 6 females, 4 males), mean age ± SD was 39.2 ± 7.8 years (range: 29–51 years; median: 40 years); in group B ($n = 10$; 4 females, 6 males) it was 38.2 ± 9.0 years (range: 25–57 years; median: 39 years). No patient was under the influence of centrally active medication or had a history of heart or cerebrovascular disease, respiratory or neurological problems, or hypertension. The study was approved by the Ethic Committee of the Beijing Hospital of TCM and carried out in compliance with the Declaration of Helsinki. All patients gave oral informed consent.

2.2. Teleacupuncture between China and Europe. 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 duration of RR-intervals is measured during a special time period (5 min), and on spectral analysis basis HRV is determined.

The researchers at the Beijing Hospital of TCM used a medilog AR12 HRV (Huntleigh Healthcare, Cardiff, UK) system from the TCM Research Center at the Medical University in Graz. The system has a sampling rate of 4096 Hz [9, 10]; the raw data are stored on a memory card, and, 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 center in Graz via internet. The ECG data were analyzed and HRV was displayed in a way to help to judge the function of the autonomic nervous system. Similar to previous teleacupuncture studies [11–15], mean HR, total HRV, and the LF (low frequency)/HF (high frequency) ratio of HRV were chosen as preliminary 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 [16]. In addition to these standard parameters, the so-called HR-HRV "dynamic acupuncture treatment score" (DATS) is introduced in this paper. DATS is defined as the ratio of HR or HRV changes at the end (post) of the acupuncture treatment in comparison to the HR or HRV alterations at the beginning (pre) of the acupuncture treatment (DATS = HRV changes post/HRV changes pre). The new score should be an estimate value to describe the dynamic effects of acupuncture treatment on HR and HRV.

Table 1: Scientific literature (as of Sep 15, 2013) concerning fatigue, burnout, and chronic fatigue syndrome and complementary medical treatment methods (acupuncture and laser acupuncture).

PubMed (http://www.pubmed.gov)	Acupuncture	Laser Acupuncture
Fatigue	232	4
Burnout	3	0
Chronic Fatigue Syndrome	50	1

2.3. Needle and Laser Acupuncture and Procedure. For manual acupuncture stimulation, sterile single-use needles (length: 30 mm, diameter: 0.3 mm; Huan Qiu, Suzhou, China) were inserted perpendicularly in the skin to standard depths at the acupoints. 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.

Laser acupuncture was performed using laser needles [17, 18]. 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 in this field of research started in 2002 at the Medical University of Graz, Austria [17, 18]. A laser needle acupuncture system based on red and infrared light is shown in Figure 1.

Multichannel laser needle acupuncture allows the noninvasive simultaneous stimulation of individual point combinations. 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 that the patient does not immediately feel the activation of the needle (30–40 mW per needle; diameter: 500 μm; duration: 20 min). More details regarding this method are described in previous studies and books [17–19].

Acupuncture points were selected by experienced Chinese acupuncturists. Each patient underwent altogether six

Figure 1: Multichannel laser acupuncture using bichromatic laser needles with red and infrared light.

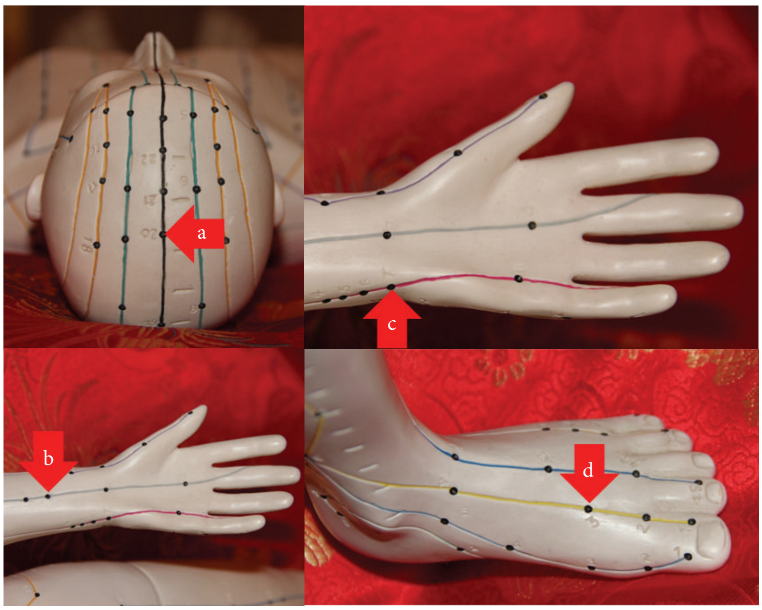

Figure 2: Acupuncture points used in the present study in burnout patients.

acupuncture sessions. The interval between the treatment sessions was 2 to 4 days.

The following acupoints were used in this study (needle and laser needle acupuncture): Baihui (GV20), Neiguan (PC6), Shenmen (HT7), and Taichong (Liv3) (see also Figures 2(a)–2(d)).

The measurement profile and measurement phases (a–f; 5 min each) are shown schematically in Figure 3. Six measurement periods were compared: one before stimulation (a), four during acupuncture (b–e), and one after acupuncture (f).

2.4. Statistical Analysis. Data were analyzed using SigmaPlot 11.0 software (Systat Software Inc., Chicago, USA). Graphical presentation of results uses box plot illustrations. Testing was performed with repeated measures ANOVA on ranks and Tukey or Holm-Sidak test. The criterion for significance was $P < 0.05$.

3. Results

Figures 4 and 5 show the mean HR and total HRV from the ECG recordings of altogether 20 patients with burnout syndrome during the six measurement phases (a–f). There was a slight decrease in HR but no significant change before, during, or after the first stimulation session with needle acupuncture. However, in the last needle acupuncture treatment session HR decreased significantly ($P = 0.003$).

In contrast to HR, HRV showed significant alterations in the first and last needle acupuncture sessions as well as in the last laser acupuncture session.

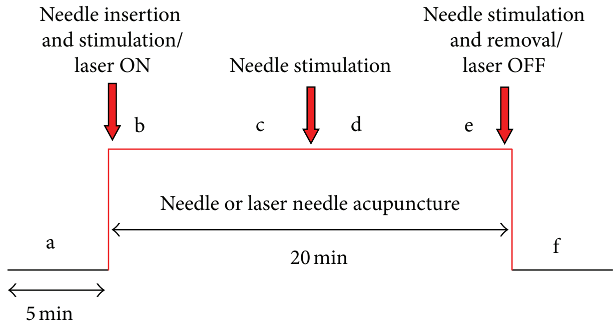

Figure 3: Experimental protocol for manual needle acupuncture and laser acupuncture.

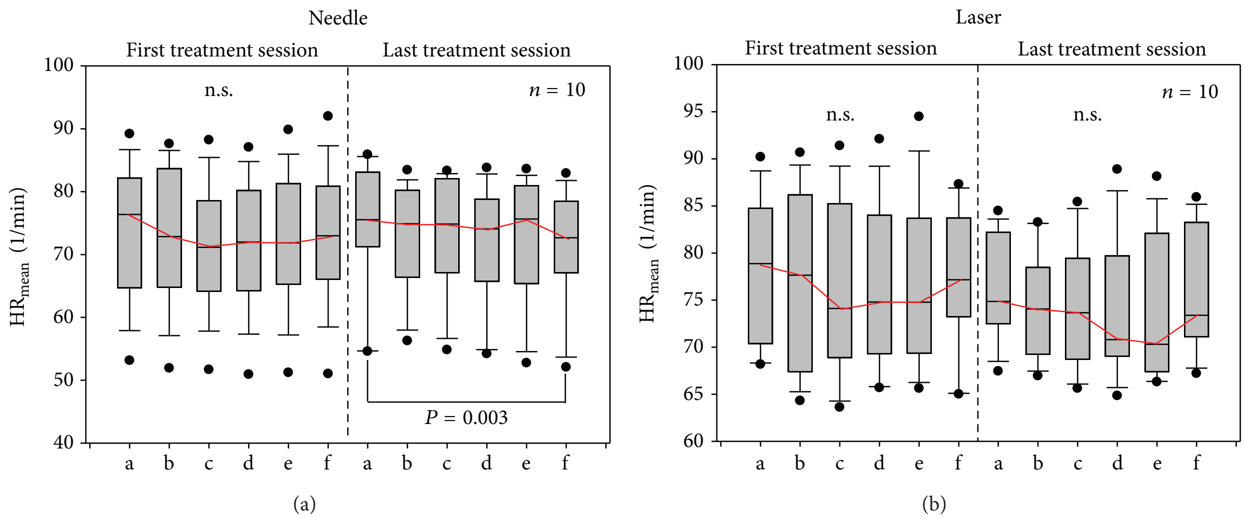

Figure 4: Box plots displaying the changes in mean heart rate (HR) of the 10 patients receiving needle acupuncture (a) and the 10 patients receiving laser acupuncture (b). HR had decreased significantly only after the last needle acupuncture session. The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles.

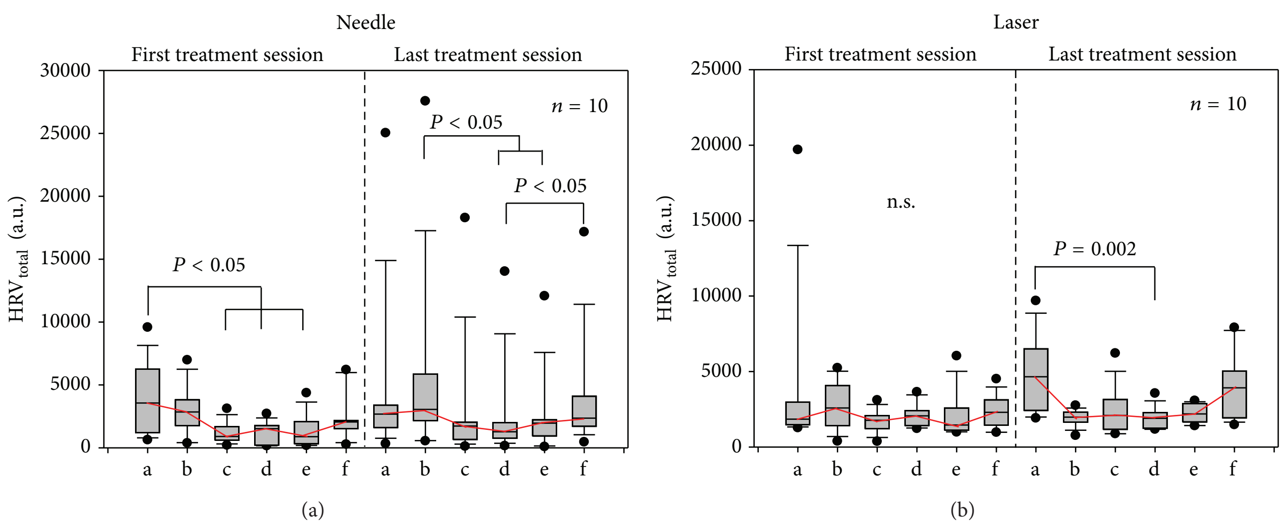

Figure 5: Changes in total heart rate variability (HRV). Needle and laser needle stimulation induced dynamic stimulation-related changes in total HRV in the twenty patients investigated in this study. For further explanations, see Figure 4.

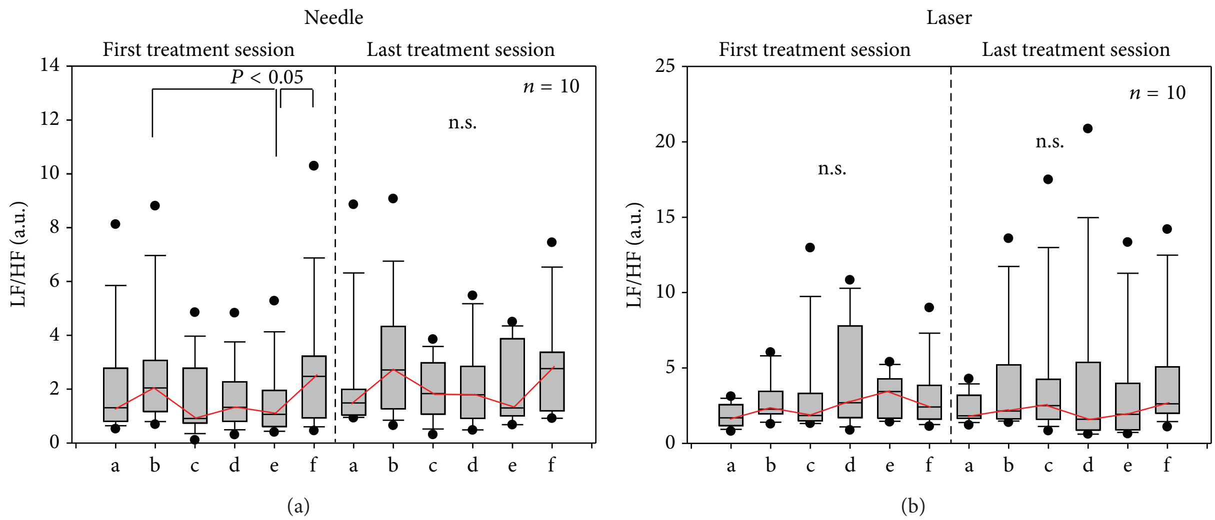

Figure 6: The low frequency (LF)/high frequency (HF) ratio did not change significantly during the three days of the investigation. For further explanations, see Figure 4.

Continuous HRV monitoring showed mainly no significant alterations in the LF/HF ratio during or after acupuncture stimulation (see Figure 6).

The analysis of the new HR-HRV DATS score revealed interesting results. The HR-DATS score was 0.6 during needle acupuncture treatment and 1.7 during laser needle treatment. The HRV-DATS score showed a value of 0.8 during needle treatment and 4.0 during laser needle treatment.

4. Discussion

Burnout can cause serious health implications. In a systematic review of the scientific literature published in 2013, burnout was found to be a risk factor for myocardial infarction, coronary heart diseases, and many other diseases [20]. Chronic stress-related disorders often fall outside the category of a “true” disease and are often treated as depression or not treated at all [20]. Symptoms of stress can be temporarily relieved with drugs, although they do not remove the cause of stress. Developing new treatment approaches therefore has priority, and invasive (needle) or noninvasive (laser needle) acupuncture is one possibility.

In 2013 a randomized, sham-controlled trial investigating acupuncture for chronic fatigue syndrome was published [21]. In this study the authors from Hong Kong saw improvements in clinical scores also after sham acupuncture. They concluded that these effects may be due to the pressure of the sham needles on the acupuncture points, in addition to the placebo effect [21]. Our present study has a completely different study design. There was no control group; we compared two different acupuncture methods in two patient groups, both with the diagnosis burnout. One method was invasive manual needle acupuncture and the other noninvasive laser needle acupuncture. Neurovegetative parameters like HR and HRV were chosen as evaluation parameters. Developing a new parameter, the so-called “dynamic acupuncture treatment score” (DATS), we were able to quantitatively describe treatment effects on the different neurovegetative responses immediately and 5 minutes after the acupuncture sessions. DATS is a simple ratio which reflects the dynamic alterations within different treatment sessions. It shows how the body (neurovegetative system) reacts to a stimulation (in our case manual needle and laser needle acupuncture). The values from the first and last sessions were compared so that one can compare changes and thus effects of the acupuncture treatment sessions. It was interesting that our new score shows stronger effects of laser needle acupuncture than of manual needle acupuncture after six treatment sessions. However, both groups had a very small sample size (10 patients each), and therefore more investigations are absolutely necessary.

In conclusion it can be stated based on new neurovegetative acupuncture treatment evaluation scores that noninvasive laser needle acupuncture, like manual needle acupuncture, has the potential to be a powerful approach for evidence-based complementary treatment of patients with burnout syndrome. Further transcontinental studies to verify or refute the preliminary findings are in progress.

Conflict of Interests

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

Acknowledgments

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Research Article

The Physical Effects of Aromatherapy in Alleviating Work-Related Stress on Elementary School Teachers in Taiwan

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People use aromatherapy to relieve the symptoms of physical and psychological stress. However, previous studies have not precisely clarified a scientific basis for the beneficial effects of aromatherapy. Therefore, the overall purpose of this study was to elucidate the beneficial effect of aromatherapy in relieving work-related stress. Twenty-nine elementary school teachers from Taiwan participated in this study. The experimental procedures comprised 2 phases. First, we verified the effect of aromatherapy by conducting 2 blind tests. We used natural bergamot essential oil extracted from plants and synthesized a chemical essential oil as the placebo to do the aromatherapy. Second, we analyzed the performance of the aromatherapy treatment on the teachers who had various workloads. We measured the teachers' heart rate variability to evaluate their autonomic nervous system activity. The results show that only the natural bergamot essential oil had an effect and that the aromatherapy treatment relieved work-related stress of teachers with various workloads. However, the aromatherapy treatment had a weak effect on young teachers who had a heavy workload. Moreover, the aromatherapy treatment exhibited no effect on teachers who belong to the abnormal body mass index subgroup having a heavy workload.

1. Introduction

In modern society, job-related stress is a substantial problem because 40%–50% of all relative work misses are related to stress [1]. Several previous studies have shown that the level of work-related stress could increase the risk of diseases such as cardiovascular disease [2, 3], neurodegenerative diseases [4], chronic diseases of aging [5], and metabolic syndrome [6]. Elementary school teachers in Taiwan must address problems related to Taiwan's low birth rate. In numerous Taiwanese families, there is only one child. Therefore, elementary school teachers must provide more time to process the child's problem except teaching. Moreover, teachers also do various administrative duties. Therefore, all elementary school teachers are at risk of suffering from work-related stress [7]. Thus, it is crucial for elementary schools to identify appropriate methods for coping with stress.

Various methods for coping with stress have been studied [8]. Aromatherapy is an appealing method because it is both effective and easy to implement [9]. Numerous previous studies have reported using essential oils to alleviate the symptoms of physical and psychological stress [10–13]. Among these studies, bergamot, lavender, and geranium were the most commonly applied essential oils, and the inhalation method was used more than other methods of delivery. These studies employed a randomized controlled trial to conduct the experiments and measured changes in the autonomic nervous systems of participants to quantify the performance of the aromatherapy treatment. During the experimental procedure, the participants were seated for 5 minutes after commencing the test, and their physiological data were collected. Subsequently, the aromatherapy group received the aromatherapy intervention for approximately 10–20 minutes, whereas the control group was either resting or inhaling from an empty diffuser. Following the test, the participants' data were collected. As discussed, the control group did not always use the placebo during the aromatherapy treatment for relieving physical stress symptoms. However, Lorig and Schwartz examined the relationship between the time-domain electroencephalograph (EEG) activity and self-reports from people exposed to various odorants [14]. The results indicate that the odors generated few perceptual or mood differences. EEG alpha and theta are the activity in the left and right hemispheres of the brain. Bagetta et al. used the brain wave spectrum power and found that bergamot essential oil correlates well with its exocytotic and carrier-mediated release of discrete amino acids endowed with the neurotransmitter function in the mammalian hippocampus [15]. Therefore, we considered the placebo a necessary factor for evaluating the effects of aromatherapy treatment.

The autonomic nervous system includes sympathetic and parasympathetic systems. When people feel physical or psychological stress, the sympathetic system becomes more active. When this stressor disappears, the parasympathetic system reduces the heart rate and the breathing rate. The response of the autonomic nervous system can be monitored using the heart rate variability (HRV), which is derived from heartbeat interval time series [16, 17]. Some studies have shown that physical tasks influence the HRV [1, 18, 19]. Psychological depressive and anxiety disorders can also affect the HRV [20, 21]. Therefore, during aromatherapy treatment, the HRV is used to verify the effects of aromatherapy on physical and psychological tasks [10–13, 22].

Chang and Shen examined the effect of aromatherapy on elementary school teachers [10]. In their study, two conditions were not considered. (a) A placebo was not used in the experiment. They analyzed the HRV of subjects before and after aromatherapy treatment. If a synthetic essential oil is used in the treatment process, can it alleviate the symptoms of physical and psychological stress? (b) The varying workloads of subjects were not considered when conducting aromatherapy. Thus, quantifying the effect of aromatherapy is difficult.

The overall objective of our study was to clarify the beneficial effects of aromatherapy in alleviating work stress. The experiment comprised two phases. The purpose of the first phase was to verify the effect of aromatherapy by using two blind tests. We used natural essential oil extracted from plants and synthetic essential oil made with chemical materials to do the aromatherapy. The second phase involved analyzing the performance of aromatherapy for participants with varying workloads. To compare our results with those obtained by Chang and Shen, we also recruited elementary school teachers in Taiwan, and natural bergamot essential oil was used during treatment.

2. Materials and Methods

2.1. Participants. We recruited 29 elementary schoolteachers who did not have asthma, hypertension, or a heart condition. Because an aromatherapy spray may induce asthma, participants with a history of asthma were excluded. Moreover, numerous studies have shown that heart conditions such as arrhythmia, myocardial ischemia, and a history of heart failure affect the HRV [23–26]. Thus, participants with a heart disease and hypertension also were excluded from this study. The Beck Anxiety Inventory (BAI) was used to estimate the anxiety degree of every participant. The anxiety degree is considered minor when the score ranges between 0 and 7. A score ranging between 8 and 15 indicates a light degree. A score ranging between 16 and 25 indicates a moderate degree, and a score between 26 and 63 indicates a serious degree.

2.2. Outcome Measures. For this study, we used 100% pure bergamot essential oil made in Italy, diluted to 2%. The placebo was a synthetic essential oil (Shunyi Chemical Co., Ltd., Taiwan) with a similar scent to the bergamot essential oil. An ultrasonic ionizer aromatherapy diffuser was used for aroma evaporation (type YHL668/I, ultrasound frequency 2.5 MHz, Nature Creart Co., Ltd, Taiwan). For the heart rate measurements and HRV analysis, we employed a handheld HRV meter (LR8Z11, made by Yunyin Co., Ltd., Taiwan). It measures one lead electrocardiogram (ECG), the sampling rate is 500 Hz, and the resolution is 12 bits. The blood pressure was measured using an electronic blood pressure monitor (type HEM-7210, made by OMRON Co., Ltd., Japan).

The HRV is derived from the heartbeat interval time series whose resampling is 4 Hz by using the further discrete Fourier transform. Low-frequency power (LF: 0.04–0.15 Hz), high-frequency power (HF: 0.15–0.4 Hz), and the logarithmic ratio low- to high-frequency power (LF/HF) were calculated. We also used the normalized LF (LF%) and HF (HF%) to indicate the response of autonomic nervous activity. LF is affected by the vagal nervous and the sympathetic nervous, and HF is affected by the parasympathetic nervous [17].

2.3. Experiment Procedure. This experiment was approved by the Asia University Medical Research Ethics Committee. This was a two-phase experiment. The purpose of the first phase was to verify the effect of aromatherapy by conducting a blind test. The second experiment involved analyzing the effect of aromatherapy on teachers with varying workloads.

2.3.1. The Effect of Aromatherapy. In this experiment, each participant underwent the aroma treatment twice. Although the scent of the natural and synthetic essential oils is similar, certain people who carefully compare them at the same time can distinguish between them. Therefore, we set an interval of one week between the two experiments. To avoid the effect of different workloads, the aroma treatment for each participant was conducted on the same weekday at the same time. In both experiments, the participant did not know which essential oil was used. The experiment procedure is described below.

Step 1. Each participant was required to complete a consent form and a BAI survey and provide personal information including gender, age, years of employment, height, weight, and relationship status. Participants were required to abstain from smoking, alcohol, and coffee 6 hours before aroma treatment. At the resting time, the participants were then asked to sit in a chair and rest for approximately 3 minutes.

Step 2. Their blood pressure was measured. We then measured the ECG lasting 3 minutes as a pretest. The participants kept their eyes open and remained still.

Step 3. The aroma treatment lasted approximately 15 minutes. The respiration rate and volume during this time were asked the same as the resting time.

Step 4. Blood pressure was again measured after completion of the aroma treatment. We then measured the ECG lasting 3 minutes as a posttest.

2.3.2. The Performance of Aromatherapy. In this experiment, we recruited the same participants. Each participant underwent aroma treatment twice: once during a heavy-workload state, and once more during a light-workload state. A heavy workload was defined as teaching more than four classes in one day, whereas a light workload was defined as teaching less than two classes in one day. All aroma treatment was conducted after the end of a day's classes. To allow independence for the two treatments, the second treatment was delayed one week from the first treatment. Natural bergamot essential oil was used. The experiment procedure is the same as in Section 2.3.1.

2.4. Statistics. We employed the SPSS 12.0 software package to conduct the one-way ANOVA analysis. Significance for the P value was set at 0.05. Descriptive statistics were represented as the mean ± standard deviation. LF, HF, LF%, HF%, LF/HF, and the R-R interval time (RRI) were used to indicate the response of the autonomic nervous system. Zhang showed that age and gender affect the HRV [27]. Jarrett et al. indicated that the HRV is affected by the body mass index (BMI) [28]. Therefore, the intergroup differences among age, BMI, and the degree of anxiety were examined using an F test.

Table 1: Participant information ($n = 29$).

Items	People number
Gender	Male: 3
	Female: 26
BAI	Minor: 20
	Light: 9
Age (years)	Elder (>40): 18
	Young (≤40): 11
BMI (Kg/m^2)	>24 : 9
	≤24 and ≥21 : 20

3. Results

Detailed participant information is shown in Table 1. We examined 3 male participants and 26 female participants. The average age was 41.4 ± 4 years, and the average BMI was 22.2 ± 3.6. The light-anxiety subgroup comprised 9 teachers, and the minor-anxiety subgroup contained 20 teachers. The older subgroup had 7 teachers, and the young subgroup had 18 teachers. Regarding BMI, the abnormal subgroup had 9 teachers, and the normal subgroup had 20 teachers.

The results obtained after natural bergamot essential oil treatment are shown in Table 2. The LF, LF%, HF%, and LF/HF were found to have a significant difference. Table 3 shows that the results obtained for the synthetic essential oil did not have any indicators with a significant difference.

Tables 4 and 5 show the results before and after natural bergamot essential oil treatment for participants with light and heavy workloads, respectively. We can find that LF, LF%, HF%, and LF/HF have significant differences. Therefore, the aromatherapy can alleviate the symptoms of physical and psychological stress at light and heavy workloads. Furthermore, after controlling the different variables, including age, BMI, and anxiety degree, we conducted an analysis of the subgroup regarding the performance of aromatherapy, the results of which are described as follows. Table 6 shows the results of the age subgroups with a light workload. LF, LF%, HF%, and LF/HF all show a significant difference for the young and older subgroups. Table 7 shows the results for heavy workload teachers. Only the older subgroup was shown to be affected by aromatherapy, with LF, LF%, HF%, and LF/HF having a significant difference. However, in the young subgroup, none of the indicators, except for LF, were found to have a significant difference. Table 8 shows the results of the BMI subgroups with a light workload. The indicators, LF, LF%, HF%, and LF/HF, have a significant difference in the normal and abnormal BMI subgroups. Table 9 shows the results for teachers with a heavy workload. Only the normal BMI subgroup was shown to be affected by aromatherapy; LF, LF%, HF%, and LF/HF have a significant difference in this subgroup. In the abnormal BMI subgroup, none of the indicators showed a significant difference. Tables 10 and 11 show the analyzed results of the anxiety-degree subgroups with light and heavy workloads. LF, LF%, HF%, and LF/HF were all found to have a significant difference.

4. Discussions

Chang and Shen examined the effect of aromatherapy on elementary schoolteachers in Taiwan [10]. Although their results were positive, certain research points were not considered clearly, such as placebo use and teachers' varying workloads. The focus of our study was on the physical effect of aromatherapy on elementary schoolteachers with different workloads. We added a placebo variable to determine whether the HRV response was actually obtained from the aromatherapy. The placebo was a synthetic essential oil that has a similar scent to natural bergamot essential oil. We then examined the physical effect of aromatherapy on alleviating the work stress of teachers with different workloads. Because the age, BMI, and anxiety degree could affect the HRV, we also analyzed these subgroups. Chang and Shen used the ANS Watch monitor to measure the HRV and blood pressure. This device measures the pulse wave of the radial artery to detect the HRV. In contrast, we used an ECG to detect the HRV. Thus, the data of certain indicators differ.

In this study, the HF indicator did not show a significant difference in the two experiments. Because the respiration

Table 2: The HRV one-way ANOVA results before and after natural bergamot essential oil treatment for all participants.

Indicators	Before (n = 29)	After (n = 29)	F	P
LF (ms^2)	9.80 ± 1.19	9.05 ± 1.31	15.21	0.00**
HF (ms^2)	8.91 ± 1.45	8.96 ± 1.36	0.04	0.840
LF/HF (nu)	0.88 ± 1.00	0.10 ± 1.10	24.34	0.00**
LF% (nu)	68.6 ± 19.4	52.2 ± 22.2	26.76	0.00**
HF% (nu)	32.3 ± 19.5	48.7 ± 22.1	26.92	0.00**
RRI (ms)	766.3 ± 90.2	781.5 ± 98.4	1.12	0.290

$^*P < 0.05$, $^{**}P < 0.01$.

Table 3: The HRV one-way ANOVA results before and after synthetic essential oil treatment for all participants.

Indicators	Before (n = 29)	After (n = 29)	F	P
LF (ms^2)	9.5 ± 1.1	9.3 ± 1.2	0.43	0.511
HF (ms^2)	8.8 ± 1.2	9.0 ± 1.2	0.99	0.322
LF/HF (nu)	0.64 ± 1.01	0.34 ± 1.13	3.27	0.075
LF% (nu)	63.25 ± 20.11	57.09 ± 22.94	3.54	0.061
HF% (nu)	37.45 ± 20.10	43.64 ± 23.05	3.54	0.061
RRI (ms)	740.1 ± 85.9	760.2 ± 85.7	2.48	0.124

$^*P < 0.05$, $^{**}P < 0.01$.

Table 4: The HRV one-way ANOVA results before and after natural bergamot essential oil treatment for all participants with a light workload.

Indicators	Before (n = 29)	After (n = 29)	F	P
LF (ms^2)	9.74 ± 1.18	8.97 ± 1.30	16.96	0.000**
HF (ms^2)	8.98 ± 1.49	8.92 ± 1.39	0.07	0.790
LF/HF (nu)	0.76 ± 0.97	0.04 ± 1.14	20.13	0.000**
LF% (nu)	65.85 ± 20.35	50.68 ± 22.57	21.69	0.000**
HF% (nu)	34.45 ± 19.88	50.27 ± 22.43	24.21	0.000**
RRI (ms)	773 ± 96.4	778 ± 102	0.09	0.763

$^*P < 0.05$, $^{**}P < 0.01$.

Table 5: The HRV one-way ANOVA results before and after natural bergamot essential oil treatment for all participants with a heavy workload.

Indicators	Before (n = 29)	After (n = 29)	F	P
LF (ms^2)	9.68 ± 1.15	9.12 ± 1.13	10.41	0.001**
HF (ms^2)	8.98 ± 1.59	9.26 ± 1.27	1.68	0.196
LF/HF (nu)	0.70 ± 1.15	−0.14 ± 1.16	23.04	0.000**
LF% (nu)	63.6 ± 22.3	47.3 ± 24.0	21.47	0.000**
HF% (nu)	37.2 ± 22.4	53.4 ± 23.9	21.42	0.000**
RRI (ms)	771 ± 114	7821 ± 109	0.36	0.548

$^*P < 0.05$, $^{**}P < 0.01$.

Table 6: The HRV one-way ANOVA results of the age subgroups with a light workload before and after natural bergamot essential oil treatment for all participants.

Indicators	Elder (n = 18)				Young (n = 11)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.74 ± 1.15	9.06 ± 1.41	7.50	0.007**	9.75 ± 1.25	8.81 ± 1.10	10.42	0.002**
HF (ms^2)	8.70 ± 1.42	8.70 ± 1.38	0.00	0.995	9.45 ± 1.52	9.29 ± 1.36	0.19	0.660
LF/HF (nu)	1.04 ± 0.82	0.36 ± 1.13	12.71	0.001 **	0.30 ± 1.02	−0.48 ± 0.94	10.41	0.002**
LF% (nu)	71.2 ± 17.5	57.1 ± 21.8	13.59	0.000**	57.2 ± 21.9	40.2 ± 19.9	10.92	0.002**
HF% (nu)	28.7 ± 16.5	43.1 ± 21.8	16.61	0.000**	43.8 ± 21.6	60.7 ± 19.6	11.04	0.001**
RRI (ms)	763 ± 74.7	771 ± 76.5	0.36	0.552	792 ± 123	790 ± 136	0.01	0.946

$^*P < 0.05$, $^{**}P < 0.01$.

Table 7: The HRV one-way ANOVA results of the age subgroups with a heavy workload before and after natural bergamot essential oil treatment for all participants.

Indicators	Elder (n = 18)				Young (n = 11)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.55 ± 1.23	9.08 ± 1.16	4.258	0.042*	9.89 ± 0.98	9.19 ± 1.10	7.32	0.009**
HF (ms^2)	8.55 ± 1.67	9.11 ± 1.30	3.71	0.057	9.67 ± 1.17	9.51 ± 1.20	0.31	0.583
LF/HF (nu)	1.00 ± 1.18	−0.03 ± 1.07	22.53	0.000**	0. 21 ± 0.91	−0.32 ± 1.30	3.71	0.058
LF% (nu)	69.0 ± 22.2	49.6 ± 22.9	20.02	0.000**	54.8 ± 19.8	43.6 ± 25.7	3.91	0.052
HF% (nu)	31.7 ± 22.4	51.2 ± 22.9	20.15	0.000**	46.1 ± 19.7	57.0 ± 25.4	3.78	0.056
RRI (ms)	759 ± 86.2	769 ± 76.4	0.46	0.498	792 ± 148	801 ± 147	0.07	0.797

$^*P < 0.05$, $^{**}P < 0.01$.

Table 8: The HRV one-way ANOVA results of the BMI subgroups with a light workload before and after natural bergamot essential oil treatment for all participants.

Indicators	BMI > 40 (n = 9)				21 ≤ BMI ≤ 24 (n = 20)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.7 ± 1.6	8.8 ± 1.6	4.59	0.037*	9.8 ± 0.9	9.1 ± 1.2	13.46	0.000**
HF (ms^2)	8.71 ± 2.09	8.63 ± 2.05	0.02	0.895	9.11 ± 1.13	9.1 ± 1.0	0.07	0.790
LF/HF (nu)	0.97 ± 0.77	0.12 ± 0.97	12.89	0.001**	0.66 ± 1.04	0.01 ± 1.21	10.16	0.002**
LF% (nu)	71.1 ± 15.4	52.3 ± 20.1	14.95	0.000**	63.5 ± 21.94	50.0 ± 23.7	10.53	0.002**
HF% (nu)	29.9 ± 15.5	48.7 ± 20.4	14.47	0.000**	36.5 ± 21.36	51.0 ± 23.4	12.53	0.001**
RRI (ms)	767 ± 139	773 ± 163	0.02	0.888	777 ± 70.1	781 ± 60.2	0.11	0.740

$^*P < 0.05$, $^{**}P < 0.01$.

Table 9: The HRV one-way ANOVA results of the BMI subgroups with a heavy workload before and after natural bergamot essential oil treatment for all participants.

Indicators	BMI > 40 (n = 9)				21 ≤ BMI ≤ 24 (n = 20)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.40 ± 1.30	9.33 ± 1.13	0.05	0.817	9.80 ± 1.06	9.02 ± 1.13	14.93	0.000**
HF (ms^2)	8.88 ± 1.93	8.96 ± 1.48	0.08	0.774	9.04 ± 1.43	9.40 ± 1.16	2.18	0.143
LF/HF (nu)	0.58 ± 0.93	0.37 ± 0.91	0.71	0.402	0.76 ± 1.24	−0.37 ± 1.20	25.60	0.000**
LF% (nu)	62.4 ± 19.1	58. 6 ± 20.1	0.50	0.481	64.2 ± 23.7	42.3 ± 24.1	25.20	0.000**
HF% (nu)	38.7 ± 19.6	42.4 ± 20.3	0.45	0.507	36.4 ± 23.7	58.4 ± 23.8	25.54	0.000**
RRI (ms)	768 ± 153	802 ± 152	0.64	0.426	773 ± 93.0	772 ± 82.9	0.00	0.989

$^*P < 0.05$, $^{**}P < 0.01$.

frequency at a resting time is within 0.1~0.4 Hz, which locates at the range of HF, the calculated power of HF will include the power of respiratory frequency [29]. Therefore, some studies of aromatherapy have used only the LH and LF/HF indicators to identify the effect of aromatherapy [10, 13]. In this study, we focused on the LF, LF/HF, LF%, and HF% indicators to analyze the effect and performance of aromatherapy.

According to Tables 2 and 3, although the participants could not distinguish between the scent of the natural essential oil and synthetic essential oil, the natural bergamot essential oil has a relaxing effect, whereas the synthetic essential oil does not. This result directly shows that the essential bergamot oil extracted from natural materials for aromatherapy could be a better choice.

Tables 4 and 5 show the effect of aromatherapy on reducing work stress according to varying workloads. The results are in agreement with those of Chang and Shen [10]. In the age subgroup, the light-workload results are in agreement with those of Chang and Shen [10]. However, the elder subgroup with a heavy workload had a better performance than the young subgroup by the aromatherapy. This result differs from the findings of Chang and Shen because Chang and Shen did not consider differences in subjects' workloads; their results for all age subgroups showed that aromatherapy caused an effect [10]. Zhang demonstrated that age had a greater impact on HRV than gender. The older age group had a consistently lower LF and HF compared with the younger group [27]. In our study, the LF% and LF/HF of the young subgroup decreased, and HF% increased before and after aroma treatment. However, none of these indicators were found to have a significant difference. Therefore, age variable may affect the performance of aromatherapy for teachers with a heavy workload. In the BMI analysis, the aromatherapy had effect on teachers with a light workload. But the HRV did not have the significant change before and after aroma treatment with a heavy workload. This is a very interesting

Table 10: The HRV one-way ANOVA results of the anxiety-degree subgroups with a light workload before and after natural bergamot essential oil treatment for all participants.

Indicators	Minor (n = 20)				Light (n = 9)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.72 ± 1.24	9.12 ± 1.36	6.27	0.014*	9.80 ± 1.08	8.62 ± 1.10	15.85	0.000**
HF (ms^2)	8.95 ± 1.54	8.89 ± 1.43	0.04	0.834	9.06 ± 1.40	9.00 ± 1.33	0.03	0.870
LF/HF (nu)	0.77 ± 1.03	0.23 ± 1.24	6.69	0.011*	0.74 ± 0.82	−0.38 ± 0.72	28.01	0.000**
LF% (nu)	65.6 ± 21.7	54.4 ± 24.4	6.94	0.010*	66.5 ± 17.4	42.3 ± 15.0	29.99	0.000**
HF% (nu)	34.3 ± 21.0	46.6 ± 24.3	8.73	0.004**	34.8 ± 17.4	58.5 ± 14.8	29.02	0.000**
RRI (ms)	778 ± 102	788 ± 115	0.24	0.626	763 ± 82.5	756 ± 64.5	0.12	0.733

*$P < 0.05$, **$P < 0.01$.

Table 11: The HRV one-way ANOVA results of the anxiety-degree subgroups with a heavy workload before and after natural bergamot essential oil treatment for all participants.

Indicators	Minor (n = 20)				Light (n = 9)			
	Before	After	F	P	Before	After	F	P
LF (ms^2)	9.62 ± 1.27	9.14 ± 1.23	4.28	0.041*	9.82 ± 0.81	9.07 ± 0.88	10.46	0.002**
HF (ms^2)	8.83 ± 1.66	9.21 ± 1.33	1.91	0.170	9.31 ± 1.40	9.38 ± 1.15	0.04	0.841
LF/HF (nu)	0.79 ± 1.18	−0.06 ± 1.25	14.82	0.000**	0.51 ± 1.08	−0.31 ± 0.95	8.74	0.005**
LF% (nu)	65.3 ± 22.9	49.2 ± 25.3	13.32	0.000**	60.0 ± 20.8	43.2 ± 20.8	8.75	0.005**
HF% (nu)	35.4 ± 23.0	51.6 ± 25.2	13.59	0.000**	41.1 ± 21.1	57.5 ± 20.7	8.30	0.006**
RRI (ms)	77.3 ± 9.5	74.7 ± 11.6	0.08	0.783	68.3 ± 9.6	66.9 ± 9.6	0.94	0.337

*$P < 0.05$, **$P < 0.01$.

result. Jarrett et al. indicated that age and BMI affect the HRV [28]. However, this was not within the scope of our study.

5. Conclusion

We conducted this trial to examine the potential of using natural bergamot essential oil in appeasing the work stress of elementary schoolteachers. The response of automatic nervous system has a significant change after the natural essential bergamot oil treatment. We also analyzed the effect of aromatherapy at different workloads. The aromatherapy may alleviate the symptoms of physical and psychological stress. The results also suggest that age and BMI factors affect aromatherapy performance when teachers have a heavy workload.

Acknowledgment

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Research Article

Auricular Acupuncture at the "Shenmen" and "Point Zero" Points Induced Parasympathetic Activation

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Purpose. Since auricular acupuncture is a diagnostic and treatment system based on normalizing the body's dysfunction, auricular acupuncture has been applied for pain relief, relaxation, and so on. These techniques would modulate the autonomic nerve system, thereby inducing the above-mentioned effects. The aim was to see the effect of auricular acupuncture applied to the "Shenmen" and "Point Zero" points on the postoperative heart rate variability (HRV). *Methods*. Twenty-six patients who underwent hemicolectomy under general anesthesia were randomized into the control or the acupuncture group. After the operation and before emergence, the acupuncture group received auricular acupuncture. An electrocardiographic unit was placed for recording the autonomic nervous activities. *Results*. The low frequency (LF)/high frequency (HF) ratio of HRV increased ($P = 0.0007$) in the control, but the ratio in the acupuncture did not change. There were significant differences between the ratios of the two groups at 3 : 00, 4 : 00, and 5 : 00. HF of the acupuncture group tended to be higher. HFs of the acupuncture group were significantly higher than those of the control group at 3 : 00, 4 : 00, and 5 : 00. *Conclusion*. Auricular acupuncture kept the LF/HF ratio at lower levels and HF at higher levels during postoperative period in the patients who had undergone hemicolectomy.

1. Introduction

Pain relief, relaxation, and the other effects are provided by acupuncture and acupressure techniques using the traditionally used acupuncture points [1–4]. Auricular acupuncture is a diagnostic and treatment system based on normalizing the body's dysfunction [5]. Also, auricular acupuncture has been applied in order to relieve postoperative pain and improve neurorehabilitation and insomnia [6–8]. Moreover, our previous case series showed that after agitated patients received auricular acupuncture at tranquilizing points, they did not show postoperative problematic behaviors such as agitation [9]. Some studies have suggested that these techniques would modulate the reticular formation and the autonomic nerve system, thereby inducing the above-mentioned effects [5].

Frequency-domain analysis of heart rate variability (HRV) is a sophisticated noninvasive tool for the assessment of autonomic nervous system (ANS) regulation of the heart [1, 3, 10, 11]. Frequency fluctuations in the range of 0.04–0.15 Hz (low frequency, LF) are considered to be markers of sympathetic nerve activity, and high frequency (HF) fluctuations in the range of 0.15–0.4 Hz are considered markers of parasympathetic nerve activity [1, 3, 10]. The LF/HF ratio is considered to show sympathovagal balance or reflect the sympathetic modulation [1, 3, 12]. Under various conditions such as physical and mental stress, the activity of the ANS changes and these parameters change.

We hypothesized that auricular acupuncture at the "Shenmen" and "Point Zero" points [9, 13] would tranquilize the mind of patients during postoperative period, thereby changing autonomic nervous activity. In the present study, we therefore investigated the effect of auricular acupuncture applied to the "Shenmen" and "Point Zero" points on the

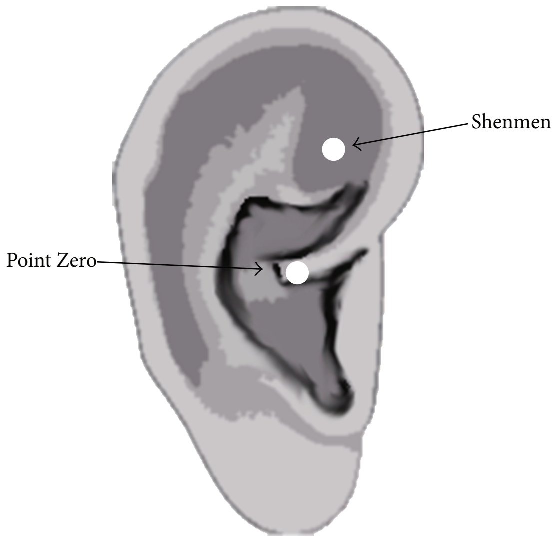

Figure 1: The locations of the “Shenmen” and “Point Zero” points.

postoperative HRV in patients who underwent hemicolectomy for an ascending or descending colon cancer.

2. Methods

After obtaining approval from the Ethics Committees of our institution and written informed patient’s consent, 26 American Society of Anesthesiologists physical status I or II patients presenting for hemicolectomy under general anesthesia combined with epidural anesthesia were enrolled in the present study from February 2010 to November 2010. Patients who had a history of central nervous system or cardiovascular system dysfunction were not invited to participate in the present study.

Patients were randomized into two groups, using sealed envelopes: the control group received no treatment; the acupuncture group postoperatively received occlusive press needles (Pyonex-small; Seirin, Japan) at the “Shenmen” and “Point Zero” points (Figure 1) [9, 13] at both auricles. On arrival in the operation room, all patients had standard monitoring in place (noninvasive arterial pressure, electrocardiogram (ECG), and pulse oximetry). With the patients in the right lateral position, a 20-gauge epidural catheter was placed after identification of the epidural space at the Th10-11 interspace using a 17-gauge Tuohy needle. Then, general anesthesia was induced with fentanyl 100 μg and propofol 160 mg. Anesthesia was maintained using 1.5–2% sevoflurane and epidural 2% lidocaine 8 mL. They received fentanyl 20 $\mu g\, h^{-1}$ and 1% lidocaine 2 $mL\, h^{-1}$ epidurally for the management of postoperative pain. After the operation and before emergence, patients of the acupuncture group received occlusive press needles. All patients were not told whether or not they had received auricular acupuncture. A palm-sized electrocardiographic unit (Active Tracer AC300, GMS, Tokyo, Japan) was placed on all patients for continuous recording of the variation of autonomic nervous activities [14, 15]. The unit was worn in a pouch at the waist with three electrodes taped to the chest until the next day of the operation.

Table 1: Demographic data of patients. Values are the median (range) or number.

	Control group ($n = 13$)	Acupuncture group ($n = 13$)	P
Age (year)	62.5 (37–72)	54 (40–77)	0.8775
Sex (M/F)	8/5	9/4	0.7534
Weight (kg)	50 (43–68)	53.5 (36–70)	0.5308

Data recorded in the palm-sized electrocardiographic unit was analyzed for heart rate variability (HRV) by the maximum entropy method (CHIRAM, Suwa Trust Japan, Japan) [14, 15]. The R-R intervals (RRI) were obtained every 5 minutes. The two components of power of the RRI (ms.ms); LF (0.04–0.15 Hz) and HF (0.15–0.5 Hz), were calculated. Heart rate (HR), the LF and the HF values, and the LF/HF ratio of HRV were analyzed.

Data are presented as the median (range), number or the median with the 25th and 75th percentiles. The demographic data was analyzed by the Mann-Whitney U test or chi-square test. The Friedman test followed by Dunn’s method for multiple comparisons was used for intragroup comparison because of the nonnormal distribution. The Mann-Whitney U test was used to analyze intergroup data. $P < 0.05$ was considered statistically significant.

3. Results

Twenty-six patients were assigned to the control, or acupuncture group. The two groups were comparable in terms of patients’ characteristics (Table 1). All patients did not show postoperative problematic behavior.

In the control group, the LF/HF ratio of HRV started to increase around 1 : 00 ($P = 0.0007$) and the ratio significantly increased at 4 : 00 and 5 : 00 compared to that at 23 : 00 (Figure 2). In contrast, the LF/HF ratio in the acupuncture group did not change ($P = 0.8489$) (Figure 2). There were significant differences between the ratios of the two groups at 3 : 00, 4 : 00, and 5 : 00. HF of the acupuncture group tended to be higher than that of the control group (Figure 3). HFs of the acupuncture group were significantly higher than those of the control group at 3 : 00, 4 : 00, and 5 : 00 (Figure 3).

4. Discussion

Since acupuncture is a diagnostic and treatment system based on normalizing the body’s dysfunction, acupuncture has been applied in order to provide pain relief, relaxation, and the other effects [1–8]. Experimental and clinical investigations have indicated that afferent input in somatic nerve fibers has a significant effect on the autonomic nervous system and hormones. Several studies have investigated the effect of acupuncture on the sympathetic nervous system. In contrast, only a few have explored its effect on the parasympathetic nervous system [16]. Some studies have suggested that these

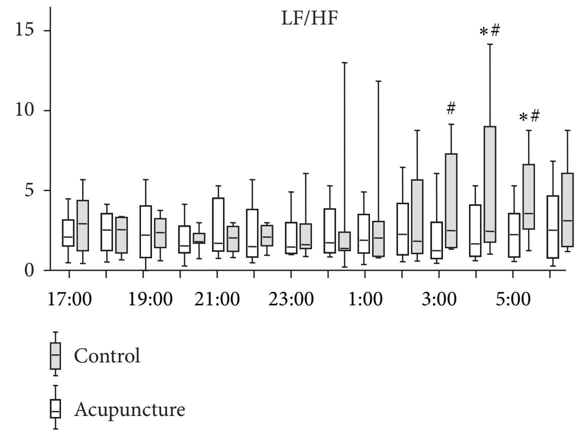

Figure 2: Time course of change in low frequency (LF)/high frequency (HF) ratio of heart rate variability. Horizontal bars represent medians, boxes represent the 25th and 75th percentile ranges, and T bars represent the 5th and 95th percentile ranges. *Different from 23 : 00 ($P < 0.05$). #Different from the acupuncture group ($P < 0.05$).

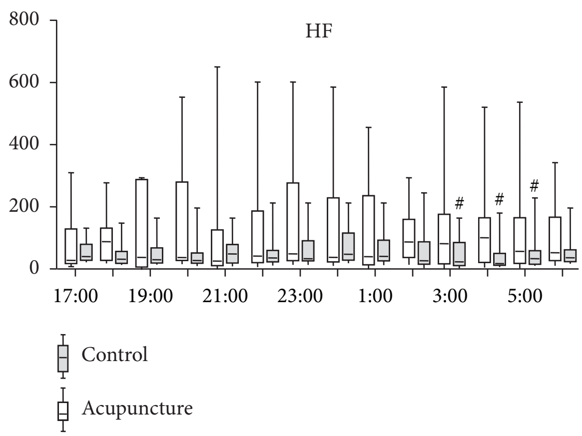

Figure 3: Time course of change in high frequency (HF) of heart rate variability. Horizontal bars represent medians, boxes represent the 25th and 75th percentile ranges, and T bars represent the 5th and 95th percentile ranges. #Different from the acupuncture group ($P < 0.05$).

techniques would modulate the reticular formation and the autonomic nerve system [5], thereby inducing the above-mentioned effects. Although there has been a controversy on the effect of acupuncture on the parasympathetic nervous activity, several studies have shown that auricular acupuncture influences parasympathetic nerve activity [17, 18].

The present study showed that auricular acupuncture kept the LF/HF ratio at lower levels and HF at higher levels during postoperative period in the patients who had undergone hemicolectomy. The data indicate that occlusive press needles at the "Shenmen" and "Point Zero" points at both auricles increased parasympathetic nerve activity, which is consistent with the above-mentioned studies.

In some pathophysiologically disturbed cases, we observe neuropsychiatric symptoms such as epileptic seizure and delirium [19, 20]. Several studies have shown that epilepsy occurs in line with autonomic imbalance, an increased sympathetic activity and a reduced parasympathetic activation [21, 22]. Also, several reports have described that induction or withdrawal of some drugs induced simultaneously delirium and autonomic dysfunction, an increased sympathetic activity [23–25]. Thus, the procedures increasing parasympathetic tone might treat and alleviate these diseases. Our previous case series showed that after agitated patients received auricular acupuncture at tranquilizing points, they did not show postoperative problematic behavior such as agitation [9]. We therefore postulate that, as the present study demonstrated, auricular acupuncture at tranquilizing points had induced the activation of parasympathetic nerve tone, thereby preventing postoperative agitation in the case series.

In conclusion, auricular acupuncture kept the LF/HF ratio at lower levels and HF at higher levels during postoperative period in the patients who had undergone hemicolectomy.

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Research Article

Laser Acupuncture: Two Acupoints (Baihui, Neiguan) and Two Modalities of Laser (658 nm, 405 nm) Induce Different Effects in Neurovegetative Parameters

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There are only few scientific publications dealing with the basic investigation of the effects of only one or two acupoints or comparing one single point with another single point, using different stimulation methods in the same persons. The aim of this needle-controlled, randomized crossover study was to investigate the neurovegetative parameters heart rate (HR) and heart rate variability (HRV) using two different acupoints, Baihui (GV20) and Neiguan (PC6), in separate sessions. We investigated 11 healthy volunteers (3 m, 8 f) with a mean age ± SD of 22.9 ± 2.8 years. The two acupoints were stimulated for 10 minutes each with manual needle acupuncture, red laser acupuncture (658 nm), and violet laser acupuncture (405 nm), in randomized order. Needle and red laser stimulation of the Baihui acupoint decreased HR significantly. Only violet laser stimulation at the Neiguan acupoint induced a significant increase of total HRV. Further studies using other neurovegetative parameters and more volunteers are necessary to confirm the preliminary results.

1. Introduction

The term “laser” is often connected with precision, future, and innovation. Only very few other areas have shown such a rapid and enormous development over the last years as medical laser applications. High-tech laser acupuncture [1], intravenous laser blood irradiation [2], and noninvasive and invasive laser needle stimulation [1–3] are only some of the future-oriented laser therapy options in medicine.

Our research group was the first to investigate violet laser acupuncture (405 nm). Important contributions on this topic were published between 2010 and 2012 [4–8]. The most important difference between violet and, for example, red laser (658 nm) is the penetration depth (405 nm: 1-2 mm; 658 nm: 3-4 cm) on the one hand and the fact that the entire energy of the violet laser is absorbed already at the skin surface on the other hand. In practical terms, this means that the red laser cannot be felt, whereas the violet laser can, thus inducing a stronger deqi sensation [9].

The aim of this needle-controlled, randomized crossover study was to investigate neurovegetative parameters like heart rate (HR) and heart rate variability (HRV) in two different sessions using two different acupoints in healthy volunteers.

2. Materials and Methods

2.1. Stimulation Methods

2.1.1. Manual Needle Acupuncture. Needle acupuncture was performed with single-use sterile needles (0.30 × 30 mm, Huan Qiu, Suzhou, China). After disinfecting the skin at the

Figure 1: Laser needle system at the Medical University of Graz.

chosen acupoint(s) (see below), the needle was inserted and stimulated clockwise and counterclockwise for 15 seconds each, with two rotations per second, resulting in 30 rotations [10]. After ten minutes, the needle was removed.

2.1.2. Red Laser. The red laser needle radiation (658 nm) was coupled into optical fibers, and the laser needles were arranged at distal ends of the optical fibers of a Laserneedle-touch system (Laserneedle GmbH, Berlin, Germany; see Figure 1). Output power of each laser needle was 40 mW. The fiber core diameter was about 500 μm. The time of irradiation was 10 minutes, resulting in an energy density of about 20 J/cm^2 per acupoint. A continuous wave (cw) mode was applied. The needles were placed vertically on the skin using special applicators, triggering painless and nonperceptible stimulation at the acupoint. The method is described in detail in previous publications [11].

2.1.3. Violet Laser. Noninvasive violet optical laser needles (wavelength: 405 nm, output power 110 mW, laser needle spot diameter 500 μm, duration: 10 min, cw-mode) were also fixed onto the skin, but not inserted. The same laser needle system described above was used. Optical energy density was very high (range: kJ/cm^2). More details concerning the technical parameters of laser needle acupuncture can also be found in recent publications [12].

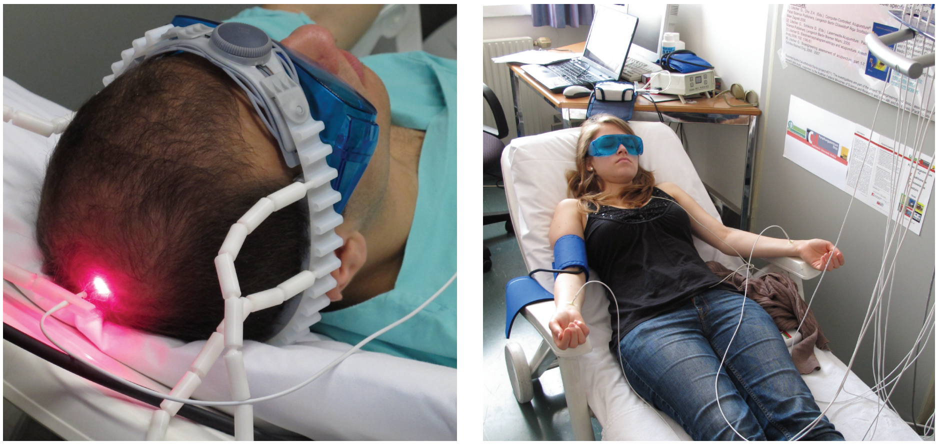
2.2. Neurovegetative Monitoring. The two neurovegetative parameters were recorded using an HRV Medilog AR12 (Huntleigh Healthcare, Cardiff, UK; and Leupamed GmbH, Graz, Austria) system. This system is designed for a monitoring period up to 24 hours. The sampling rate of the recorder is 4096 Hz, so that R waves can be detected extremely accurately. Three “Skintact Premier F-55” ECG electrodes (Leonhard Lang GmbH, Innsbruck, Austria) were fixed on the chest. All raw data are stored digitally on special memory cards.

HRV is measured as the percentage change 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 and is used also in acupuncture research [13–23]. 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]. To the best of our knowledge, there are no scientific results concerning laser acupuncture and long-lasting thermoregulatory effects which might be reflected in HRV.

2.3. Volunteers. The investigations were performed in eleven healthy volunteers (M/F, 3/8) with a mean age ± SD of 22.9 ± 2.8 years. Body height was 172.9 ± 7.4 cm and body weight 66.0 ± 10.4 kg. None of the subjects was under the influence of any medication. The registration of the non-invasive parameters was approved by the local ethics committee and in accordance with the Declaration of Helsinki of the World Medical Association. All persons provided written informed consent.

2.4. Acupuncture Points. The following acupuncture points were used in our study: Baihui (GV20) and Neiguan (PC6) (see Figures 2(a) and 2(b)). Baihui is one of the most important acupoints of the Du meridian (governing vessel) and commonly used in neurology and psychiatry [24]. It is located on the continuation of the line connecting the lowest and highest points of the ear, on the median line of the head, 7 cun above the posterior hairline, and 5 cun behind the anterior hairline [25]. Neiguan is located on the palmar side of the forearm, on the line connecting Quze (PC3) and Daling (PC7), 2 cun proximal to the transverse crease of the wrist, and between the tendons of m. palmaris longus and m. flexor carpi radialis [26]. This point was stimulated bilaterally (see Figure 2(b)). Its main indications are cardiac pain, palpitation, oppressed feeling on the chest, vomiting, epilepsy, mania, and febrile diseases.

2.5. Measurement Procedure. The procedure was divided into three parts: violet laser, red laser, or needle acupuncture, which were performed in the same subjects in a randomized order. Between the different stimulation modalities, there was a break of at least 10 min. Each of the acupoints described above was tested in all subjects in separate sessions on two different days (cross-over), also in randomized order. The participants were lying comfortably on a bed during the entire investigation.



(a) (b)

Figure 2: Acupuncture points Baihui (GV20; (a)) and Neiguan (PC6; (b)).

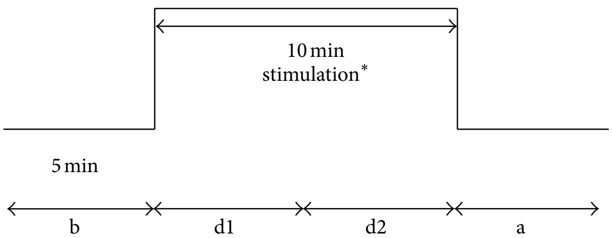


Figure 3: Recording profile. *Manual needle stimulation, red laser (658 nm), and violet laser (405 nm), in randomized order. (b) before stimulation; (d1, d2), two phases during stimulation; (a) after stimulation.

The measurement procedure, and the 5-minute segments (altogether 20 min) are shown in Figure 3.

2.6. Statistical Analysis. Data were analyzed with one-way repeated measures analysis of variance (ANOVA; SigmaPlot 12.0, Systat Software, Chicago, IL, USA), and the Tukey test was used for *post hoc* analysis. The level of significance was defined as $P < 0.05$.

3. Results

Figure 4 shows HR values from the measurements. During and after stimulation of the Baihui acupoint, HR decreased; however, the effect was significant only during and after needle and red laser stimulation. Stimulation of Neiguan did not induce any significant changes in HR.

The values of total HRV of all 11 healthy volunteers are summarized in Figure 5. Neither needle nor red laser nor violet laser stimulation at the Baihui acupoint induced significant changes in HRV, although HR decreased during and after all three stimulation modalities (cf. Figure 4). However, violet laser stimulation of the Neiguan acupoints showed a significant increase during the second half of the stimulation phase (d2) in comparison to the baseline values (b) and also to the first half of the stimulation phase (d1). It is interesting that needle and red laser stimulation did not induce similar effects.

4. Discussion

Acupuncture, an oriental medicine technique that can be traced back at least 2,500 years, is gaining popularity as an alternative and complementary intervention in the Western world. According to traditional Chinese medicine (TCM), acupoints are distributed along meridians beneath the body's surface [27]. In TCM, the single acupuncture points will often have different effects, although they are used in the treatment of the same disease. When investigating the effectiveness of acupuncture on certain diseases, acupoint combinations or schemes are usually used. There are only few scientific publications dealing with the basic investigation of the effects of only one or two acupoints. There are even fewer studies comparing one single point with another single point, using different stimulation methods in the same persons, as performed in this study.

Baihui (GV20) is one of the most important acupoints of the entire meridian acupoint system. It was reported that electroacupuncture stimulation at GV20 increased the cerebral perfusion in the cerebral cortex which was suppressed in endothelial nitric oxide synthase knockout mice [28] and also increased cerebral blood flow in a model of ischemic brain injury in rats [29]. There were also reports of acupuncture stimulation of GV20 working well in lowering blood pressure not only in hypertension patients [30] and healthy subjects [31] but also in spontaneously hypertensive rats [32]. Similar effects may be relevant for decreasing abnormally elevated glutamate and acetylcholine levels in the lesioned side of the striatum [33]. Another aspect of this study on the effect of GV20 was that the point was ascribed the ability to calm and stabilize emotions [33]. In the clinical procedure for patients with sleep disturbances, Baihui is the most commonly used acupuncture point [34, 35]. Acupuncture on GV20 mitigated the anxiety symptoms of women undergoing

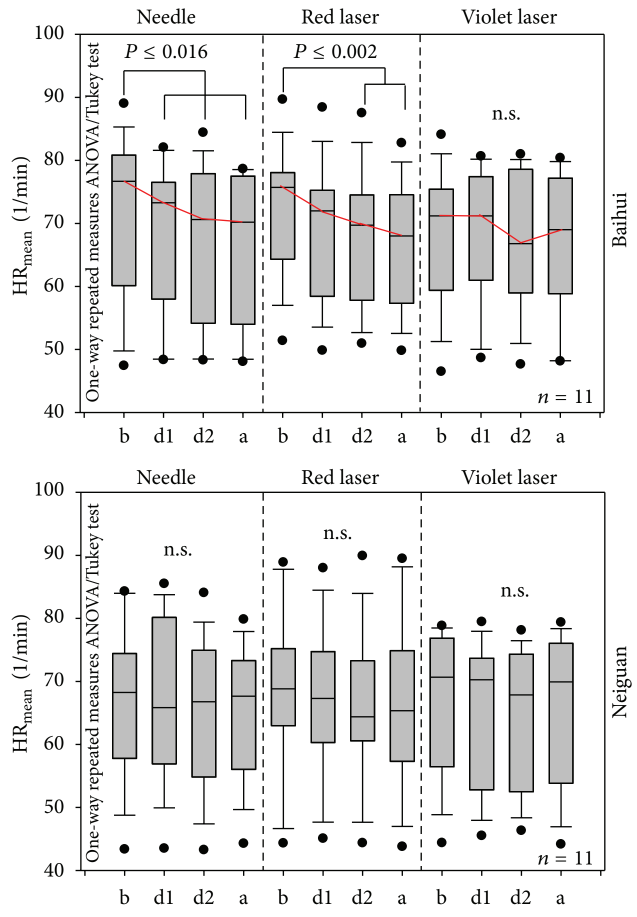


Figure 4: Box plot presentation of HR results. Note the significant decrease of HR (needle and red laser acupuncture). The lines in the boxes represent the median; the ends of the boxes, the 25th and 75th percentile; the error bars, the 10th and 90th percentile; and the dots, the outliers. (b) Before stimulation; (d1, d2), two phases during stimulation; (a) after stimulation.

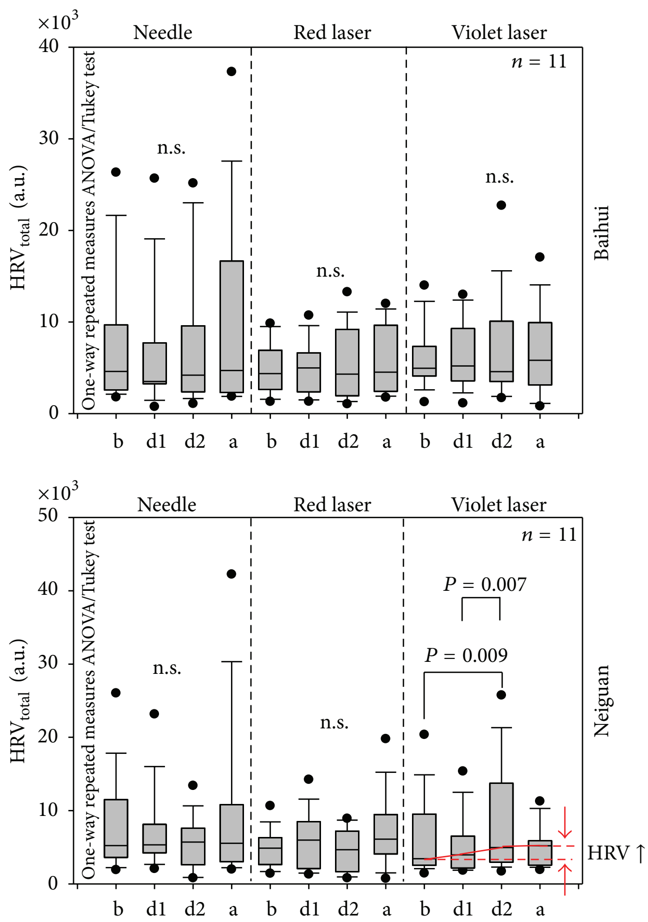


Figure 5: Changes in total HRV. A significant increase occurred only during violet laser stimulation of the Neiguan acupoint. For further explanations, see Figure 4.

in vitro fertilization [36] and patients during dental treatment [37]. GV20 was found to have the ability of vasodilatation and reduction of sympathetic activity in the stress response [28–32]. In the present study, we found GV20 to have the effect of decreasing HR, which is in accordance with the previously mentioned effects.

Neiguan (PC6) is also a classic acupuncture point in TCM. It is considered to be effective when treating cardiovascular disorders. Accumulating scientific evidence has recently shown that PC6 could modulate cardiovascular functions, possibly through activation of the rostral ventrolateral medullar (RVLM) area [38, 39]. It was also reported that stimulation of PC6 decreased the extent of myocardial ischemia by means of reducing the myocardial oxygen demand in animals, and it reduced sympathoexcitatory cardiovascular reflex responses, partly through an effect on the RVLM [40, 41]. Another study suggested that PC6 and ST36 both affected cardiac activities in healthy volunteers [42]. In reports from patients suffering from circadian rhythm disorders, laser acupuncture stimulation applied on PC6 increased vagal activity and suppression of cardiac sympathetic nerves [43]. However, another study suggested that HRV was not influenced by laser needle acupuncture at the Neiguan point (PC6) [44]. This is similar to the observations in our experiment. Further studies concerning possible long-term effects of different kinds of lasers on neurovegetative parameters are desirable.

5. Conclusions

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

(i) Needle and red laser stimulation of the Baihui acupoint decrease heart rate in human subjects significantly.

(ii) Only violet laser stimulation at the Neiguan acupoint induces a significant increase of total heart rate variability. This is even more interesting because of the fact that at the same time HR did not change significantly.

Conflict of Interests

The authors declare to have no conflict of interests.

Acknowledgments

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Research Article

Effects of Acupuncture on Heart Rate Variability in Beagles; Preliminary Results

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Evidence-based animal experimental research concerning the effects of acupuncture on autonomic function was performed by two research teams from China and Austria. This study describes measurements in beagles. Heart rate variability (HRV) recordings were performed under stable conditions in Beijing, China, and the data analysis and interpretation were completed in Graz, Austria. The electrocardiograms were recorded during bilateral body acupuncture (PC6, Neiguan). Power of the low frequency (LF), high frequency (HF), and the ratio (LF/HF) changed significantly during acupuncture stimulation in beagles after injection of atropine and β-blocker. However, there was no significant change in HF power after needling the Neiguan acupoint when a cervical vagotomy has been performed. Our findings show that acupuncture can mediate the HRV even after pharmaceutical blocking of autonomic function. Acupuncture effects on HRV should rely not only on autonomic nervous system but on complete central nervous system.

1. Introduction

Acupuncture is being recognized as an effective treatment for various autonomic disorders; however, most of the mechanisms of this therapeutic method still remain unclear. The results obtained using good experimental designs are well documented and are important for general acceptance of this traditional Chinese medical treatment in the Eastern and Western worlds.

This study represents different acupuncture measurements performed on beagles. The main goal was to investigate the effects of acupuncture stimulation on heart rate variability (HRV) in dogs under stable conditions. The data were recorded in Beijing, China, and the data analysis and interpretation were completed in Graz, Austria.

2. Materials and Methods

2.1. Animals. Three beagles (weight 27.5 kg, 25 kg, and 30 kg) were investigated. Each dog was housed in a well-ventilated facility in a single cage and fed twice a day with commercial dry food. The animals were anesthetized with an intraperitoneal injection of 2.5% pentobarbital sodium (1.0 g/kg). 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 Committee.

The exact anatomical features, especially the centers in the beagles' brain responsible for the modulation of HRV, are described in detail in special books [1].

2.2. Electrocardiographic Monitoring. The electrocardiogram (ECG) was monitored using a Zymed 1410-type 3-lead dynamic ECG system (La Honda, CA, USA). After fast Fourier transformation (FFT) into heart rate spectra, low frequency (LF; 0.04–0.15 Hz) was used to represent the usual regulating region by sympathetic and vagus nerve activity, and high frequency (HF; 0.15–0.40 Hz) represented the vagus nerve activity. In addition, the LF/HF ratio was calculated. In order to minimize the number of animals, we analyzed altogether nine measurement phases in three dogs. The measurements were repeated two times in all dogs.

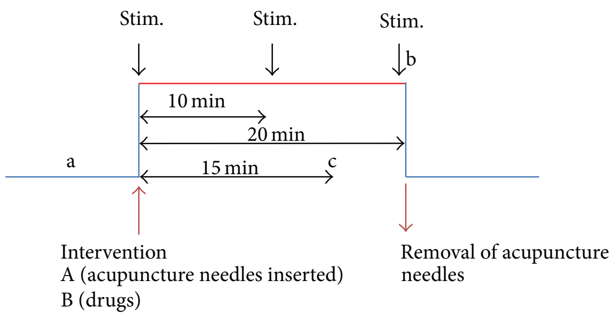

Figure 1: Measurement procedure of the study.

2.3. Acupuncture Stimulation, Drugs, and Procedure. Once the data of the ECG monitoring had reached a steady state, acupuncture stimulation was performed. 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 Neiguan acupoint (PC6) on both sides. The needles were stimulated clockwise and counterclockwise for 15 sec each, with two rotations per second, resulting in 30 rotations per stimulation. The stimulation was performed immediately after inserting the needle, 10 min later, and before removing the needle, always by the same expert. After 20 min, the needles were removed. All points were identified by anatomical marks based on descriptions in textbooks [2]. Briefly, PC6 is located proximal to the accessory carpal pad of the forelimb between the flexor carpi radialis and palmaris longus ligaments. It is one of the most prominent acupuncture points. The following measurement periods were analyzed: one before intervention (a) (acupuncture (A) or drugs (B)), one after 30 sec at the end of three acupuncture stimulations (b), and 15 min after injection of drugs (c), respectively (Figure 1). As already mentioned, the dogs were also investigated under the influence of propanolol (0.2 mg/kg body weight, partial β-adrenergic blockade) and atropine (0.1 mg/kg body weight, partial vagal blockade).

2.4. Statistical Analysis. The data were analyzed using one-way repeated measures analysis of variance (ANOVA) (SigmaPlot 11.0, Systat Software, 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

Tables 1–3 demonstrate the total results from the parameters (mean values ± standard deviation) of HRV in the nine measurements after drug application, after acupuncture, and after vagotomy.

After injection of atropine and propranolol dinitrite, HRV shows the changes of LF, HF, and LF/HF. Both drugs induced a significant decrease in LF ($P < 0.05$). The injection of propranolol led to a significant ($P < 0.01$) increase in HF which contributed to the significant ($P < 0.01$) decrease in LF/HF. The atropine decreased the HF significantly ($P < 0.01$), which led to a significant ($P < 0.01$) increase in LF/HF (see Table 1).

Table 1: Drug effect on HRV.

Phase	LF (bpm^2)	HF (bpm^2)	LF/HF
Control	37.27 ± 16.20	45.02 ± 25.32	0.91 ± 0.32
Propranolol	19.25 ± 8.24*	66.83 ± 26.26**	0.68 ± 0.35**
Atropine	19.88 ± 20.10*	5.03 ± 6.44**	6.89 ± 3.19**

$^*P < 0.05$; $^{**}P < 0.01$ (LF: low frequency band; HF: high frequency band; LF/HF: ratio; bpm: beats per minute).

Table 2: Effect of acupuncture on HRV after blocking the autonomic function.

Phase	LF (bpm^2)	HF (bpm^2)	LF/HF
Control	37.27 ± 16.20	45.02 ± 25.32	0.91 ± 0.32
Atropine/propranolol	13.97 ± 5.72*	14.15 ± 9.30**	1.83 ± 1.77**
Acupuncture	14.44 ± 5.75*	22.97 ± 17.45**	1.57 ± 1.05**

$^*P < 0.05$; $^{**}P < 0.01$ (LF: low frequency band; HF: high frequency band; LF/HF: ratio; bpm: beats per minute).

Table 3: Effect of acupuncture on HRV after cervical vagotomy.

Group name	LF (bpm^2)	HF (bpm^2)	LF/HF
Control	37.27 ± 16.20	45.02 ± 25.32	0.91 ± 0.32
Cervical vagotomy	1.60 ± 0.04**	0.28 ± 0.03**	5.63 ± 0.22**
Acupuncture	1.86 ± 0.86*	0.28 ± 0.16	6.38 ± 1.46**

$^*P < 0.05$; $^{**}P < 0.01$ (LF: low frequency band; HF: high frequency band; LF/HF: ratio; bpm: beats per minute).

The effects of acupuncture on HRV after blocking the autonomic function by atropine and propranolol dinitrate can be seen in Table 2. After giving atropine and propranolol dinitrate, the LF and HF of the animals decreased significantly compared with the control measurement ($P < 0.05$), and LF/HF was increased significantly ($P < 0.05$). After the acupuncture treatment, both LF and HF increased, but only HF increased significantly ($P < 0.01$), which led to significant ($P < 0.01$) changes in LF/HF (see Table 2).

Table 3 summarizes the effect of acupuncture on HRV after cervical vagotomy. Continuous ECG monitoring showed substantial and significant decreases in both the LF and HF band ($P < 0.01$) and significant increases in LF/HF ratio ($P < 0.01$). After acupuncture stimulation, both LF/HF and LF increased significantly ($P < 0.01$, $P < 0.01$, resp.), but there was no significant change in HF.

4. Discussion

There are several human and animal HRV investigations concerning the mediation of this physiologic parameter. On the one hand the HRV can be blocked through vagotomy of the Nn vagi [3] but also atropine can depress HRV depending on the dosage [4, 5] which is also demonstrated in this study. On the other hand a β-receptor blockade has no or only minimal effect on HRV [4, 5]. Investigations in animal models show that changes in the heart rate after stimulation of the sympathicus are independent of the cardiac cycle. In contrary after vagal stimulation there is a manifestation in relationship to the heart cycle with an irregular, discontinuous function of the vagal stimulus frequency. This could be found in the ECG as alterations between the different activities of the heart chambers [6]. These results show clearly that the HRV is mainly influenced via parasympathetic activity; only the low frequency parts will be mediated from sympathetic and parasympathetic nervous system simultaneously [5, 7]. These activities are mainly based on cardio circulatory centers in the medulla [8, 9].

There are several animal experimental studies in the field of HRV in dogs available in the scientific literature [10–15]. The HRV reduction in our study was mainly due to the reduced vagus nerve activity and increased sympathetic nerve activity. Vagus nerve's function is to protect the heart [16]. Our report supports the idea that vagus nerve activation can increase the LF and reduce the HF and HRV, which coincides with previous studies. The present experiment shows clearly that needling the acupuncture point Neiguan can significantly improve the HRV of beagles with low HRV level induced by atropine. So needling Neiguan acupuncture point plays a role of drug intervention effect resistance which can inhibit the sympathetic nervous system and activate vagus nerve activity at the same time. It is interesting that needling PC6 can also regulate sympathetic nervous system when the sympathetic nerve activity was inhibited by β-blocker. HRV decreased significantly when both sympathetic nervous system and vagus nerve activity were blocked, and the effects of acupuncture on HRV were inhibited significantly, suggesting that complete autonomic system is necessary in acupuncture treatment to balance the function of sympathetic nervous system and vagus nerve activity. Considering the insignificant regulation after needling Neiguan acupoint and blocking both ways of sympathetic nervous system and vagus nerve, we deduce that acupuncture still works through the humoral coordination. Acupuncture mechanism seems to work in two directions. It can help the organism return to its own balance.

The data mainly suggest that acupuncture can only mediate sympathetic nerve function after vagotomy.

There are some limitations within this pilot study. The number of animals was small; however as already mentioned we wanted to minimize the number of animals and therefore we analyzed altogether nine measurement phases. In addition there was no control group with a control nonacupuncture point.

Of course, HRV can be calculated not only in the frequency domain, but also in the time domain. However, with this kind of analysis it is not possible to differentiate between the single frequency components, and therefore it was not used in our present study [8, 17].

As already mentioned, Neiguan (PC6) is a prominent and classic acupuncture point in traditional Chinese medicine. It is considered to be effective in the treatment of cardiovascular disorders. In a recently published review article [18], the authors focused on the neurophysiological bases of the effects of PC6 stimulation on cardiovascular mechanisms. They stated that experimental studies have shown that the hypothalamic rostral ventrolateral medulla, arcuate nucleus, and ventrolateral periaqueductal gray are involved in acupuncture-induced attenuation of sympathoexcitatory cardiovascular reflex responses [18]. This long-loop pathway also appears to contribute to the long-lasting, acupuncture-mediated attenuation of sympathetic premotor outflow and excitatory cardiovascular reflex responses [18]. Thus, the authors conclude that acupuncture of PC6 modulates the activity in the cardiovascular system, an effect that may be attributed to an attenuation of sympathoexcitatory cardiovascular reflex responses [18].

After bilateral cervical vagotomy needling and stimulation of the acupuncture point Neiguan can still evoke HRV changes. We deduce that acupuncture can also work through humoral coordination. This could be an interesting topic for future investigations.

5. Conclusions

The following conclusions can be drawn from the results of this transcontinental experimental animal acupuncture study.

(i) Both propranolol and atropine can affect the HRV of beagles significantly.

(ii) Acupuncture can mediate the HRV even after pharmaceutical blocking of autonomic function.

(iii) Acupuncture effects on HRV should rely not only on autonomic nervous system but on complete central nervous system.

Conflict of Interests

No conflict of interests is declared.

Authors' Contribution

H. Wang and G. Litscher, the first two authors, contributed equally.

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Research Article

Intravenous Laser Blood Irradiation, Interstitial Laser Acupuncture, and Electroacupuncture in an Animal Experimental Setting: Preliminary Results from Heart Rate Variability and Electrocorticographic Recordings

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This is the first study to investigate intravenous (i.v.) laser blood irradiation, interstitial (i.st.) laser acupuncture, and electroacupuncture (EA) in combination with heart rate variability (HRV) and electrocorticogram. We investigated 10 male anesthetized Sprague-Dawley rats under the three conditions mentioned previously in Beijing, China, and data analysis was performed in Graz, Europe. For i.v. laser stimulation in the femoral vein and i.st. laser acupuncture at Neiguan (PC6), we used a European system (Modulas needle, Schwa-Medico, Germany; 658 nm, 50 mW, continuous wave mode), and for EA at Neiguan, a Chinese system (Hanshi-100A; Nanjing Jisheng Medical Technology Company, China; 15 Hz, 1 mA). HR, HRV, and electrocorticogram were recorded using a biophysical amplifier AVB-10 (Nihon-Kohden, Japan). HR changed significantly during i.st. laser acupuncture stimulation of Neiguan in anesthetized rats. Total HRV increased insignificantly during i.v. and i.st. laser stimulation. The LF/HF ratio showed significant changes only during i.v. laser blood irradiation. Integrated cortical EEG (electrocorticogram) decreased insignificantly during EA and i.v. laser blood irradiation. Further studies concerning dosage-dependent alterations are in progress.

1. Introduction

Intravenous (i.v.) laser blood irradiation was accomplished for the first time approximately 25 years ago in the former Soviet Union [1–3]. Laser light was brought directly into the blood stream through a one-way catheter. In vitro tests showed that biological soft laser irradiation of white blood cells caused various positive effects, in particular expression of immunoglobulins, interferons, and interleukins. After the introduction of the new method, several clinical studies were published, showing additional effects on various metabolic pathways [4–6].

A new technique, percutaneous interstitial (i.st.) laser therapy (using a sterile catheter), allows penetration of laser light into deeper tissues for successful treatment of, for example, herniated disks or spinal stenosis [7]. With this technique, it is possible to irradiate the inside of damaged joints directly, which can lead to better therapeutic results [7]. Penetration depths of up to 10 cm can be reached. Besides red and infrared lasers, also green and violet (blue) lasers, which are normally absorbed directly by the tissue, can be applied deep in the joints or acupuncture points and develop their positive microcirculatory effects [8, 9]. Furthermore, it offers the option to treat tumors with combined photodynamic therapy [10].

In addition to i.v. and i.st. laser therapy, of interest predominantly in Western medicine, we included electroacupuncture (EA) as a treatment modality in our present study.

EA is a well-known method which has been used and investigated over the last 25 years [11].

In the present study, we explored, for the first time i.v., i.st. laser acupuncture and EA in anesthetized rats under stable conditions and analyzed the effects on physiological neurovegetative parameters and bioelectrical brain activity. Similar to previous studies [12, 13], the data were recorded in 10 rats in Beijing, China, and the data analysis was performed in Graz, Austria.

2. Animals and Methods

2.1. Sprague-Dawley Rats. Ten male healthy Sprague-Dawley rats (weight: 260–300 g) were kept in an animal house maintained at 24 ± 1°C, with a 12-hour light-dark cycle and free access to food and water for seven days before the experiment. The animals were initially anesthetized with an intraperitoneal injection of 10% urethane (1.2 g/kg, Sigma-Aldrich, St. Louis, USA). Additional sodium pentobarbital was administered if necessary to prolong the anesthetic state. Animals were sacrificed by an overdose of anesthetics after the study. The study was approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences and was in accordance with the National Institutes of Health guidelines.

2.2. Intravenous Laser Irradiation. Under anesthesia, the rat was fixed in supine position. The skin in the right groin was cleaned and sterilized with 2% iodophor. Skin incision was performed along the right groin, and the femoral vein was exposed and separated about 1.0–1.5 cm. The proximal femoral vein was clipped by a bulldog clamp, and the distal femoral vein was ligated. The laser needle was inserted into the femoral vein and fixed. The bulldog clamp was removed, and the skin incision was purse string sutured.

The laser needle for i.v. laser irradiation (length 35 mm, diameter 0.55 mm) was a Modulas needle (type: IN-Light, Schwa-Medico, Ehringshausen, Germany). It emits red laser light in continuous wave mode with a wavelength of 658 nm and an output power of 50 mW (Figure 1).

2.3. Interstitial Laser Acupuncture. Apart from the i.v. irradiation, we stimulated the acupoint Neiguan (PC6) on the left side using i.st. laser acupuncture, using the same type of equipment as for the intravenous irradiation. The laser needle was inserted about 3 mm in the acupoint Neiguan. This acupoint is located proximal to the accessory carpal pad of the forelimb, between the flexor carpi radialis and palmaris longus ligaments.

2.4. Electroacupuncture. Electroacupuncture was also performed at the left Neiguan acupoint. One needle was inserted about 3 mm into the acupoint, and the other needle was inserted into a point 2 mm from the acupoint. The pair of needles was connected to an EA device (Hanshi pain healing device, Hanshi-100A; Nanjing Jisheng Medical Technology Company, Nanjing, China). The current intensity was 1 mA, and the pulse frequency was 15 Hz, since studies by Han have

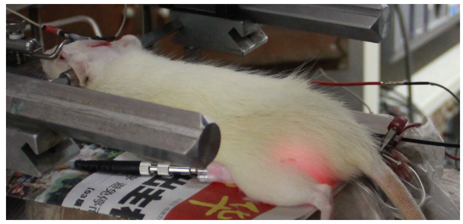

Figure 1: Active i.v. laser blood irradiation.

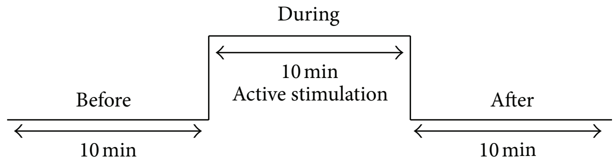

Figure 2: Experimental procedure for the different stimulation methods.

shown that low frequencies yield better results; however, in future studies also 100 Hz will be investigated.

2.5. Procedure. The measurement profile is shown in Figure 2. Three measurement periods were compared: one before, one during, and one after stimulation. This scheme was used for all three conditions (i.v. laser blood irradiation, i.st. laser acupuncture, and EA) in the same rat. The order of the stimulation methods was randomized, and the time between the separate measurements was at least 30 min.

2.6. Measurement Parameters. We registered electrocardiographic (ECG) and electroencephalographic (EEG) parameters using a biophysical amplifier AVB-10 (Nihon-Kohden, Japan). For the ECG, we evaluated heart rate (HR), heart rate variability (HRV), and the LF (low frequency)/HF (high frequency) ratio of HRV. EEG was registered directly on the brain; high cutoff frequency was 100 Hz, and the low cutoff frequency was 0.5 Hz.

2.7. Statistical Analysis. The data were analyzed using one-way repeated measures analysis of variance (ANOVA) (SigmaPlot 12.0, 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

From a technical point of view, the data quality was very good in all 10 rats with a minimum of artifact. Figure 3 shows an example of the raw data of the EEG and the ECG.

The analysis of the HR of all 10 rats is summarized in Figure 4. Note the significant ($P = 0.026$) decrease of HR after i.st. laser acupoint stimulation. It is also interesting that already in the phase during i.v. laser stimulation HR decreases, whereas HR decreased only in the phase after the two acupuncture stimulation methods.

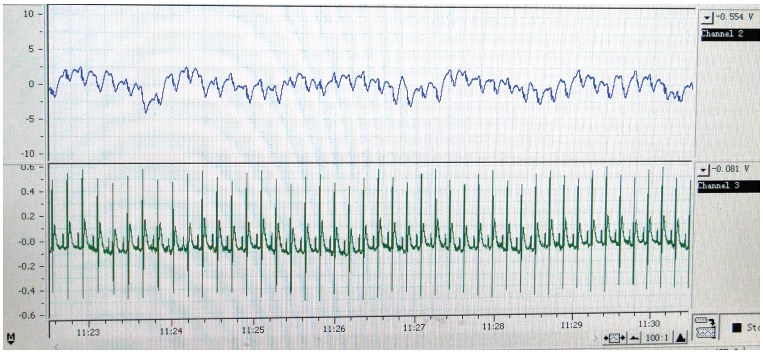

Figure 3: EEG (upper panel) and ECG (lower panel) raw data.

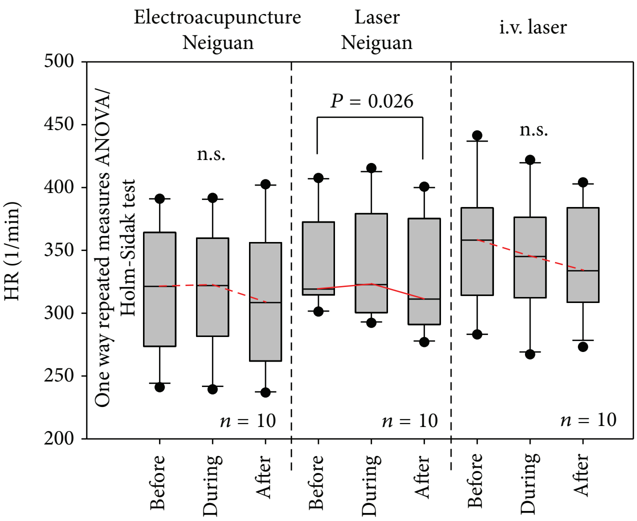

Figure 4: Box plots displaying the mean heart rate (HR) of the 10 rats. The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles. The different measurement phases (before, during, and after stimulation; compare with Figure 2) and different stimulation methods are indicated.

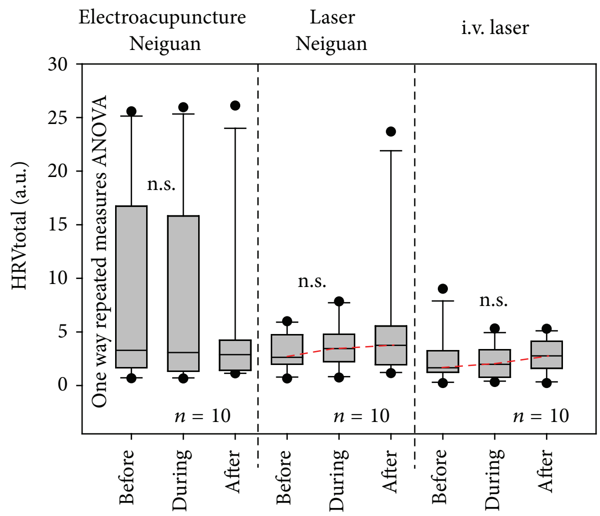

Figure 5: Changes in total heart rate variability (HRVtotal) before, during, and after the three stimulation procedures. For further explanation, compare with Figure 4.

In contrast to HR, total HRV increased slightly (insignificantly) during the two laser stimulation methods. No changes in total HRV were seen under EA (Figure 5).

Figure 6 shows the changes of LF/HF. Significant changes were only found in the i.v. laser session.

Analysis of the bioelectrical brain activity (EEG, Figure 7) revealed the following trends. There was a marked decrease in the slow frequencies during EA and i.v. laser stimulation, which, however, did not reach the level of statistical significance. Absolutely no changes were found in the i.st. laser acupuncture session.

4. Discussion

Currently, there are about 150 publications in the database PubMed concerning i.v. laser irradiation of blood. However, only two papers on the topic "i.v. laser blood irradiation and EEG" can be found [14, 15]; both are in Russian language. One paper [14] describes i.v. laser therapy for circulatory encephalopathy, and the other [15] presents clinical data on therapeutic effects of i.v. laser blood irradiation in severe alcohol intoxication, complicated by alcohol coma. We did not find a publication in PubMed concerning "HRV and i.v. laser blood irradiation" or "electrocorticogram and i.v. laser blood irradiation." However, this present publication is not the first one dealing with the topic i.v. laser irradiation in animal experiments, especially in rats. From 1988 to 2012, altogether 12 publications deal mainly with investigations of photodynamic therapy in rats [16–27]. Concerning humans, there are 86 publications on i.v. laser blood irradiation investigating different parameters; 80% of these studies are in Russian language. Since i.v. laser blood irradiation is already being often used in clinical practice, one of the goals of this study was to test its impact on neurovegetative parameters—a question hitherto unaddressed in research—in an animal experimental setting.

In our study, we recorded for the first time electrocardiogram and electrocorticogram simultaneously and continuously during i.v. laser blood irradiation. We found a decrease in HR and also in the integrated corticogram and a small increase in HRV. Of course both EEG and HRV parameters

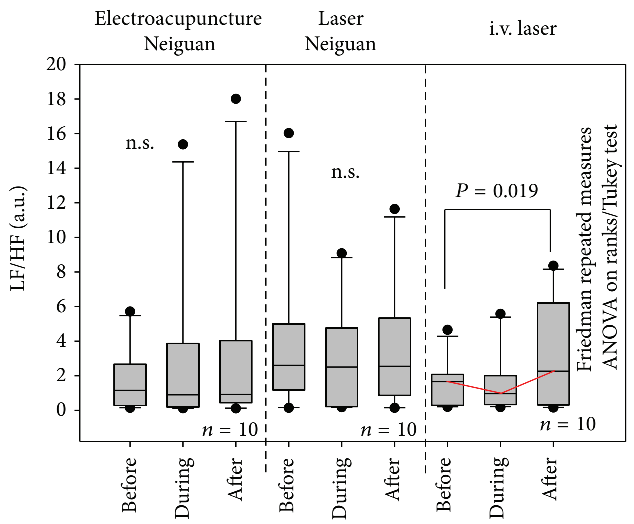

FIGURE 6: LF/HF of the 10 investigated rats. For further explanation, see Figure 4.

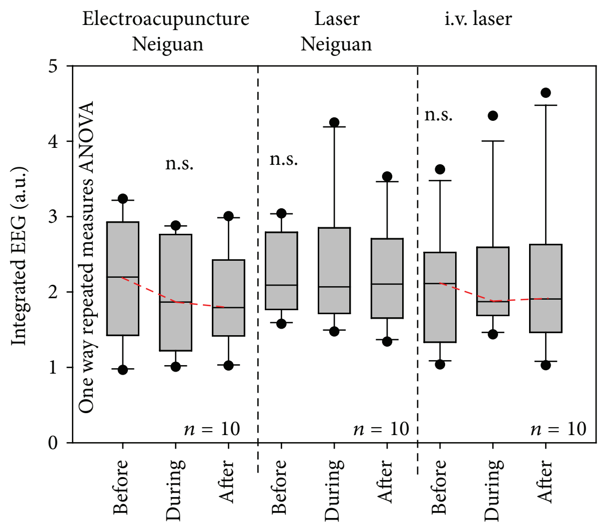

FIGURE 7: Statistical box plot analysis of the electrical rat brain activity. Note the (insignificant) decrease of the integrated EEG during EA and i.v. laser.

are somewhat attenuated following the use of anesthetics, but since we are interested in changes in these parameters under otherwise steady-state conditions, this does not influence the conclusions that can be drawn from the results. Previous studies showed that some EEG components might be associated with the autonomic nervous modulation of the subject during positional change. It was suggested that there might be a mechanism located in the brain-stem which jointly controls both autonomic influences on heart rate and EEG activation [28]. In sleep apnea patients, the results have shown that EEG delta, sigma, and beta bands exhibited a strong correlation with cardiac HRV parameters at different sleep stages [29].

Our research group could show in previous studies that acupressure at the Yintang acupoint can induce EEG effects in humans [30]. Similar investigations with acupressure on the Extra 1 point by other authors found significantly reduced EEG spectral entropy in both genders, but LF/HF was affected only in females [31]. Manual stimulation on (Hegu) LI4 seems to lead to specific changes in alpha EEG frequency and in HRV parameters. A linear relationship between the HRV parameters and the alpha EEG band might point to a specific modulation of cerebral function by vegetative effects during acupuncture [32]. The relationship among specific sensations induced by acupuncture manipulation, effects on sympathetic and parasympathetic autonomic functions, and EEG changes has been investigated by Sakai et al. [33]. The authors demonstrated that acupuncture manipulation significantly decreased the LF spectral component of HRV and significantly reduced LF/HF, which is an index of sympathetic activity. They also found a significant negative correlation between changes in LF/HF and the number of specific acupuncture sensations reported and a significant positive correlation between HF of HRV and the number of acupuncture sensations. In addition, analyses of EEG data indicated that acupuncture manipulation nonspecifically increased power of all spectral bands except the gamma band. Furthermore, changes in HF (index of parasympathetic activity) and total power (overall activity of the autonomic nervous system) of HRV were positively correlated with changes in theta, alpha, and gamma power. These results are consistent with the suggestion that autonomic changes induced by manipulation inducing specific acupuncture sensations might be mediated through the central nervous system, especially through the forebrain as shown in EEG changes [33]. In our study, we only analyzed integrated EEG, and we did not perform detailed frequency analyses of EEG. This will be done in further studies with multichannel EEG (electrocorticogram, resp.) recordings.

As mentioned previously, the clinical use of i.v. laser blood irradiation is already widespread; yet there are only few studies dealing with basic research on this topic. Our present study is the first one comparing i.v. laser stimulation with acupuncture laser stimulation and comparing EA and laser acupuncture in rats. It is well known that the effectiveness of laser acupuncture depends upon dosage [34, 35]. We used a red laser (658 nm) with an output power of 50 mW, which results in a very high dosage. This dosage is also time dependent. In followup studies with this experimental rat model, it would be highly interesting to perform investigations with, for example, half the output power (25 mW), but twice the irradiation time (20 min instead of 10 min as used in this study). This is a very interesting topic which, to the best of our knowledge, has not been investigated in an experimental study design.

5. Conclusions

The following conclusions can be drawn from the results of this animal experimental study.

(i) HR changes significantly during i.st. laser acupuncture stimulation of Neiguan in anesthetized rats.

(ii) Total HRV increased insignificantly during i.v. and i.st. laser stimulation.

(iii) LF/HF showed a significant increase only during i.v. laser blood irradiation, indicating an increase in sympathetic tone.

(iv) Integrated cortical EEG (electrocorticogram) decreased insignificantly during EA and i.v. laser blood irradiation.

Authors' Contribution

W. He and G. Litscher contribute equally to this study.

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Research Article

Ear Acupressure, Heart Rate, and Heart Rate Variability in Patients with Insomnia

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This high-tech "teleacupuncture study" describes a neurovegetative ear acupressure effect in patients with chronic insomnia by using heart rate variability analysis. Heart rate (HR) and heart rate variability (HRV) measurements in 31 patients (mean age ± SD: 54.3 ± 10.6 years) were performed under standardized conditions in Harbin, China, and the data analysis was performed in Graz, Austria. Similar to our previous clinical and basic teleacupuncture research works, the electrocardiograms (ECGs) were recorded by an HRV Medilog AR12 system during ear acupressure of the Shenmen point on the left ear. HR decreased significantly ($P < 0.05$) during and after acupressure stimulation. The effect was not visible after the first stimulation, rather it appeared in the phase following the second acupressure stimulation (10 min after the first stimulation). Total HRV showed significant stimulation-dependent increases ($P < 0.05$), immediately after each acupressure stimulation with a maximum after the third stimulation (20 min after the first stimulation), but there was no long-lasting effect. The present results can serve as a solid basis for the further investigations of auricular point stimulation for noninvasive complementary use in treating insomnia.

1. Introduction

The term "teleacupuncture" was first mentioned worldwide by our research group of the Medical University of Graz, Austria, Europe, at the International Symposium "Modernization of Traditional Chinese Medicine" in May 2009. Today (December 21, 2012) a google search for "tele-acupuncture" yields 650,000 results.

This high-tech clinical and basic acupressure study using a "transcontinental teleacupuncture" design deals with the acute effects of ear acupressure on heart rate (HR) and heart rate variability (HRV) in patients with chronic insomnia. HR and HRV have been applied to understand autonomic changes during sleep and insomnia. One definition of insomnia is "difficulties initiating and/or maintaining sleep, or nonrestorative sleep, associated with impairments of daytime functioning or marked distress for more than 1 month" [1]. It has become a global health problem. The aim of this study was to test the hypothesis that patients with insomnia will demonstrate acute neurovegetative effects such as decreased HR and increased HRV during and after ear acupressure treatment as measured by continuous electrocardiographic monitoring and spectral analysis techniques.

In the scientific database PubMed (http://www.pubmed.gov/), there are more than 2,400 reviews on this topic. A research group from Taiwan published a randomized controlled trial in 2010 which showed effectiveness of acupressure for residents of long-term care facilities with insomnia [2].

2. Materials and Methods

2.1. Patients. In total, 31 patients (6 male, 25 female) with a mean age of 54.3 ± 10.6 (SD) years (range: 39–82) were investigated in this transcontinental clinical and basic research study. They all presented themselves at the hospital due to chronic insomnia. The Athens Insomnia Scale (AIS) was used for classification of the disease [3] (inclusion criteria: AIS ≥ 7). The scores ranged from 7 to 22, resulting in a mean value of 14.7 ± 4.4 (SD). The subjects had no obvious history of heart disease, cerebrovascular disease, or 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 including the registration of the noninvasive parameters was approved by the local ethics committee and was in accordance with the Declaration of Helsinki of the World Medical Association.

2.2. Electrocardiographic Monitoring. An HRV Medilog AR12 (Huntleigh Healthcare, Cardiff, UK, and Leupamed GmbH, Graz, Austria) system was used for bioelectrical cardiographic (ECG) recording. The data were analyzed using the "Fire of Life" software (Huntleigh Healthcare, Cardiff, UK) [4, 5]. The sampling rate of the recorder is 4096 Hz, allowing R waves to be detected extremely accurately. All raw data are stored on a compact flash memory card. The data are then read by an appropriate card reader connected to a standard computer and sent to the Stronach Research Unit at the Medical University in Graz. Biosignal ECG registration was performed in Harbin with three adhesive electrodes (Skintact Premier F-55; Leonhard Lang GmbH, Innsbruck, Austria) applied to three standard positions on 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 [5–7]. These parameters are recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [8]. Similar to previous studies of our research team, the mean HR, total HRV, LF (low frequency) and HF (high frequency) bands, and the LF/HF ratio of the HRV were evaluated [9–11].

2.3. Ear Acupressure Stimulation and Procedure. Auricular acupoint Shenmen on the left ear was selected for acupressure stimulation. The ear point Shenmen is located at the lateral third of the triangular fossa, in the bifurcating point between superior and inferior crura of antihelix (see Figure 1). This point is indicated mainly for insomnia, pain, and skin itching. Its action is thought to ease the mind, relieve pain, and subdue inflammation [12].

Semen vaccariae (Wang Bu Liu Xing, cowherb seed; globes of ~1.5 mm in diameter; surface: smooth; color: black; Hebei, China) was applied unilaterally onto the acupoint Shenmen (TF 4) of the left ear by adhesive plaster.

The auricular acupressure was performed for 15 seconds each time, with two pressure movements per second, resulting in a total of 30 pressure movements per stimulation. Acupressure was applied every 10 minutes, for a total of 3 times during the entire measurement period of each patient.

The measurement profile and measurement times (a–h) are shown schematically in Figure 2. Eight measurement periods were compared: two phases before stimulation (a, b), four phases during which acupressure stimulation was performed (c–f), and two phases after acupressure (g, h).

Figure 1: Location of the auricular acupoint Shenmen.

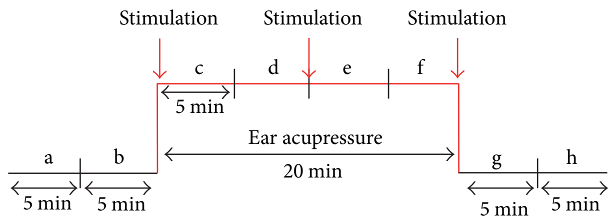

Figure 2: Experimental protocol for auricular acupressure at the Shenmen ear acupuncture point.

2.4. Statistical Analysis. The data were analyzed using Friedman repeated measures analysis of variance (ANOVA) on ranks (SigmaPlot 12.0, Systat Software Inc., Chicago, USA). Post hoc analysis was performed using the Tukey test. The level of significance was defined as $P < 0.05$.

3. Results

Mean HR is shown in Figure 3. In this figure, the results from 31 patients for measurement phases a–h (before, during, and after stimulation of the Shenmen auricular acupoint) are documented. There was a significant ($P < 0.05$) decrease in HR, starting after the second acupressure stimulation (phase e) and lasting until the end of the recording period compared to the first control interval (a) before stimulation. After the third acupressure stimulation, the HR reached its minimum (phase g). In the last phase (h), five minutes after the last stimulation, HR increased again slightly; however, it was still significantly reduced compared to the baseline value before stimulation (phase a).

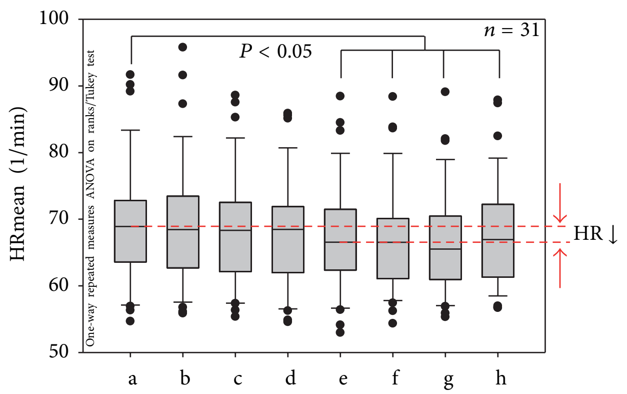

FIGURE 3: Box plots displaying the mean heart rate (HR) of the 31 patients. Note the significant decrease beginning in phase (e). The ends of the boxes define the 25th and 75th percentiles with a line at the median and error bars defining the 10th and 90th percentiles. The different measurement phases (a–h; cf. Figure 2) are indicated.

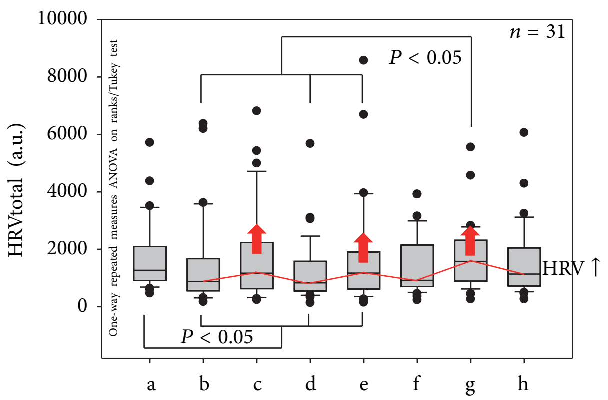

FIGURE 4: Statistical analysis and box plot illustration of HRVtotal of the 31 patients with insomnia. Note the stimulation-dependent increases in HRVtotal (red arrows). For further explanations, see Figure 3.

Figure 4 shows stimulation-dependent increases of total heart rate variability (HRVtotal). The maximum increase of HRVtotal occurred after the third acupressure stimulation at the ear. This increase was not only significant compared to the control phase before stimulation, but also compared to the phase after the second acupressure stimulation ($P < 0.05$).

The analysis of the LF/HF ratio did not show any significant differences between the phases before, during, and after acupressure stimulation.

4. Discussion

Acupressure is one of the most commonly used treatment methods in China. It is especially used for very young and old patients. Persons can use it by themselves, or assisted by family members, at home. There are courses in China on TV or in newspapers on how to perform acupressure. There is still a need for scientific investigations concerning acupressure especially ear acupressure. In the scientific database PubMed (http://www.pubmed.gov/), one can find about 30 times more articles on acupuncture than on acupressure (December 21, 2012). There are more clinical reports on acupressure than basic research on this topic. Up to now, there are no standard stimulation parameters which are easy to reproduce and/or compare; one only needs to mention the subject-dependent pressure applied on the skin. Usually, acupressure is performed manually, but there are also some innovative approaches and developments to apply vibration or acupressure stimulation via electronic devices. First results concerning this high-tech acupressure stimulation have been presented by our group in the field of ear acupressure [13].

As another treatment modality of traditional Chinese medicine, auricular acupressure is characterized by easy manipulation, sustained local stimulation, a wide range of appropriate indications, and cost efficiency with effective results. With its many benefits, ear acupressure has the potential to become one of the most commonly applied treatments intending to promote relaxation, relieve pain and discomfort in the body, and treat insomnia.

Various studies have shown that acupressure is effective for treating insomnia. In 2005, a research group from the College of Nursing Science at Kyung Hee University in Korea designed a triangulation study to observe the effects of auricular acupuncture on insomnia in Korean elderly [14]. Another group in Taiwan conducted a study in 2011 which found the relationship of subjective sleep quality and cardiac autonomic nervous system in postmenopausal women with insomnia under auricular acupressure [15].

Single-point treatment, which has been more frequently used in acupuncture investigations nowadays, might be important to prove the point specificity by using different means of acupoint stimulation.

We have already investigated the effects of Shenmen (H7) on the left hand on HRV in patients with insomnia in one of our previous teleacupuncture studies between Harbin and Graz [11]. In order to compare the complementary effects and also the specificity of different single acupoints in the treatment of insomnia patients, ear acupressure on Shenmen on the left ear is conducted in this study on chronic insomnia in adults of different ages.

Similar results have been obtained in our recently published Sino-European high-tech acupuncture study. By using single-point acupuncture, this study has shown decreased HR and increased HRV acute effects on insomnia patients during and after acupuncture treatment [11].

Our Study Has Some Limitations. First, there is no clinical outcome data of the treatment's effect on patients' insomnia correlating with the data of HR and HRV. Secondly, there is no control group in this study. Without a sham point control that has no known effect on insomnia, it is not clear whether our results were due to a specific effect of the classically used acupuncture point for insomnia, or merely due to a nonspecific effect. In future investigations, we will use another ear point (e.g., ear point tonsil, *biantaoti*) as a sham point.

5. Conclusions

The following conclusions can be drawn from the results of the present first transcontinental teleacupressure study in patients with insomnia.

(i) Heart rate decreased significantly during and after acupressure stimulation of the Shenmen acupuncture point on the left ear. The effect was not visible after the first stimulation, rather it appeared in the phase following the second acupressure stimulation (10 min after the first stimulation).

(ii) Total HRV showed significant stimulation-dependent increases, immediately after each acupressure stimulation with a maximum after the third stimulation (20 min after the first stimulation), but there was no long-lasting effect.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

L. Wang, W. Cheng, and G. Litscher have equally contributed to the study.

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