

## Original Article

# The influence of cardiac autonomic nerve plexus on the electrophysiological properties in canines with atrial fibrillation

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**Abstract:** Background: This study sought to examine the effect of the cardiac autonomic nerve plexus, which originates from the vagus nerve trunk, on atrial vulnerability. Methods: Dogs in group I (n = 6) underwent ganglionated plexi (GP) sequential ablation following six hours of left atrial appendage rapid atrial pacing (RAP). The monophasic action potential duration at 90% of repolarization (APD90), effective refractory period (ERP), and the atrial fibrillation inducing rate of bilateral atria and pulmonary veins were recorded at baseline, 1 h, 3 h and 6 h after pacing, as well as after sequential ablation (RAGP + RIGP ablation, LSGP + RIGP ablation). Dogs in group II (n = 6) received vagus nerve stimulation following six hours of left atrial appendage RAP. APD90, ERP and atrial fibrillation inducing rate of bilateral atria and pulmonary veins were recorded at baseline, 1 h, 3 h and 6 h after pacing, as well as after GP sequential ablation (RAGP + RIGP ablation, LSGP + RIGP ablation). Results: In group I, APD90 and ERP progressively shortened and atrial fibrillation inducing rate increased in various sites 1 h, 3 h and 6 h after RAP ( $P < 0.05$ ). APD90 and ERP shortened significantly and atrial fibrillation inducing rate was significantly higher in the left atrial appendage and bilateral pulmonary veins than in other sites ( $P < 0.05$ ). Following GP sequential ablation, APD90, ERP and atrial fibrillation inducing rate were not significantly different from baseline levels ( $P > 0.05$ ). In group II, APD90 and ERP progressively shortened in various sites over pacing time period, and the atrial fibrillation inducing rate increased 1 h, 3 h and 6 h after RAP + VNS ( $P < 0.05$ ). APD90 and ERP shortened significantly and atrial fibrillation inducing rate was significantly higher in the left atrial appendage and right superior/inferior pulmonary veins when compared with other sites ( $P < 0.05$ ). After GP sequential ablation, APD90, ERP and atrial fibrillation inducing rate were not significantly different from baseline levels ( $P > 0.05$ ). Compared with group I, APD90 and ERP shortened significantly, while atrial fibrillation inducibility increased significantly at baseline and 1 h, 3 h, and 6 h after pacing in group II ( $P < 0.05$ ). After ablation of the four major cardiac GPs, no significant differences were observed in the two groups with respect to APD90, ERP and atrial fibrillation inducing rate ( $P > 0.05$ ). Conclusion: GP activation, as a result of vagal nerve stimulation, alters MAP90, ERP and atrial fibrillation inducing rate of the atrium and pulmonary veins and promotes the occurrence of RAF in the early stage of atrial fibrillation, resulting in increased atrial vulnerability and triggering the occurrence and maintenance of atrial fibrillation.

**Keywords:** Atrial fibrillation, vagal nerve stimulation, ganglionated plexi, cardiac autonomic nervous, rapid atrial pacing, atrial vulnerability

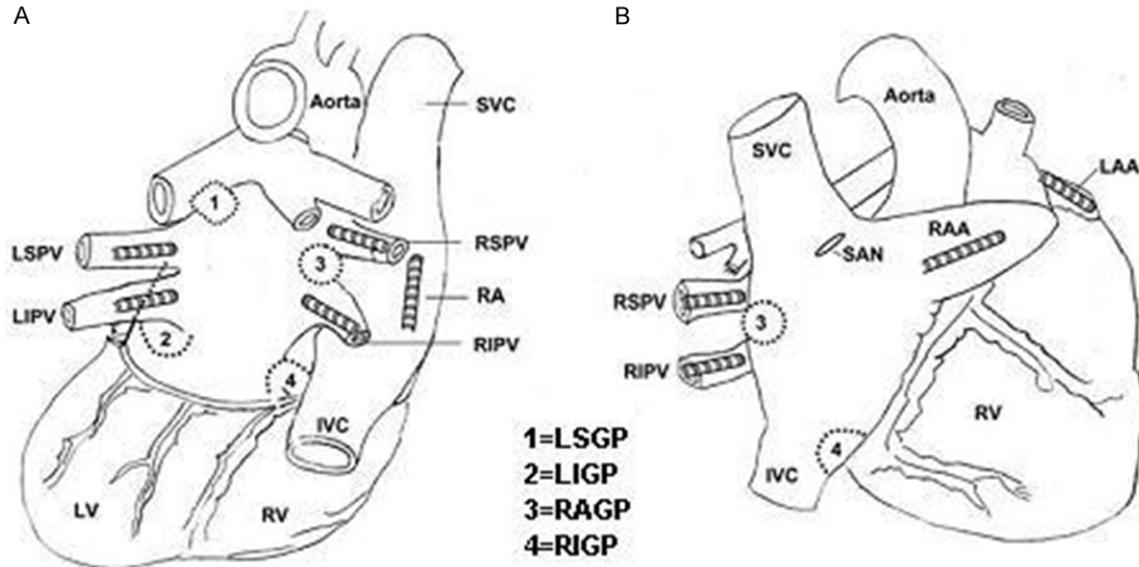
## Introduction

Numerous basic and clinical studies have shown that the cardiac autonomic nervous system may play an important role in the initiation, maintenance, and termination of atrial fibrillation [1-3]. The cardiac autonomic nervous system can be divided into the intrinsic cardiac autonomic nervous system (ICANS) and the extrinsic cardiac autonomic nervous system (ECANS) [4]. The intrinsic cardiac autonomic nervous system is affected by the extrinsic

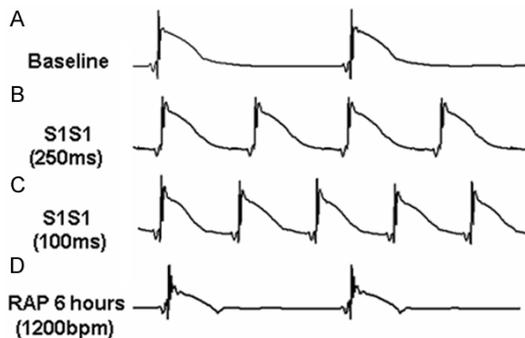
autonomic nervous system [5]. A complex network exists among ganglionated plexi (GPs), which innervate surrounding heart muscle and regulate the electrophysiology of distal atrial myocardium. The autonomic nervous system can alter atrial conduction and refractory period to induce atrial fibrillation. Conversely, atrial fibrillation can also trigger autonomic nervous system.

Atrial vulnerability represents an important research area in determining the underlying

## Atrial vulnerability



**Figure 1.** Schematic diagram and catheter positions in the atria and pulmonary veins. A: Posterior–anterior view; B: right anterior oblique view. Multi-electrode catheters were sutured to left superior pulmonary vein (LSPV), left inferior pulmonary vein (LIPV), left atrial appendage (LAA), right superior pulmonary vein (RSPV), right inferior pulmonary vein (RIPV) and right atrial appendage (RAA). SVC, superior vena cava; LPA, left pulmonary artery. LV: left ventricle; RV: right ventricle.



**Figure 2.** MAP patterns under different stimulations. A: Left atrial appendage MAP at baseline; B: MAP with S1S1 stimulation at a cycle length of 250 ms; C: MAP with S1S1 stimulation at a cycle length of 100 ms; D: MAP after 6-hour S1S1 stimulation at a cycle length of 50 ms.

mechanisms of atrial fibrillation. Extensive research has been performed in this field on topics such as arrhythmia, vagus nerve stimulation, and the effects of physiological indicators like atrial size and atrial tension on atrial vulnerability. Repetitive atrial firing (RAF) is an important electrophysiological feature of atrial vulnerability and is closely related to the occurrence of atrial fibrillation. In this study, we recorded left and right atrial action potentials in dogs under autonomic nerve stimulation

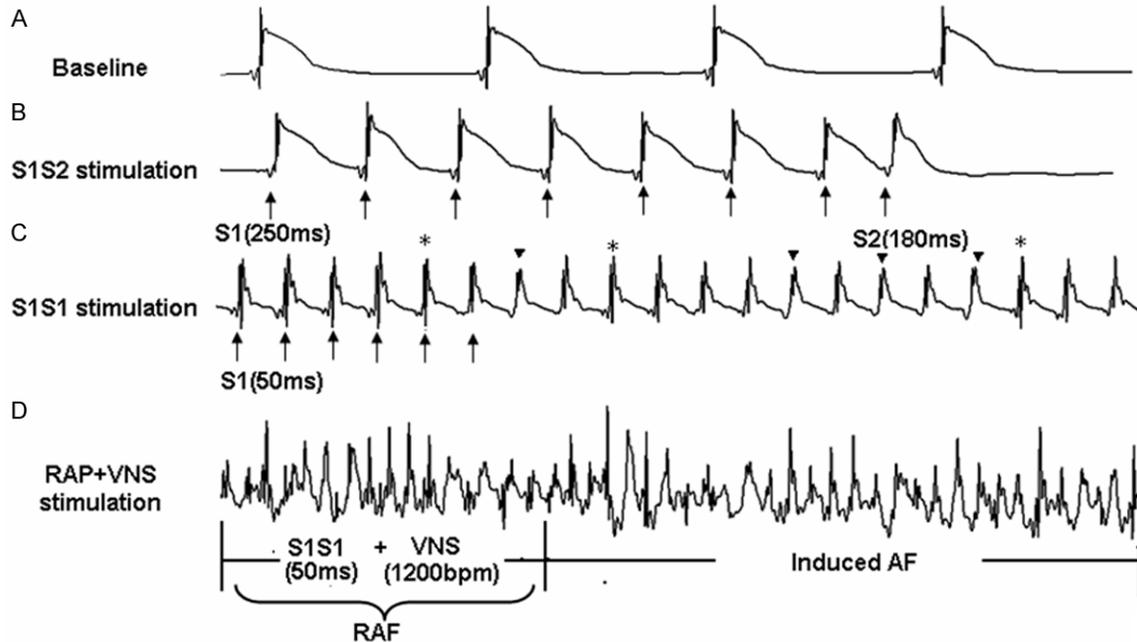
using monophasic action potential recording. We simultaneously recorded the effective refractory periods of pulmonary veins and atrial muscle. This study investigated the effect of the intrinsic cardiac autonomic nerve plexus, which originates from the vagus nerve trunk, on atrial vulnerability as well as the underlying mechanisms of autonomic nerve-induced changes in triggering and maintaining atrial fibrillation.

### Methods

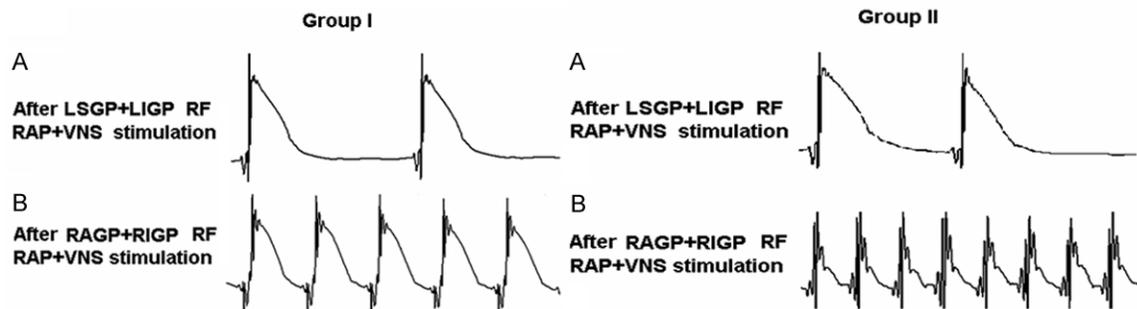
#### Animal preparation

All animal studies were reviewed and approved by the animal experimental administration of Xiniang Medical University, China. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Twenty adult mongrel dogs weighing 18-25 kg were anaesthetized with Na-pentobarbital, 30 mg/kg, and followed by additional dose of 2 mg/kg at the end of each hour. All dogs were ventilated with room air by a positive pressure ventilator. Core body temperature was maintained at 36.5~37°C. The chest was entered via both left and right-sided thoracotomy at the 4th intercostal space.

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**Figure 3.** Changes in left atrial appendage MAP under different stimulations. A: Left atrial appendage MAP at baseline; B: MAP with programmed stimulation (S1 = 250ms; S2 = 200 ms; step = 5 ms); C: MAP with burst stimulation (S1 = 50 ms; 8V), \* and ▼ denote repetitive waveforms; D: Varying MAP patterns with VNS stimulation (1200 times/min; 6V) + S1S1 stimulation (50 ms; 8 V).



**Figure 4.** Changes in left atrial appendage MAP in the two groups after GP ablation. A: Left atrial appendage MAP after LSGP + LIGP sequential ablation. B: Left atrial appendage MAP after RAGP + RIGP ablation. RAP (1200 times/min, 8 V) + VNS (20HZ, 6 V).

Eight multi-electrode catheters were sutured to allow pacing and recording at the left and right atrial appendage (LAA, RAA), left and right superior pulmonary vein (LSPV, RSPV), and left and right inferior pulmonary vein (LIPV, RIPV) (Figure 1). A standard ECG limb lead was continuously recorded, filtered at 0.1-250 Hz. All tracings from the electrode catheters were amplified and digitally recorded using a computer-based Lab System (Lead 2007, Jingjiang, Inc, China), filtered at 30-500 Hz. All pacing and stimulation were performed with a battery powered Medtronic stimulator.

### *The monophasic action potential*

The monophasic action potential was recorded epicardially by a custom-made Ag-AgCl catheter as described in detail in previous reports. The tip electrode protrudes to form a smooth spherical surface 1 mm in diameter. The reference electrode is 0.5 mm in diameter and 5 mm proximal to the tip electrode. The two electrodes were made from cylindrical pellets of sintered Ag-AgCl. The action potential electrograms were filtered at 1-1200 Hz. The pacing was subsequently delivered to each site by the

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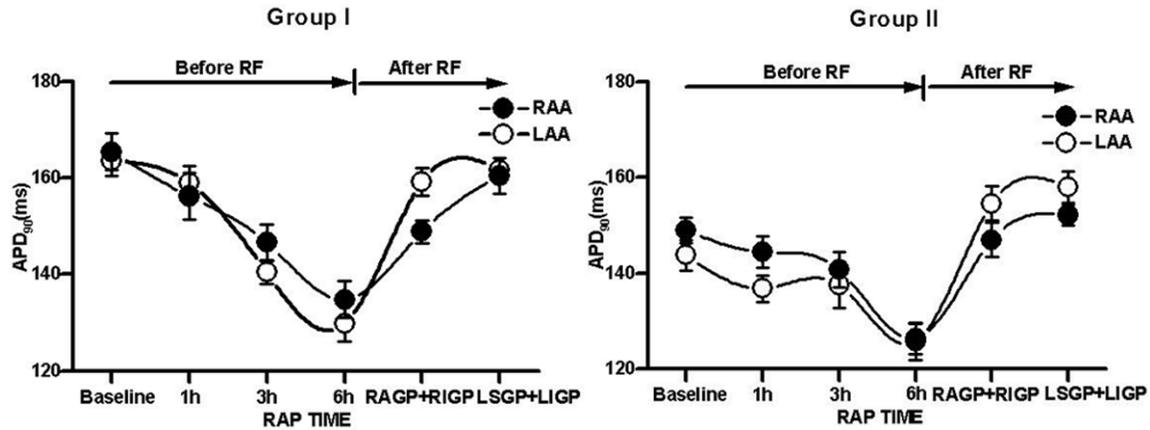


Figure 5. Changes in APD90 in the left and right atrial appendages.

multiple-electrode catheters, and the pacing and action potential recording were always at the same site. A dynamic steady-state pacing protocol (S1S1) was applied with a series of pulse trains at constant pacing cycle length (PCL). The pulse train was delivered at an initial PCL just slightly shorter than the sinus cycle length and maintained for 30 s to ensure a steady state. After each pulse train was delivered, the PCL was shortened in 10 ms steps until AF occurred. The pacing was interrupted for 30 s to minimize the pacing memory effects before the next pacing train. The action potential recording and the dynamic pacing protocol were subsequently performed at the LAA, LSPV, LIPV, RAA, RSPV, and RIPV. The monophasic action potential catheter was continuously shifted, and the sites where APD was recorded were marked so that the measurements could be repeated at the same sites after interventions, i.e. vagal nerve stimulation (VNS) and 6-h rapid atrial pacing. The action potential and ECG recordings were analyzed by the LEAD 2007 work station system. The APD at RAA and LAA site was measured at 90% repolarization (APD90).

### AF inducibility

AF inducibility was evaluated by the similar dynamic pacing protocol as for constructing of the APD restitution curve. The pulse train was delivered at an initial PCL just slightly shorter than the sinus cycle length and lasted for 5 s. If AF was not induced after termination of pacing, the PCL was shortened in 10 ms steps until AF occurred. The AF threshold was defined as the longest PCL (in 10 ms steps) inducing AF. Based

on multiple atrial and PV recordings, AF was defined as irregular atrial activation (500 b.p.m., .5 s) associated with irregular atrioventricular conduction after the interruption of rapid pacing or MAP polymorphism.

### GP mapping and ablation

The ablation electrode probe was arranged in the GP. HFS (20 Hz, 0.1 ms duration, 0.5-5.0 V) stimulation caused the heart rate lower more than 50% or atrioventricular block as GP site, GP was repositioned and then ablated (30 W, 60 s). After ablation, HFS (20 Hz, 0.1 ms duration, 12 V) stimulation could not cause obviously decrease in heart rate and/or atrioventricular block, GP completely successful ablation.

### Protocols

12 dogs were divided into 2 groups.

**Group I (n = 6): 6 h rapid atrial pacing induced AF:** Six dogs underwent rapid atrial pacing (1200 b.p.m., 2× threshold, 2 ms in duration) deliver from the LAA catheter for 6 h. The APD90, ERP and the AF inducibility were measured at each site at baseline, after pacing (the end of 1 h, 3 h, 6 h) and after GP RF.

**Group II (n = 6): vagally mediated AF:** 6 hours RAP and right VNS electrical stimulation (20 Hz) at the same time at voltages 50% below the threshold which slowed sinus rate or AV conduction. Right cervical vagal nerves were dissected and a pair of Teflon-coated silver wires (0.1 mm diameter) was inserted into the vagal nerves for electrical stimulation. The VNS was

performed at 20 Hz and 0.1-10 V. The strength for VNS was set at the voltage level inducing a 50% decrease in sinus rate or 2:1 atrioventricular conduction block. The APD90, ERP and the AF inducibility were measured in each site in the presence of VNS.

### *Statistical analysis*

Data were expressed as mean standard error. Repeated-measures analysis of variance was used for the comparison of repeated measures for ERP data points acquired at different time intervals in two groups. Fisher exact test of proportions was used to compare the induction of ERP in two groups. *P* values of  $\leq .05$  were considered statistically significant.

## Results

### *RAF and MAP recordings*

In group I, a stable monophasic action potential (MAP) first appeared with an increase in the rapid pacing frequency of the left atrial appendage. A further increase in the pacing frequency and pacing duration led to a shortened atrial refractory period and MAP duration shortened. In group II, under vagus nerve stimulation, rapid atrial firing (RAF) was recorded prior to the onset of atrial fibrillation, which was induced by S1S1 (50 ms) stimulation. Following atrial fibrillation induction, MAP showed slower and distorted depolarization of varying amplitudes. The repolarization phase was terminated prematurely by ensuing depolarization at different levels of repolarization, and diastolic time intervals between continuous and regular MAPs disappeared. MAP was slightly prolonged after right atrial ganglionated plexus (RAGP) + right inferior ganglionated plexus (RIGP) ablation and returned to normal after subsequent left superior ganglionated plexus (LSGP) + left inferior ganglionated plexus (LIGP) ablation. The results are shown in **Figures 1-4**.

### *APD90 recordings in the two groups*

APD90 progressively increased at 3 h and 6 h in group I and group II ( $P < 0.05$ ). After RAGP + RIGP and LSGP + LIGP sequential ablation in the two groups, APD90 differed insignificantly from baseline levels ( $P > 0.05$ ).

APD90 was higher at baseline and 1 h, 3 h and 6 h after pacing in group II when compared to

group I ( $P < 0.05$ ). Following RAGP + RIGP and LSGP + LIGP sequential ablation, APD90 was not significantly different between the two groups ( $P > 0.05$ ). The results are shown in **Figure 5**.

### *ERP and AF inducibility in group I*

In group I, ERP progressively shortened and the atrial fibrillation inducibility increased in various sites at 3 h and 6 h after RAP ( $P < 0.05$ ). In addition, ERP shortened significantly and the atrial fibrillation inducibility significantly increased in the left atrial appendage and bilateral pulmonary veins compared to other sites ( $P < 0.05$ ). After GP sequential ablation, ERP and atrial fibrillation inducibility were not significantly different from baseline levels ( $P > 0.05$ ). These results are shown in **Figures 6, 7**.

### *ERP and AF inducibility in group II*

In group II, ERP progressively shortened and the atrial fibrillation inducing rate increased in various sites at 3 h and 6 h after RAP + VNS ( $P < 0.05$ ). In addition, ERP shortened significantly and the atrial fibrillation inducibility was significantly higher in the left atrial appendage and right superior/inferior pulmonary veins compared to other sites ( $P < 0.05$ ). After GP sequential ablation, ERP and the atrial fibrillation inducibility in various sites were not significantly different compared to baseline levels ( $P > 0.05$ ). Compared with group I, ERP was shortened and the atrial fibrillation inducibility was increased at baseline and 1 h, 3 h, and 6 h after pacing when compared to group II ( $P < 0.05$ ). Following ablation of the four major cardiac GPs, no significant difference was observed in ERP and atrial fibrillation inducibility between the two groups ( $P > 0.05$ ). These results are shown in **Figures 6, 7**.

## Discussion

The intrinsic cardiac autonomic nervous system (ICANS) originates from the vagus nerve trunk and is a neural network consisting of multiple GPs and interconnected nerves. High-frequency vagus nerve stimulation plus rapid pacing of atrial tissue stimulated GPs and increased atrial vulnerability, resulting in focal rapid electrical activity. GP sequential ablation reduced atrial vulnerability and suppressed the induction of atrial fibrillation. Thus, GP-targeted

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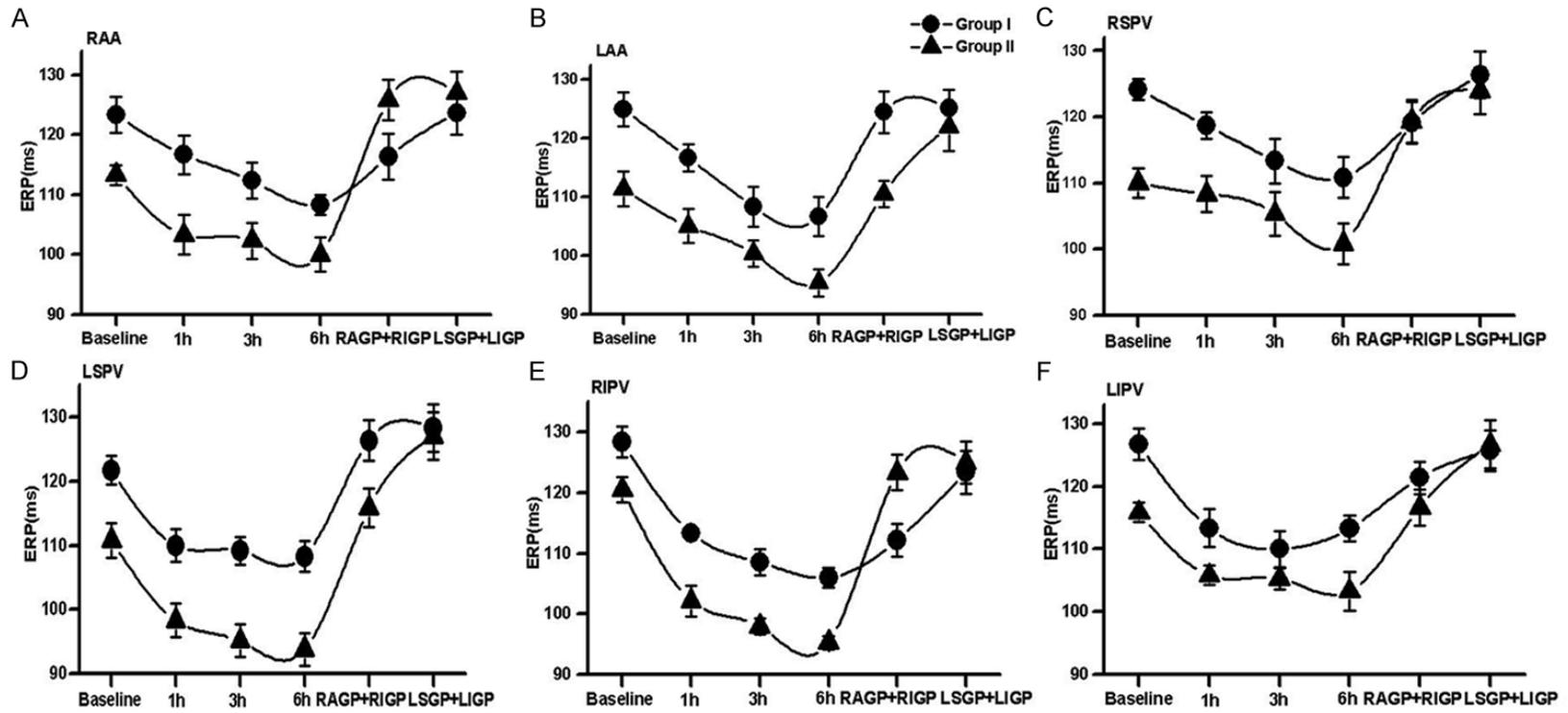


Figure 6. Change of ERP value the pulmonary vein between two groups the pulmonary vein and atrial each part.

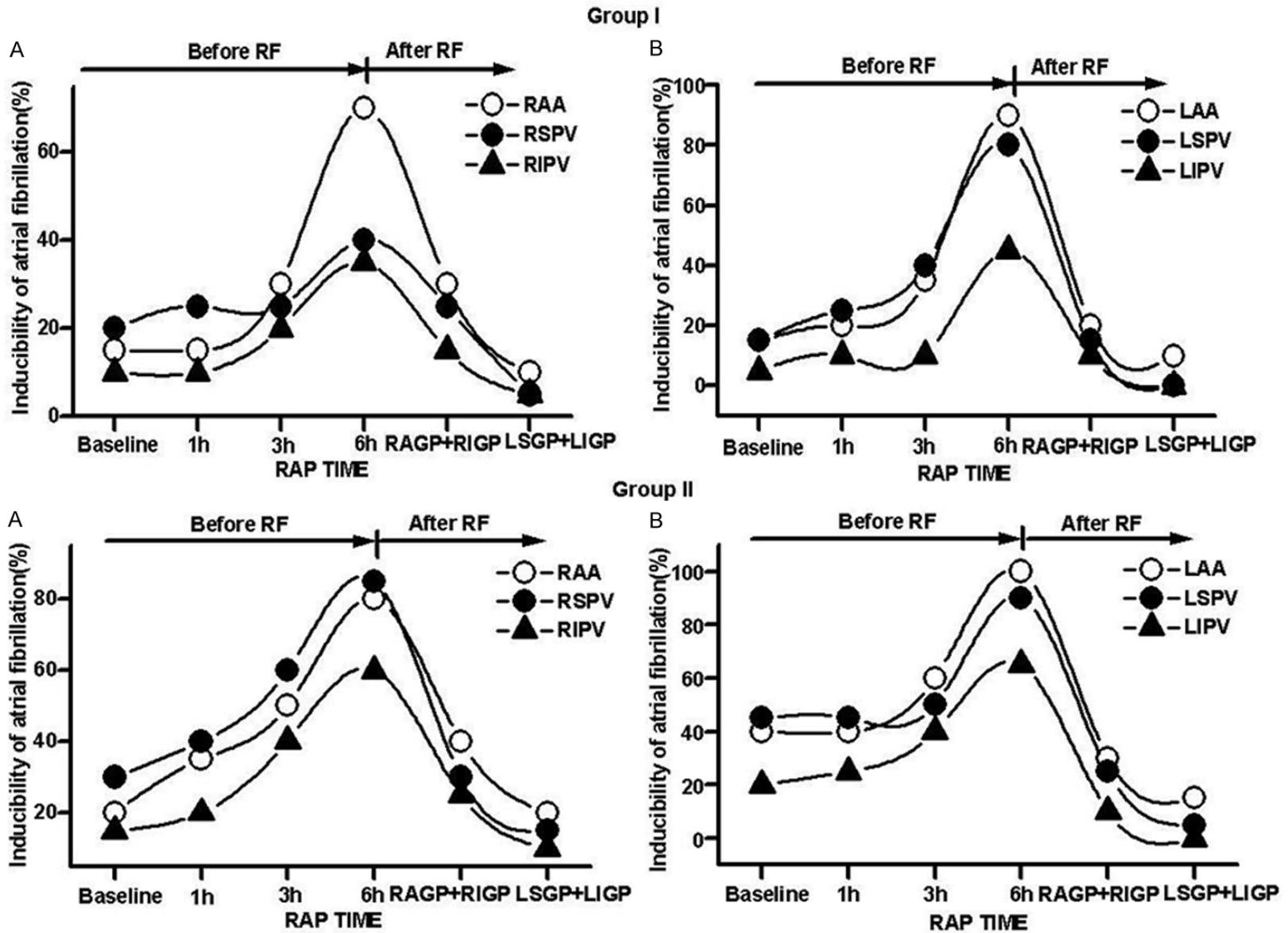


Figure 7. Change of AF inducibility between two groups the pulmonary vein and atrial each part.

regulation of the intrinsic cardiac autonomic nervous system activity plays an important role in the treatment of atrial fibrillation.

### *MAP and APD90 following 6 h-pacing*

After 6-hour left atrial appendage rapid pacing and right vagus nerve stimulation in group I, the MAP pattern and A wave of the epicardial left atrial appendage and right atrial appendage appeared in the early stage of vagus nerve stimulation-induced atrial fibrillation. In addition, the P waves were in disarray, showing various shapes and sizes. The repolarization phase was terminated prematurely following depolarization, and diastolic time intervals between continuous and regular MAPs disappeared. After 6-hour rapid pacing of the left atrial appendage in group II, the MAP90 duration shortened and MAP amplitude decreased in the absence of vagus nerve stimulation. Lubinski *et al.* investigated ERP and MAP90 in patients with organic heart disease before burst stimulation and after termination of atrial fibrillation [7]. Their results showed that burst stimulation-induced atrial fibrillation shortened ERP and MAP90, which were reversed upon termination of atrial fibrillation.

### *ERP following 6-h pacing*

RAP + VNS stimulation shortened ERP and increased the atrial fibrillation inducibility in the right atrium and right pulmonary vein. Left atrial appendage RAP shortened ERP and increased the atrial fibrillation inducibility in the left atrium and left and right superior pulmonary veins. Lu *et al.* found that 6-hour RAP significantly decreased ERP and increased the vulnerability [10]. Cervical vagus nerve stimulation can induce atrial fibrillation. In addition, endocardial nerve plexus stimulation can induce atrial fibrillation, which is similar to focal paroxysmal atrial fibrillation seen in the clinic. Electrophysiological changes due to vagus nerve stimulation are similar to electrical remodeling during atrial fibrillation. Shorter atrial APD and ERP contribute to electrophysiological conditions that trigger and maintain atrial fibrillation.

### *MAP and APD90 following GP sequential ablation*

Sequential ablation of four GPs significantly restored MAP morphology and action potential

duration in both group I and group II. In addition, following GP ablation, vagus nerve stimulation failed to induce atrial fibrillation. These results suggest that stimulation of the extrinsic autonomic nervous system increases atrial vulnerability and induces atrial fibrillation via GP stimulation. Previous studies have defined atrial vulnerability as the ability to induce atrial fibrillation with atrial extrinsic stimulation. Jais *et al.* found that atrial fibrillation patients show characteristic RAF rhythms with gradual advancements of a premature beat, indicating its important role in the initiation of atrial fibrillation [13]. Saksena *et al.* found that RAF due to premature beats progressed to atrial fibrillation following a period of time [14]. In addition, Ndrepepa *et al.* confirmed that RAF, as an intermediate rhythm, plays an important role in atrial fibrillation initiation [15]. Premature beat stimulation causes activation capture during repolarization, slowing premature activation transmission and causing conduction block and conduction delay [16]. Meanwhile, continuous activations further contribute to the development of arrhythmias. In short, RAF often occurs before the onset of atrial fibrillation, suggesting that RAF is a characteristic marker predisposing to paroxysmal atrial fibrillation and represents an indicator of atrial vulnerability.

### *ERP following GP sequential ablation*

After RIGP + RAGP ablation in group I, ERP and the atrial fibrillation inducibility decreased in bilateral superior pulmonary veins. Following LIGP + LSGP ablation, ERP in the left and right atria and pulmonary veins was restored to baseline levels. In addition, RAGP + RIGP ablation decreased ERP and atrial fibrillation inducibility in the right superior and inferior pulmonary veins in group II. Furthermore, after subsequent LSGP + LIGP ablation, ERP was prolonged and atrial fibrillation inducibility restored to baseline levels in the left and right atria and pulmonary veins. Our results indicated that the ERP changes in the left atrium and bilateral pulmonary veins in group I may be related to cardiac nerve density. Chevalier *et al.* demonstrated that the cardiac area with the highest nerve density is situated at the juncture between the pulmonary vein and left atrium [17]. In addition, Tan *et al.* showed that the cardiac area with the highest density of adrenergic and cholinergic

nerves occurs 5 mm within the junction between the pulmonary vein and left atrium, with the upper wall of left superior pulmonary vein and antero-superior wall of the right superior pulmonary vein having more adrenergic and cholinergic nerves than their contralateral walls [18]. Ablation of the right GP resulted in uneven distribution of GPs bilaterally and an imbalance in GP-innervated atrial function. However, ERP and atrial fibrillation inducibility was not improved after the ablation of right GPs. The sequential ablation of left GPs resulted in the loss of GP innervation and recovery of ERP and atrial fibrillation inducibility.

In group II, ERP was changed in the right pulmonary vein. The superior and inferior GPs on the right originate from the right vagus nerve trunk and act mainly on the region around the right pulmonary vein. Right vagus nerve stimulation altered atrial electrophysiological properties through regulating intrinsic autonomic nerve activity following extrinsic autonomic nerve stimulation. The ablation of right GPs led to significantly prolonged ERP and lower atrial fibrillation inducibility. Sequential ablation of left GPs returned ERP and atrial fibrillation inducibility to baseline levels. Extrinsic stimulation of intrinsic GPs altered electrophysiological features in different cardiac areas and led to atrial vulnerability.

Dispersion of atrial refractoriness and decreased conduction velocity predispose patients to atrial fibrillation and represent electrophysiological markers of atrial vulnerability. It has been shown that vagus nerve trunk stimulation induces atrial fibrillation. However, studies investigating vagus nerve stimulation on atrial vulnerability are limited. Lu *et al.* found that both vagal block and GP ablation effectively reduced atrial vulnerability [20]. In addition, they found that vagus nerve stimulation significantly shortened ERP in the pulmonary veins and reduced the threshold for atrial fibrillation [21]. Zhang *et al.* applied vagus nerve trunk stimulation, which served as extrinsic nerve stimulation, and ganglion nerve stimulation, which served as intrinsic nerve stimulation, to the right upper pulmonary vein and the right atrial appendage, and then compared atrial vulnerability in the two areas [22]. They showed that vagus nerve stimulation produced a more significant impact on the vulnerability of the

right atrial appendage than ganglion stimulation on the susceptibility of the right upper pulmonary vein. Based on these findings, they proposed that the heart is subject to intrinsic innervation and extrinsic innervation, which are distributed to different parts of the heart. Hou *et al.* investigated the ventricular rate during sinus rhythm and atrial fibrillation in RAGP, RIGP and LSGP following unilateral stimulation on the left or right vagus nerve [23]. Their study showed that right vagus nerve stimulation resulted in arrhythmogenic effect than left vagus nerve stimulation. Moreover, they also found that right vagus nerve stimulation induced atrial fibrillation and broadened atrial vulnerability, whereas left vagus nerve stimulation only shortened ERP, indicating that the heart is subject to regulation by extrinsic autonomic and intrinsic autonomic nerves. Sheng *et al.* found that low-intensity vagus nerve stimulation could improve atrial vulnerability markers [24]. This improvement may have been mediated by the inhibition of intrinsic cardiac autonomic nervous system, offering a new target for atrial fibrillation prevention and treatment. A number of radiofrequency ablation techniques, including fragmented potential ablation [25], pulmonary vein isolation ablation [26], ligament of Marshall ablation [27], epicardial fat pad ablation [28], circumferential pulmonary vein ablation and linear left atrial ablation [29], have been shown to improve atrial vulnerability markers. Our study indicates that epicardial GP sequential ablation can also improve atrial vulnerability markers.

### *Clinical significance*

Cardiac ganglions are an important part of the cardiac autonomic nervous system and are important for the transmission of external neural information into the heart. This study showed that maintenance of atrial fibrillation maintenance is dependent on atrial APD and ERP shortening, which is primarily mediated by acetylcholine in vagal nerve terminals. Radiofrequency ablation of epicardial GPs can selectively abolish vagal innervation of the atrium, sinoatrial node and atrioventricular node, thereby preventing the occurrence of atrial fibrillation. This study also showed that the ablation of major cardiac GPs can abrogate vagal-mediated atrial APD and ERP shortening,

prevent decline in the atrial fibrillation inducibility, and reduce atrial fibrillation vulnerability.

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### Disclosure of conflict of interest

None.

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