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RESEARCH ARTICLE

A Naturalistic Study of the Effect of Acupuncture on Heart-Rate Variability

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Abstract
Objectives: To study the effect of acupuncture on heart rate variability (HRV) by using linear and non-linear methods of analysis.
Methods: 40 patients were recruited consecutively, including patients with insomnia, stomachache, diarrhea, dizziness, cervical syndrome, lower back pain, gonarthritis, peripheral facial paralysis, post-traumatic organic brain syndrome and urinary retention. Different acupoint prescriptions were used, according to the textbook for 5-years' education on traditional Chinese medicine specialty, which is used in Chinese Universities. HRV was recorded before, during, and after acupuncture.
Results: Acupuncture substantially reduced variability, causing a 41% reduction in the standard deviation. Using a Fourier analysis, the variances both in the low frequency (LF) and the high frequency (HF) ranges were markedly reduced, but the LF/HF ratio (an indication of sympathovagal balance) was not altered. The HR was unchanged. The sample entropy, which is a measure of the complexity of time series, was significantly increased (+35%).
Conclusions: Acupuncture produced a pattern of changes different from that seen in pathological conditions, where increased variability and reduced complexity is expected.

1. Introduction

The autonomic nervous system (ANS) is an important regulator of many physiological functions [1]. The functional status of the ANS is related to stress and a number of different diseases [2,3]. Acupuncture has been shown to produce clear central nervous system effects [4], but it is also able to alter the functional status of the ANS both in humans [5,6] and in laboratory animals [7]. The most consistent findings have been increased vagal activity and reduced heart rate (HR) [8,9].

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Recording the HR and then calculating the HR variability (HRV) by using Fourier analysis are easy non-invasive methods to investigate autonomic balance [10,11]. The variance in the high-frequency (HF) range (0.15—0.40) Hz is thought to primarily reflect vagal activity while the variance in the low-frequency (LF) range (0.04—0.15 Hz) is influenced both by the vagus and the sympathetic nervous system [10]. Analysis of the HR and the HRV is increasingly used to investigate cardiovascular regulation both under physiological and pathological conditions [12]. Alterations in HRV have been shown to be important in the evaluation of heart diseases [10,11,13], as well as other somatic [13—15] and psychiatric [16—18] disorders. Especially interesting, are findings of reduced vagal activity that has been associated with different pathological states [19,20]. As vagus nerve stimulation has been used to treat epilepsy [21], depression [22], and heart failure [19], it is conceivable that other procedures that increase vagal activity could be used to treat diseases. Acupuncture has been used to treat patients with autonomic dysfunction related to gastrointestinal and cardiovascular disorders [8,23,24], and increased vagal activity may be one reason for the effectiveness of acupuncture. However, there is still insufficient knowledge on how such a treatment, by stimulation of specific points on the skin, may mobilize the resources of the body, alter HRV measures, and alleviate both painful and non-painful conditions [4—6,24].

In recent years, non-linear methods such as approximate entropy and sample entropy have been used to analyze biological time series, giving information in addition to that obtained by traditional methods such as the standard deviation and the Fourier analysis [25]. In the field of cardiology, reduced complexity, as measured with sample entropy, has been found to be a predictor of cardiac death [26,27], and entropy-based features have been employed to detect epileptic seizures [28]. Goldberger and Goldberger et al [29,30] postulated that increased stereotypy and reduced complexity of different physiological systems may be a characteristic of many diseases.

Because both reduced vagal activity and reduced complexity may be related to disease states, in the present study, we wanted to use an analysis of HRV to address two questions. Firstly, we wanted to see if acupuncture could alter vagal activity in a clinical setting, in patients having different clinical conditions. Secondly, we wanted to see if acupuncture could change the complexity of the HR time series, as measured with sample entropy. Our hypotheses were that acupuncture would: (1) increase vagal activity; and (2) increase complexity. We think that answering these two questions will help us better understand the mechanism for the action of acupuncture. If the conjectures are correct, they would provide a rationale for using acupuncture for treating pathological conditions characterized by reduced vagal activity and reduced complexity.

2. Materials and methods

2.1. Patients

Without taking the actual symptomatology of the patients into consideration, we consecutively recruited 40 patients (24 men and 16 women; mean age = 29 ± 10.6 years (SD), range = 17—55 years) seeking treatment at the outpatient clinic at the Acupuncture Department, South Hospital, Guangzhou, China. To enforce the naturalistic setting of the study, the only exclusion criteria were as follows: (1) patients with known cardiovascular disorder; (2) patients using β-blockers; (3) patients using digitalis; and (4) patients using other drugs that might affect cardiac activity.

In order to avoid possible effects related to the novelty of the situation [31], the HR was recorded when the patients had their fourth treatment session. The study was approved by the local ethical committee, and all patients gave informed consent. The study started in January 2008 and was completed by the end of April 2008.

2.2. Acupuncture

The same senior acupuncturist provided acupuncture to all the individuals under ordinary clinical conditions. Acupoints were conventionally sterilized by using iodine-alcohol, and needles (Global Brand, 0.30 × 40 mm) were inserted to a depth of between 10 mm and 20 mm. The stimulation method was as follows: after the needles had been inserted, they were stimulated by using the lifting-thrusting and twirling method to cause the needling sensation. Then, the needles were stimulated with even manipulation for 30 seconds (frequency of 60 times/minute). The needles then remained for 10 minutes without any other stimulation.

2.3. Recording of HRV

To record HRV, we used a commercially available device, the Actiheart, a combined HR monitor and movement sensor, manufactured by Cambridge Neurotechnology (Cambridge, UK). The Actiheart is 7 mm thick, with a diameter of 33 mm and a total weight of 8 g. It is clipped onto two standard ECG electrodes. The sensitivity is 250 μV, and the sampling frequency is 256 Hz. The measurable range of HR is 35—250 beats per minute (BPM). The Actiheart has been shown to be technically reliable and valid, as compared to standard ECG recordings [32]. The device is easy to use, with little discomfort for the patients or test persons, and the supplied software allows data to be extracted for further analyses. Individuals were quietly resting for 20 minutes before the electrodes (Ambu blue sensor VL-00-S, Ambu A/S, Ballerup, Denmark) were attached and the Actiheart connected [33]. The same clinician carried out all recordings.

After the Actiheart had been connected, recordings of the HRV were performed, and the following time periods were used for further analyses: (1) the last 5 minutes before acupuncture, i.e., when individuals were resting in a supine position before the needles had been inserted; (2) the last 5 minutes during the acupuncture session, i.e., when individuals were in a supine position with needles inserted; and (3) the last 5 minutes of the recording, i.e., after acupuncture, when the individuals were still in a supine resting position, but after the needles had been extracted.

2.4. Data analysis

HR data were first analyzed using standard time-domain and frequency-domain indices. This was performed with the software supplied by the manufacturer. The time-domain indices were BPM, standard deviation (SD), and root-mean-square successive differences (RMSSD). The frequency-domain indices obtained using Fourier analysis were the variances (powers) in the LF (0.04—0.15 Hz) and the HF (0.15—0.40 Hz) ranges, and the LF/HF ratio. The values were transformed to a logarithmic scale (ln). We also calculated sample entropy (http://www.physionet.org), which is a nonlinear measure developed to compute the regularity (complexity) of the HR and other time series [34]. The sample entropy is the negative natural logarithm of an estimate of the conditional probability that two sequences that are similar for m points, within a tolerance (r), remain similar at the next point. Data were normalized before analysis. According to Richman and Moorman [25], we chose the following parameters: $m = 2$ and $r = 0.2$.

Indices obtained from the three time periods (A, B, and C) were compared by using one-way ANOVAs, with post-hoc Bonferroni tests. Correlations were analyzed according to Pearson. SPSS version 15.0 (SSPS, Chicago, IL, USA) was used for the statistical analyses. The level of significance was set at $p < 0.05$.

3. Results

3.1. Baseline information

Baseline information on the patients is shown in Table 1, including the acupoints used.

3.2. Effects of acupuncture on HRV

Results from the Actiheart recordings before, during, and after acupuncture are shown in Table 2. There were no significant alterations in BPM, normalized values for LF and HF, and the LF/HF ratio. During acupuncture, however, the recordings showed substantial reductions in SD (41%) and RMSSD (38%). Similarly, the powers (expressed in logarithmic units) in the LF (lnLF) and HF (lnHF) ranges were markedly reduced. After acupuncture, these values increased, but were still somewhat lower than before acupuncture had started. The sample entropy showed a different pattern, with a pronounced increase during acupuncture (+35%) and a return towards the baseline afterwards. Even though the magnitudes of the changes varied from patient to patient, a consistent pattern showing reduced variability and increased sample entropy during acupuncture, despite the variety of symptoms treated, was seen with 9/10 patients.

When analyzed according to gender, results for males and females were similar and were significant for sample entropy (31% and 43% increase, respectively) and SD (37% and 48% reduction, respectively), whereas the reduction in RMSSD was only significant for females (55%, $p = 0.035$), but not for males (25%). Negative correlations existed

Table 1 Clinical data.

Diseases	*N*	Sex (M/F)	Age(y), mean ± SD	Main acupoints
Insomnia	4	3/1	31.2 ± 10.7	Shenmen (HT7), Neiguan (PC 6), Baihui (DU20), Taichong (LR3)
Stomach ache	4	2/2	27.8 ± 12.5	Zhongwan (RN 12), Neiguan (PC 6), Zusanli (ST 36), Taichong (LR 3), Yanglingquan (GB 34), Neiting (ST 44)
Diarrhea	4	3/1	25.7 ± 12.5	Tianshu (ST 25), Zusanli (ST 36), Dachangshu (BL 25),Quchi (LI 11)
Dizziness	3	1/2	31.0 ± 12.6	Taichong (LR 3), Xingjian (LR 2), Zusanli (ST 36), Yintang (EX-HN 3), Fengchi (GB 20), Taixi (KI 3), Shenshu (BL 23)
Cervical syndrome	4	3/1	26.2 ± 8.9	Fengchi (GB 20), Tianzhu (BL 10), Dazhui (DU 14), Jiaji points (EX-B2)
Lower back pain	3	2/1	31.3 ± 11.3	Shenshu (BL 23), Dachangshu (BL 10), Jiaji points (EX-B2), Weizhong (BL 40)
Gonarthritis	3	2/1	36.1 ± 8.6	Heding (EX-LE 2), Douber- Xiyan (EX-LE 5), Xuehai (SP 10), Liangqiu (ST 34), Zusanli (ST 36), Yinlingquan (SP 9), Juegu (GB 39)
Peripheral facial paralysis	6	4/2	25.2 ± 7.9	Yingxiang (LI 20), Xiaguan (ST 7), Fengchi (GB 20), Dicing (ST 4), Jiache(ST 6), Yangbai (GB 14), Taiyang (EX-HN 5)
Post-traumatic organic brain syndrome	5	3/2	24.8 ± 12.3	Jianyu (LI15), Quchi(LI11), Waiguan (SJ5), Hegu (LI4), Liangqiu (ST34), Zusanli (ST36), Juegu (GB39)
Urinary retention	4	1/3	30.7 ± 8.8	Zhongji (RN 3), Qihai (RN 6), Tianshu (ST 25), Yinlingquan (SP 9), Fenglong (ST 40), lateral III line of the forehead
Total	40	24/16	29.0 ± 10.6	

Table 2 Heart rate variability before (A), during (B), and after (C) acupuncture.

	A	B	C	ANOVA
Heart rate (BPM)	70.3 ± 9.5	68.0 ± 9.3	70.2 ± 10.2	NS
SD	90.9 ± 37.3	53.8 ± 28.2***	70.7 ± 39.2	<0.001
RMSSD	77.6 ± 55.6	48.2 ± 37.6	67.0 ± 51.5	0.027
ln LF	7.61 ± 1.05	6.69 ± 1.27**	7.43 ± 1.37	0.003
ln HF	7.16 ± 1.39	6.28 ± 1.43	7.07 ± 1.53	0.013
LFn (LF/LF + HF)	0.602 ± 0.17	0.590 ± 0.16	0.580 ± 0.16	NS
HFn (HF/LF + HF)	0.398 ± 0.17	0.410 ± 0.16	0.420 ± 0.16	NS
LF/HF	2.07 ± 1.56	1.90 ± 1.37	1.83 ± 1.37	NS
Sample entropy	1.18 ± 0.45	1.59 ± 0.29***	1.28 ± 0.49	<0.001

Post hoc Bonferroni tests: ** $p < 0.01$, *** $p < 0.001$, comparing A with B.

between sample entropy and all the measures of variability: SD (-0.402, $p < 0.001$), RMSSD (-0.382, $p < 0.001$), lnLF (-0.256, $p = 0.005$), and lnHF (-0.226, $p = 0.013$). Baseline values for BPM, SD, LF/HF ratio, lnLF and lnHF were not correlated to age, whereas sample entropy was negatively correlated to age (-0.518, $p = 0.001$) and RMSSD had a positive correlation (0.328, $p = 0.039$). The increases in sample entropy were similar for persons above ($n = 8$) and below ($n = 32$) 40 years of age. The reduction in RMSSD was less pronounced in the youngest age group, but this difference was not statistically significant.

4. Discussion

The main findings from this study were that acupuncture treatment in a naturalistic setting, using a general clinical sample, had a very pronounced effect on HRV measures, reducing variability and at the same time increasing the complexity of the time series. The pronounced effect on variability measures was seen despite there being no significant alterations in HR or in the balance between sympathetic and parasympathetic activity, as expressed in the LF/HF ratio.

Previous studies, involving both healthy volunteers and patients, have shown that acupuncture may be able to change the autonomic balance, reduce HR, and increase vagal activity [5,6,35,36]. In animal experiments, electroacupuncture either increased the vagal or the sympathetic activity, depending on the point being stimulated [37]. The functional state of the ANS in humans is influenced by mental stress and fatigue [15]. Therefore, one important consideration is that changes in HRV measures induced by acupuncture may depend on the physical state of the person being investigated. Li et al [38] found that a state of fatigue significantly influenced HRV results. Fatigued persons experienced reduced HR and reduced LF/HF ratio after acupuncture, while those not fatigued showed none of these changes. We have tested patients with a range of somatic conditions while other studies have investigated only healthy volunteers [5,6,38]. Therefore, direct comparisons of those results with ours are difficult. However, changes in mental state or in the degree of fatigue may conceivably have contributed to the different findings.

Both age and gender may influence measures of HRV [39]. RMSSD showed a modest correlation to age (0.328), and the reduction in RMSSD was only evident in the oldest patients. Sample entropy values were more strongly and negatively correlated with age (-0.518) whereas the increase in sample entropy was similar in young and older patients. No other HRV measures were correlated with age. Effects of acupuncture may be different in males and females. Arai et al [40] found increased vagal activity (reduced LF/HF ratio) after acupressure in females, whereas we found a reduction in RMSSD that was significant only in females.

It should be observed that the changes we have presented relate to the acupuncture treatment per se. We do not have data for longer time periods, and have not looked at effects resulting from longer series of acupuncture treatment.

Variance, as measured with the SD, is increasingly being recognized as an important parameter in a wide range of conditions and disorders. Increased intra-individual variability may be a central characteristic of attention deficit hyperactivity disorder [41], and increased variability may be seen before critical transitions in many different systems, including disease states [42]. It is, therefore, interesting that acupuncture is able to reduce variability, and this may be related to the beneficial effect of such treatment. Similarly, the finding of increased complexity during acupuncture is opposite to what is seen in various disease states [29]. It is thus tempting to speculate that reduced variability and increased complexity may be related to the positive effect of acupuncture. Even though reduced HRV has been associated with cardiac diseases [10], such disorders are at the same time associated with reduction of complexity, as seen in studies finding reduced sample entropy before ventricular tachyarrhythmias [27] or atrial fibrillation [43].

Five-minute windows of measurement are most often used in studies of HRV [44], and usually linear indices are employed. To evaluate the complexity of the biological time series, sample entropy is probably a better measure than approximate entropy [25,45]. Non-linear measures of HRV may be difficult to use, but may be more stable than linear measures in test-retest experiments [44]. An indication of the usefulness of sample entropy in the present experiment, is the fact that the SD of sample entropy during acupuncture (in percent of mean) was substantially

lower than the SD of the linear measure RMSSD (18% vs. 78%).

Because our study was a naturalistic study, it has clear limitations. The patients recruited had a number of different medical conditions, and a range of separate acupuncture points were used. Furthermore, we did not use a control group, so we do not know if these effects on HRV will also be seen in healthy persons. A further limitation is that we have not tested non-effective acupuncture points, and we have not administered non-effective acupuncture. Therefore, we do not know how specific the effects are. There were too few patients with each condition to make meaningful comparisons between the different diseases or disease categories. Similarly, we were not able to make statistical comparisons between the different acupuncture points. However, our findings indicate that the pattern of reduced variability and increased complexity may be a general effect of acupuncture, and not depend on the specific location of the needle nor on a specific type of medical problem. However, in further studies, we acknowledge the need to compare different acupuncture points more specifically, to use non-acupuncture points and to include healthy controls. It would also be interesting to study other groups of patients, for instance, patients with anxiety and depressive disorders.

There are no immediate clinical applications of the present findings. They are primarily interesting as clues as to how acupuncture may alter the function of the ANS and heal pathological conditions. More detailed studies of such mechanisms are clearly needed.

In conclusion, acupuncture, given in a naturalistic setting under normal clinical conditions, induced pronounced alterations in HRV without altering either the HR or the sympatho-vagal balance. This demonstrates that acupuncture may alter the physiological parameters of the human body. On the basis of the present study, we are not able to explain these findings. It is intriguing, however, that acupuncture induced changes in HRV and complexity opposite to those seen in a number of pathological conditions of the human body. Further studies are needed to determine if these changes are related to beneficial effects of acupuncture treatment.

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