

# Effects of Electroacupuncture at PC6 and ST36 on Heart Rate Variability in Anesthetized Mice

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## ABSTRACT

**Objective:** To observe the change of heart rate variability in anesthetized mice after electroacupuncture on PC6 and ST36, and compare the difference between these points.

**Methods:** A total of 33 C57BL/6 mice were randomly divided into control, PC6 and ST36 groups with 11 mice in each group. The electrocardiogram was recorded by two needle electrodes. The HRV data were analyzed by time and frequency analysis with heart rate, Standard Deviation of R-R Intervals and LF/HF Ratio.

**Result:** During the EA at PC6, SDRR was significantly increased ( $P < 0.05$ ) and maintained with a significantly higher level at the end of experiment, and the LF/HF ratio was gradually increased throughout the experiment. During the EA at ST36, SDRR was significantly increased ( $P < 0.05$ ) and there was no observable tendency of the change in LF/HF ratio during the experiment. In comparison with control, the HR of group PC6 mice decreased significantly during EA stimulation ( $P < 0.01$ ) and continued declining throughout the experiment ( $P < 0.05$ ). Time spectral analysis of the ECG recordings showed that control mice had significantly lower SDRR compared to group PC6 and group ST36 after EA stimulation ( $P < 0.05$ ). SDRR was significantly increased when measured at 90mins after EA in group PC6 in comparison with control ( $P < 0.01$ ). EA at PC6 caused a significant increase than ST36 in SDRR when measured at Stim. ( $P < 0.05$ ) and 90mins after EA ( $P < 0.01$ ).

**Conclusion:** EA at PC6 and ST36 protected anesthesia mice against decline of HRV. In comparison with ST36, the effect of EA at PC6 was more significant, which was caused by the increase of the sympathetic nerve activities from the postganglionic fibers with the same spinal cord segments to heart.

**Key words:** electroacupuncture, acupoint, HRV, anesthesia

## INTRODUCTION

Heart Rate Variability (HRV) is a measure of the naturally occurring beat-to-beat changes in heart rate<sup>[1]</sup>. Clinically, HRV provides a noninvasive method to quickly examine the activity of the autonomic nervous system<sup>[2]</sup>. Most researchers considered that acupuncture modulates the autonomic nervous system, and these modulations can be measured by HRV<sup>[3-5]</sup>. Wang et al. reported that acupuncture enhances cardiac vagal activity and suppresses sympathetic activities in healthy humans<sup>[6]</sup>. Many studies reported the effect of acupuncture at specified acupoints on HRV. Significant decreases in heart rate (HR) after verum intervention at PC6, CV4 and YinTang were found<sup>[7,8]</sup>.

The autonomic nervous system is composed of two branches, 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<sup>[9]</sup>. The change of HRV reflects the balance of autonomic nervous system, the change which related with physiological, hormonal, and emotional factors. In the research of acupuncture on HRV in normal subjects under fatigue and non-fatigue states, it was concluded that the modulating effect of acupuncture on heart rate variability not only depended on the points of stimulation such as acupuncture or non-acupuncture points but also

on the functional state of the subject<sup>[10]</sup>, whereas Wright and Aickin reported essentially no support for a relationship between HRV and acupuncture intervention in the patients of menopausal syndrome<sup>[11]</sup>.

Therefore, the present study was to observe HRV in anesthesia mice with the long-time data after electroacupuncture on Neiguan (PC6) and Zusanli (ST36). Base on the results, the difference between the effect of electroacupuncture (EA) on PC6 and ST36 was determined.

## METHODS

### 1. Animal Preparation

33 adult male C57BL/6 mice (8 weeks old; 21-30 g; purchased from the Institute of Animals, China Academy of Chinese Medical Sciences) were used for this study and randomly divided into control, EA at PC6, and ST36 groups with 11 rats in each group. The animals were kept in a temperature-controlled room ( $23 \pm 1$  °C) with a 12-hour light-dark cycle and free access to food and water. The animals were anesthetized with an intraperitoneal injection of 10% urethane (1.2 g/kg, Sigma-Aldrich, St. Louis, USA) and 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. Electrocardiographic (ECG) acquisition

To collect electrocardiographic (ECG) data in mice, two needle electrodes were placed separately in subcutaneous muscles, in the left forelimb and the right hind limb separately; one needle grounding electrode was placed in the proximal end of the tail. ECG data were acquired throughout the duration of the experiment by software Chart 5 (AD Instruments, Colorado Springs, CO, USA), and raw ECG signals were analyzed by Chart5 with the HRV plug-in. Heart rate (HR) was determined from R-R intervals. Power spectral analysis of ECG in the frequency domain was accomplished using low frequency (LF) and high frequency (HF) ranges that were previously published for C57BL/6 mice<sup>[12]</sup>. LF power ranged from 0.15 to 1.5 Hz and HF power from 1.51 to 5.0 Hz.

## 3. Electroacupuncture (EA)

The stimulation electrode was placed at PC6 and ST36, PC6 is a forelimb point and ST36 is a hind limb point. The points was bilaterally stimulated with a 1mA pulse of 0.5ms duration at a frequency of 20Hz for 10mins by a pair of needle electrodes inserted 3mm deep into the skin. The electrical current for somatic stimulation was generated by a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan).

## 4. Experimental Procedure

ECG monitoring had begun at the 10mins after intraperitoneal injection of anesthetic drugs (10% urethane) and EEG recording had begun at 20mins after anesthesia. There was electroacupuncture with duration of 10mins at the 40mins after anesthesia. ECG recording was continuing to 90min after electroacupuncture. All the data of the ECG recording

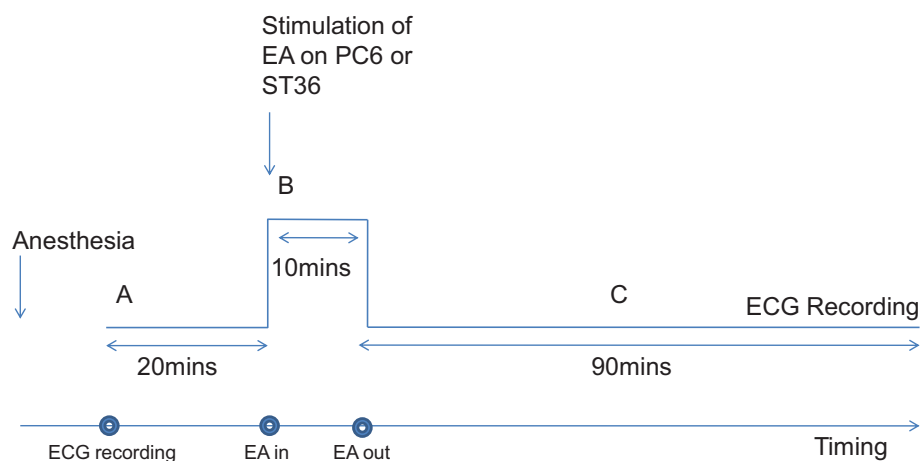
(120mins) were analyzed by 10mins (Figure 1). The control group finished the recording without EA stimulation.

## 5. Data Analysis

The HRV data were analyzed by frequency analysis to calculate LF/HF Ratio. In time analysis, Heart Rate (HR) and Standard Deviation of R-R Intervals (SDRR) were calculated in the unit of ten minutes. Results were given as mean values  $\pm$  SE. For comparisons between values obtained before and after EA, Paired-samples T-test was used. A one-way ANOVA with repeated measures was used to compare values between the three groups. This was followed by Least Significant Difference (LSD) post hoc testing when appropriate. Differences were considered significant at  $P < 0.05$ .

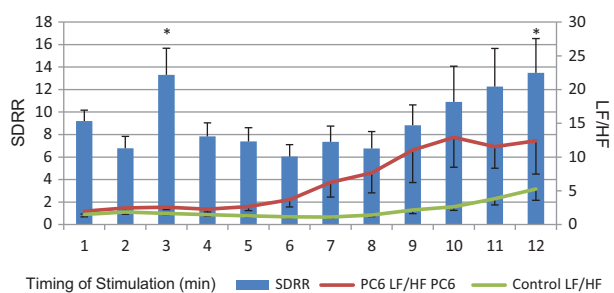
## RESULT

- 1 During the EA at PC6, the values of SDRR was significantly increased (10mins before EA:  $6.78 \pm 1.05$ , during the stim:  $13.30 \pm 2.38$ ;  $P < 0.05$ ) and maintained with a significantly higher level at the end of experiment (10mins before EA:  $6.78 \pm 1.05$ , 90 mins after EA:  $13.48 \pm 3.04$ ;  $P < 0.05$ ). The LF/HF ratio was gradually increased throughout the experiment.
- 2 During the EA at ST36, the values SDRR was significantly increased (10mins before EA:  $5.39 \pm 0.30$ , during the stim:  $7.55 \pm 1.26$ ;  $P < 0.05$ ). There was no observable tendency of the change in LF/HF ratio during the experiment.
- 3 In comparison with control, the HR of group PC6 mice decreased significantly during EA stimulation (control:  $311 \pm 11$  beats/min, PC6:  $270 \pm 8$  beats/min;  $P < 0.01$ ) and continued declining throughout the experiment (at 90mins after stimulation, control:  $315 \pm 13$  beats/min, PC6:  $259 \pm 21$  beats/min;  $P < 0.05$ ) (Figure 4A). Time spectral analysis of the ECG recordings showed that

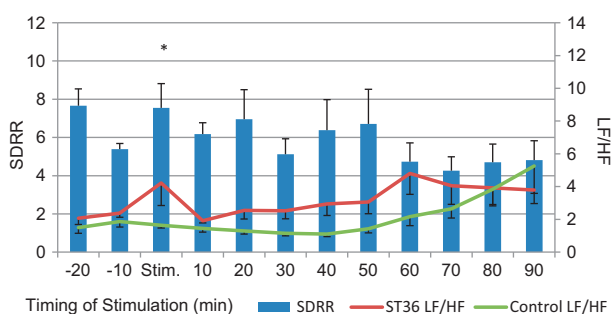


- A. Before EA stimulation, record the initial state of HRV (20mins)
- B. Duration of EA stimulation (10mins)
- C. Continued recording after EA stimulation (90 mins)

Figure 1. Measurement procedure of study



**Figure 2.** Effect off EA at PC6 on the ECG and SDRR, power spectral analysis of ECG for LF/HF. Paired-samples T-test showed significant difference between 10mins before EA and during EA, 10mins before and 90 mins after EA stimulation. \*  $P < 0.05$ .

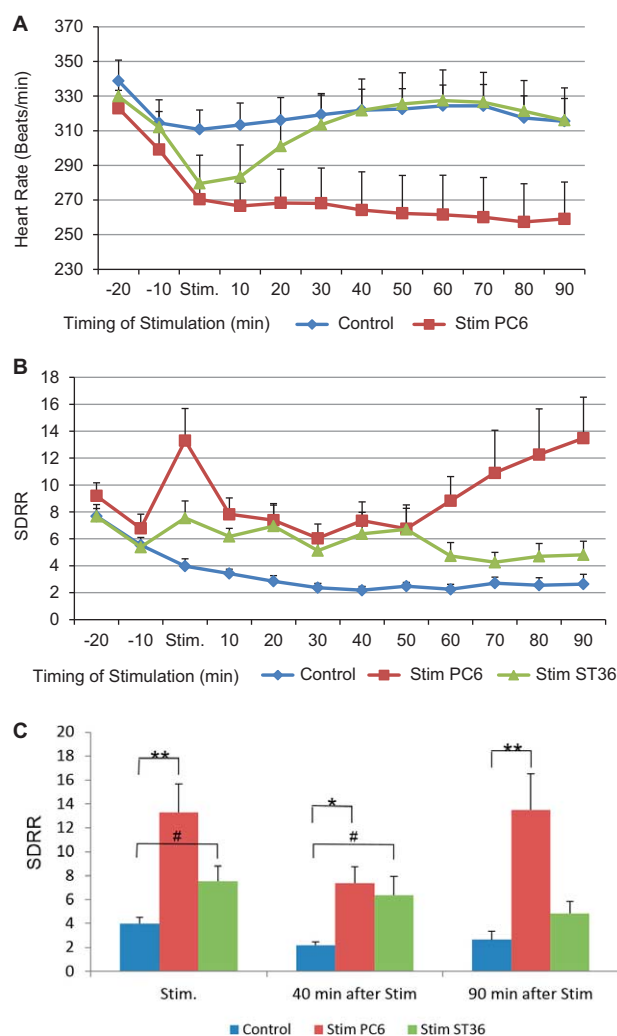


**Figure 3.** Effect of the EA at ST36 on the ECG and SDRR, power spectral analysis of ECG for LF/HF. Paired-samples T-test showed significant difference between 10mins before EA and during EA. \*  $P < 0.05$ .

control mice had significantly lower SDRR compared to group PC6 and group ST36 after EA stimulation (at 10mins after stimulation, control:  $3.42 \pm 0.32$ , PC6:  $7.83 \pm 1.21$ ; ST36:  $6.18 \pm 0.60$ ;  $P < 0.05$ ) (Figure 4B). The SDRR was significantly increased in group PC6 and ST36 on the process of EA stimulation and 40mins after stimulation in comparison with control ( $P < 0.05$ ). SDRR was significantly increased when measured at 90mins after EA in group PC6 in comparison with control ( $P < 0.01$ ) (Figure 4C). EA at PC6 caused a significant increase than ST36 in SDRR when measured at Stim. ( $P < 0.05$ ) and 90mins after EA ( $P < 0.01$ ).

## Discussion

The autonomic nervous system dynamically controls the response of the body to a range of external and internal stimulations, providing physiological stability<sup>[13]</sup>. HRV analysis reveals the interaction between the sympathetic and parasympathetic activities by modulation of heart beat interval<sup>[14]</sup>. Therefore, the change of HRV reflects the balance of autonomic nervous system, which related with physiological, hormonal, and emotional factors. These factors lead to uncertainty in the HRV study of acupuncture in human<sup>[15]</sup>. It was the reason for us to choose mice with less effect of emotional and physiological factors as the object in the study. As we observed in the experiment, SDRR of control group significantly declined during the process of anesthesia. And there was report that HRV diminishes during anesthesia, for dexmedetomidine the HRV started decreasing right after loss



**Figure 4.** Characteristics of HRV changes after EA stimulation at PC6 and ST36. Effect of EA on heart rate (A) and SDRR (B) over 90mins time course (n=11 for each group). (C) Repeated measure ANOVA showed significant effects of EA at PC6 and ST36 to control in the process of EA and 40 minutes after EA (\*\* $P < 0.011$ , \*  $P < 0.05$ , #  $P < 0.05$ ). EA at PC6 caused a significant increase than ST36 in SDRR when measured at Stim. and 90mins after EA ( $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$ ).

of consciousness<sup>[16]</sup>. The nociception-analgesia balance is a direct determinant of HRV during surgical anesthesia<sup>[17]</sup>. In the experiment design, we also avoided the timing with significant change of HR and HRV (0–40 mins after intraperitoneal anesthesia, Figure 4A–B), and selected the relatively stable period with HRV changes for EA stimulations.

In TCM, the pericardial meridian (PC) is usually used for cardiovascular diseases and PC 6 is the most commonly selected for clinical practice<sup>[18,19]</sup>. There were reports of EA or laser stimulation at the PC6 induced a significant increase of total HRV<sup>[20,21]</sup>. It was consistent with the results which obtained in present study. Cui et al. reported after cholera toxin subunit B (CTB) was injected into PC8 (the point is near to PC6) in rats, CTB labeled sensory neurons was distributed from cervical (C)6 to thoracic (T)1 dorsal root ganglia (DRG) and the labeled motor neurons were located on the dorsolateral part of the spinal ventral horn ranging from the C6 to T1 segments<sup>[22]</sup>. Cervical and upper thoracic

spinal cord is the projected segments with postganglionic fibers from heart in metasympathetic nervous system. Correspondingly, in present study the LF/HF ratio was gradually increased after EA PC6, which means increase of the sympathetic nerve activities. We speculated that the decrease of HR was caused by peripheral artery contraction with sympathetic activation.

Whereas Point ST36 without the direct nerve contact to heart, was reported with the facilitatory effect on gastric motility, which was mediated via the parasympathetic pathway<sup>[23]</sup>. Kaneko et al. reported stimulation on ST36 increased HRV and superior mesenteric artery blood flow volume, which with the increase vagus nerve activity<sup>[24]</sup>. In present study, we also observed the significant increase of SDRR after EA ST36, and there was no observable tendency of the change in LF/HF ratio.

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