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A Pilot Study of Thermal Effect of Low-intensity Focused Ultrasound on Blood Pressure Modulation

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***Abstract*—Our recent study showed that low-intensity focused ultrasound stimulation (FUS) of the vagus nerve is capable of lowering blood pressure (BP). However, it remains unknown that what is the underlying mechanisms of BP modulation with FUS. In our preliminary experiments, we noticed that there was temperature elevation accompanied the FUS. Thus, to verify whether the thermal effect of ultrasound contributes in the BP-lowering effect, this study compared the BP response under the FUS (with thermal effect and mechanical effect) and the alternative heating source treatment (AHST) (with thermal effect only) of left vagus nerve. Six Sprague Dawley rats were randomly divided into two groups (FUS, *n*=3 and AHST, *n*=3). *In vivo* temperature measurements were conducted to evaluate the heating performance of the FUS and the AHST. Blood pressure (BP) waveform was continuously recorded from the right common artery and was used for analyzing systolic BP (SBP), diastolic BP (DBP), mean BP (MBP), and heart rate (HR). The results showed that the SBP, DBP, MBP and HR decreased during the 15-min FUS. However, most of the SBP, DBP, MBP and HR increased during the 15-min AHST, which had the approximate temperature elevation of the FUS. Thus, the thermal effect of ultrasound probably does not contribute in the BP-lowering effect induced by low-intensity FUS of the vagus nerve.**

***Keywords*—Low-intensity focused ultrasound stimulation, vagus nerve, blood pressure modulation, thermal effect.**

I. Introduction

Hypertension is a crucial risk factor of cardiovascular diseases. Although antihypertensive medications are commonly used in the clinical treatments, refractory hypertension that is resistant to medications is still a great challenge to be solved [1]. In efforts to develop alternative non-drug physical treatments for refractory hypertension, we have recently proposed and demonstrated that a low-intensity focused ultrasound stimulation (FUS) of the vagus nerve can effectively lower blood pressure (BP) in normotensive rabbits [2]. While the underlying mechanisms are still unknown.

Ultrasound is a kind of acoustic energy source delivered by pressure waves. In the past decades, focused ultrasound has been shown capable of non-invasively modulating neural activity and target organ function [3][4]. Although the exact mechanisms are not fully understood, it has been shown that the neuromodulation ability of FUS was mediated by the interaction between thermal and/or mechanical (non-thermal) effects of ultrasound and biological tissues [3][5]. Specifically, a number of numerical and experimental models have revealed that low-intensity FUS can reversibly excite/inhibit the neural activity by exerting the mechanical radiation force on the mechanosensitive ion channels [3][6]. By using FUS for peripheral neuromodulation, some studies have reported that the mechanical effect of FUS was related to the excitation of neural activity [7][8], while other studies have contradictorily demonstrated that the mechanical effect was in connection with an inhibition of evoked potentials [9][10]. And some studies have shown that the thermal effects of FUS was associated with the inhibition of neural activity [11-13]. Hence, further studies are still needed to investigate the underlying mechanisms of low-intensity FUS on target nerve regulation [12-13].

In our preliminary experiments, we noticed that there was temperature elevation accompanied the FUS. Thus, to verify whether the thermal effect of ultrasound contributes in the BP-lowering effect induced by low-intensity FUS of the vagus nerve, this study compared the BP response under the FUS (with thermal effect and mechanical effect) and alternative heating source treatment (AHST) (with thermal effect only) of left vagus nerve.

II. Methods

The experiments were performed in two stages: (1) *In vivo* temperature measurement to evaluate the heating performance of the low-intensity pulsed FUS and the AHST; (2) BP modulation experiment under the FUS and AHS treatment, respectively. The experiments were conducted on six Sprague Dawley rats (all male, body weight 250-360 g). The rats were randomly divided into two groups: FUS (*n*=3) and AHST (*n*=3) groups. Rats in the FUS group were treated with a 15-min low-intensity pulsed FUS of left vagus nerve. While rats in the AHST group were heated in left vagus nerve by an electric soldering iron with calibrated temperature for 15 min. All the experimental procedures were approved by

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the Institutional Animal Care and Use Committee (IACUC) of Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences (SIAT-IACUC-190801-YGS-LWH-A0454-01). The details of each process are described in the following subsections.

A. Animal Preparation

Before the experiment, each rat was anesthetized with 2 % pentobarbital sodium intra-peritonelly at a dose of 2~3 ml/kg, followed by maintenance doses of 0.8~1.2 ml/kg per hour. The rat was placed on a platform in supine position with their neck hair shaved off. Then, left vagus nerve, the target nerve of BP modulation, was surgically exposed and retained in saline solution. After that, the right common carotid artery was exposed and catheterized for BP recording.

B. Focused Ultrasound Stimulation

The driven signal generated by a functional generator (SDG 1032X, SIGLENT, China) and amplified by a 75 W power amplifier (A075, E&I, USA) was sent to a focused ultrasound transducer with a fundamental frequency of 3.7 MHz and targeted to the vagus nerve. Detail descriptions of the ultrasonic stimulation system could be referred to our previous study [2]. The ultrasound stimulation sequence was consisted of a 2 ms pulse with a duty cycle of 20 % repeated at 100 Hz. Each ultrasonic pulse cycle consisted of a sonication duration of 1 s and an inter-stimulation interval of 5 s. The spatial-peak pulse-average intensity (I_{sppa}) was 18 W/cm^2 and the spatial-peak time-average intensity (I_{spta}) was 3.6 W/cm^2 measured by a 3D acoustic scanning system (UMS3, Precision Acoustics, UK) equipped with a calibrated needle-type hydrophone (HNP-0400, Onda, USA) in degassed water. For each rat in the FUS group, the acoustic stimulation lasted for 15 min.

C. Alternative Heating Source Treatment

According to a previous study [12], the heat we used in this study is generated by a 180 W electric soldering iron and then indirectly transmitted to the vagus nerve through a metal wire connected between the electric soldering iron and the targeted vagus nerve. The heating temperature of the electric soldering iron was calibrated to 50, 60, 70, 80, and 90 °C, so that the measured temperature conducted to the vagus nerve was between 34 and 38 °C, which approximately matched the *in vivo* temperature generated by FUS. For each rat in the AHST group, the heating treatment lasted for 15 min.

D. In Vivo Temperature Measurement

The *in vivo* temperature was monitored using a T-type needle thermocouple (ADInstruments, Australia) and a data acquisition system (PowerLab, ADInstruments, Australia) sampling at 1000 Hz. The tip of the needle thermocouple was placed under the exposed vagus nerve and adjusted to ensure its alignment with the FUS/AHS focus region. In this pilot study, we evaluated the *in vivo* heating performances of AHS at five calibrated temperatures (50, 60, 70, 80, and 90 °C) and compared the results with the FUS-induced temperature elevation (Fig. 1). The transient peak temperature (T_{peak}) is defined as the highest peak value within the duration for both FUS and AHST groups. The average temperature ($T_{average}$) is defined as the temperature averaged within a single pulse cycle for FUS group, and the temperature averaged within the duration for AHST group.

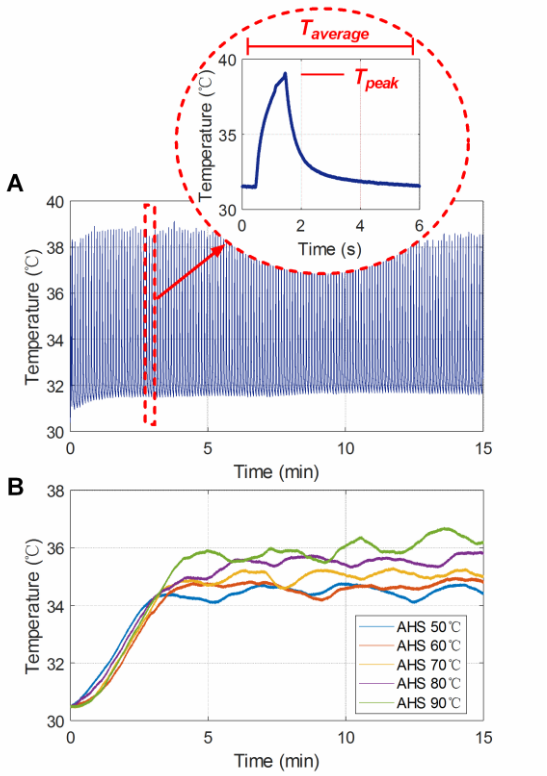

Figure 1. The *in vivo* temperature changes during (A) the 15-min focused ultrasound stimulation (3.6 W/cm^2 I_{spta}, 20% duty cycle, 100 Hz pulse repetition frequency, 1 s sonication duration, 5 s inter-stimulation interval), (B) the 15-min alternative heating source (AHS) stimulation with calibrated temperature at 50, 60, 70, 80, and 90 °C.

E. Blood Pressure Measurement

During the FUS and AHST, BP waveform was continuously measured in the exposed right common carotid artery using a commercial data acquisition system (PowerLab, ADInstruments, Australia). The sampling rate was 1000 Hz. Beat-to-beat systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and heart rate (HR) were calculated from the recorded BP waveform using a software (LabChart toolbox, ADInstrument, Australia). SBP and DBP are defined as the amplitude of the peak and trough of BP waveform, respectively. MAP represents the average blood pressure within a single cardiac cycle. HR is defined as the number of heart beats in a minute.

F. Histological Analysis

To evaluate the biological safety of low-intensity FUS and AHST to the vagus nerve, a histological analysis was conducted using hematoxylin-eosin (H&E) staining. For each rat in FUS and AHST groups, the stimulated left vagus nerve and the controlled right vagus nerve without stimulation were both drawn after the experiment. The materials were incubated in 4% paraformaldehyde overnight and then embedded in optimum cutting temperature (OCT) medium (Sakura Finetek USA Inc) and stored frozen at −80 °C for 2~7 days. The OCT-embedded tissue was sectioned into 5-um-thick slices and then stained with H&E. Finally, the photographed stained nerve slices were examined by an experienced pathologist.

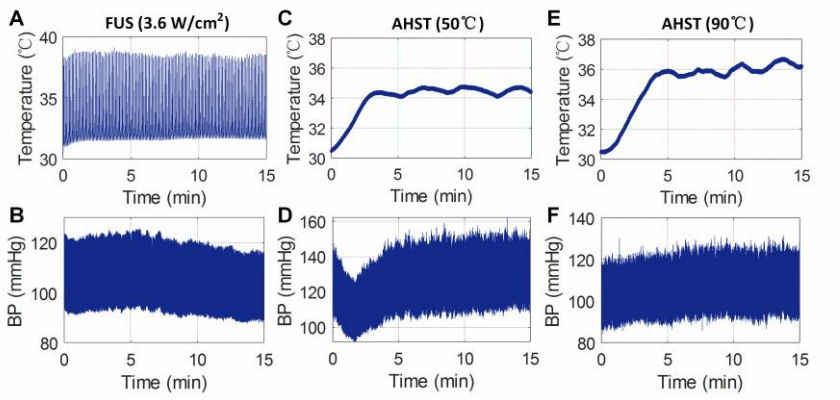

Figure 2. The typical recordings of the *in vivo* temperature and blood pressure (BP) waveform during (A, B) the 15-min focused ultrasound stimulation (FUS) at 3.6 W/cm^2 I_{spta}, (C, D) the alternative heating source treatment (AHST) with calibrated temperature at 50 °C, and (E, F) the AHST at 90 °C.

III. Results

A. BP Responses and Temperature Changes under FUS and AHST of the Vagus Nerve

Fig. 2 shows the typical recordings of BP waveform and *in vivo* temperature of two rats (one for each group) during the 15-min of FUS and AHST, respectively. As can be seen from the figure, BP gradually decreased during the low-intensity FUS at 3.6 W/cm^2 I_{spta} (Fig. 2A, B). During the AHST, two typical patterns of BP responses were observed. One is as observed in Fig. 2C, D, the BP decreased acutely when the temperature began to change, and increased back when the temperature elevated to a constant degree. The other one is as shown in Fig. 2E, F, the BP gradually increased during the AHST.

Table 1 summarizes the relative changes of the *in vivo* average temperature ($T_{average}$) and transient peak temperature (T_{peak}), as well as the change percentage of SBP, DBP, MAP, and HR of the FUS group and the AHST group, respectively. Values were expressed as mean ± standard deviation (STD). As shown in the table, for FUS group, the SBP, DBP, MAP, HR decreased by 8.3 ± 0.6%, 13.0 ± 2.4%, 11.1 ± 1.4%, and 0.9 ± 13.7%, respectively, with *in vivo* $T_{average}$ elevations of 3.1 ± 0.4 °C and T_{peak} elevations of 9.0 ± 1.1 °C. Whereas, for the AHST group, the SBP, DBP, MAP, and HR averagely changed by -0.9~13.0%, -0.6~18.8%, -0.7~16.6%, and -0.6~5.3%, respectively, under different calibrated temperatures. The mean in vivo $T_{average}$ elevations were 3.7~7.2 °C and the T_{peak} elevations were 4.7~9.8 °C. It can be seen that most BP and HR in the AHST group were elevated.

Fig. 3 shows the overall changes of BP and HR, and the elevations of $T_{average}$ responding to the FUS and AHST. It can be seen from the figure that most of the BP decreased in the FUS group while increased in the AHST group. It is noted that although AHST at calibrated temperature of 50 °C has a similar $T_{average}$ elevation with FUS (3.7 vs 3.1 °C), there is however an increase in SBP, DBP, and MAP under the 15-min AHST (Fig. 3 and Table 1).

B. Histological Analysis

Results of H&E-stained histological analysis showed that for rats in both FUS and AHST groups, the stimulated left vagus nerve and the controlled right side revealed normal histology, without necrosis or inflammatory response (Fig. 4).

Table 1. Relative temperature change in terms of average temperature ($T_{average}$), transient peak temperature (T_{peak}) measured *in vivo* and the change percentage of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) during the focused ultrasound stimulation (FUS) and alternative heating source treatment (AHST) of the vagus nerve in rats.

Stimulation parameter		Relative temperature change (vs. $T_{average}$, ℃)	Relative temperature change (vs. T_{peak}, ℃)	SBP change (%)	DBP change (%)	MAP change (%)	HR change (%)
FUS intensity (W/cm^2)	3.6	3.1 ± 0.4	9.0 ± 1.1	-8.3 ± 0.6	-13.0 ± 2.4	-11.1 ± 1.4	-0.9 ± 13.7
AHS heating temperature (℃)	50	3.7 ± 1.2	4.7 ± 1.5	6.5 ± 6.3	5.2 ± 5.5	5.7 ± 5.8	-0.6 ± 3.2
	60	4.1 ± 0.4	5.2 ± 0.6	-0.9 ± 0.5	-0.6 ± 1.9	-0.7 ± 1.3	2.3 ± 5.1
	70	5.6 ± 2.0	7.3 ± 2.9	4.5 ± 2.9	4.4 ± 2.5	4.5 ± 2.6	5.3 ± 8.1
	80	6.2 ± 1.3	8.0 ± 1.9	13.0 ± 16.6	18.8 ± 25.4	16.6 ± 22.0	2.2 ± 9.3
	90	7.2 ± 2.4	9.8 ± 3.1	6.0 ± 0.1	5.7 ± 1.1	5.8 ± 0.7	4.9 ± 1.1

Values are expressed as mean ± standard deviation (STD). Positive value of relative temperature change indicates that the measured *in vivo* temperature is increased during the FUS/AHST. In addition, positive/negative value of BP and HR change indicates that the measured BP and HR is increased/decreased during the FUS/AHST, respectively.

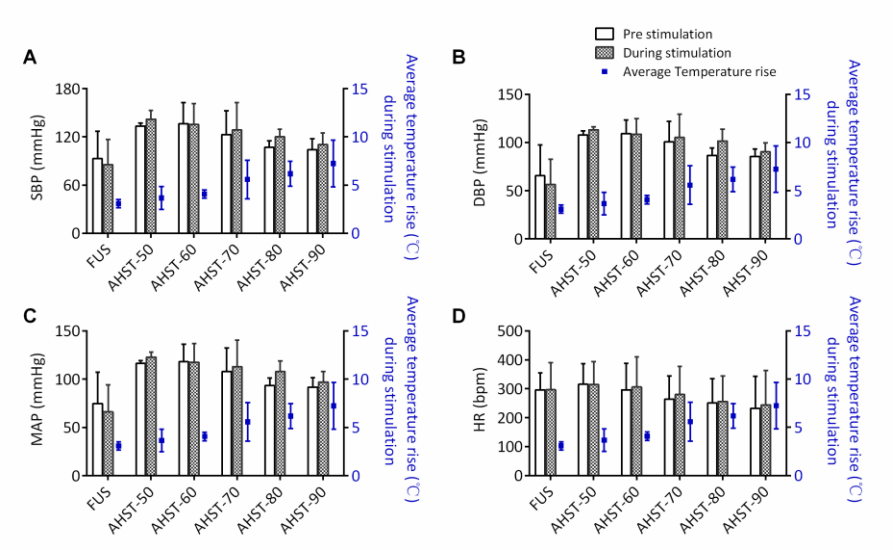

Figure 3. The overall changes (n=3 for FUS group, n=3 for AHST group) in (A) systolic blood pressure (SBP), (B) diastolic blood pressure (DBP), (C) mean arterial pressure (MAP), and (D) heart rate (HR) responding to the focused ultrasound stimulation (FUS) and alternative heating source treatment (AHST) of the vagus nerve, with corresponding average temperature rise during stimulation (blue dot). White bar represents value before stimulation, grey bar represents value during stimulation. Values are plotted as mean ± standard deviation (STD).

IV. Discussion and Conclusion

In this study, BP response under the low-intensity FUS (with thermal effect and mechanical effect) and the AHST (with thermal effect only) of the vagus nerve were investigated. The results showed that the SBP, DBP, MBP and HR decreased under the 15-min of FUS at 3.6 W/cm^2 I_{spta}. However, most of the SBP, DBP, MBP and HR increased under the AHST with $T_{average}$ elevation of 3.7~7.2°C, which approximately matched the $T_{average}$ elevation induced by FUS. Thus, it can be inferred that the thermal effect of ultrasound probably does not contribute in the BP-lowering effect induced by low-intensity FUS of the vagus nerve. The BP-lowering effect is most likely due to the mechanical effects of the ultrasound. Further studies are needed to prove this.

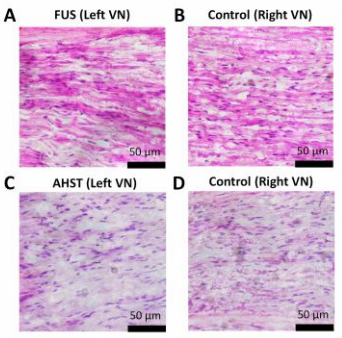

Figure 4. A typical example of the hematoxylin and eosin (H&E) staining histological analysis of (A) the left rat vagus nerve (VN) tissue treated by focused ultrasound stimulation (FUS) and (B) the right control VN, (C) the left rat VN tissue treated by alternative heating source (AHST) and (D) the right control VN tissue. The results confirmed that there was no tissue damage or hemorrhage associated with the FUS and AHST.

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