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Original Article

# Continuous vagal nerve stimulation affects atrial neural remodeling and reduces atrial fibrillation inducibility in rabbits

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ABSTRACT

*Background:* The effects of continuous vagal nerve stimulation (VNS) on atrial neural remodeling during atrial fibrillation (AF) remain unclear.
*Objective:* To test the hypothesis that VNS affects atrial neural remodeling and reduces AF inducibility.
*Methods:* Twenty rabbits were randomly divided into two groups: rapid atrial pacing (RAP) group and RAP with VNS group. AF inducibility studies and atrial histologic analyses were performed after 4 weeks.
*Results:* Five rabbits of RAP group (5/10) in the RAP group developed sustained AF. None of rabbits in RAP with VNS group had developed AF. The incidence of sustained AF in VNS group was significant lower than that in rapid pacing group ($P<.01$). Treatment with VNS resulted in a significant reduction in atrial neural remodeling and AF duration ($P<.01$).
*Conclusions:* Atrial neural remodeling plays an important role in the initiation and maintenance of AF. Modulating autonomic nerve function with VNS can contribute to AF control.



## 1. Introduction

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias. The number of patients with AF in the United States is anticipated to increase to 9.4–11.7 millions in the year 2030, many of whom will have drug-resistant AF [1]. It is highly desirable to develop a less invasive therapy, which can be easily terminated, without causing permanent damage to the autonomic structures, to treat such a large population of patients with AF. Rapid pacing of left atrial can be used in the animal laboratory as a method to induce or maintain sustained AF [2]. Vanoli et al. [3] showed that chronic vagal nerve stimulation (VNS) can prevent ventricular fibrillation and sudden cardiac death in conscious dogs with a healed myocardial infarction. Plenty of studies have shown that VNS might be used to attenuate heart failure development in dogs [4], rats [5], and humans [6–8]. However, no histological data were presented in that article to document the presence of increased sympathetic innervation. The purpose of the present study was to use immunohistochemical techniques to observe atrial sympathetic nerve density in a rabbit model of persistent AF produced by rapid atrial pacing (RAP). The results were set to investigate the potential new therapeutic options for AF that target atrial neural remodeling.

## 2. Methods

### 2.1. Animals

Thirty New Zealand rabbits, either male or female, weighing about 2.0–2.5 kg, were purchased from the Experimental Animal Department, Shanghai Jiaotong University School of Medicine, and received humane care. The experiment was approved by Shanghai Administrative Committee for Laboratory Animals, and the procedures were carried out strictly in compliance with the guide for the care and use of laboratory animals published by the National Institutes of Health in 1996. Thereafter, the animals were randomly divided into two groups: RAP group (Group P) and RAP with VNS group (Group V).

### 2.2. RAP models of AF

Rabbits were anesthetized with intravenous injection of 3% pentobarbital sodium (30 mg/kg) and were ventilated through a cuffed endotracheal tube with a Bird Mark 8 ventilator. The left thoracic cavity was opened via the third intercostal space, and then the heart was exposed by a dilator. A custom-designed set of electrodes, comprising a pair of electrodes with a distal hook for pacing, were sutured to the epicardial surface of the left atrium (LA). The distal ends of these electrode leads were tunneled subcutaneously and exposed on the back and connected to a pacemaker (output of 6 V with 1.0 ms pulse duration; Guangzhou Academy of Sciences, China). The pacemaker was programmed to provide RAP at 900 ppm, and this pacing rate was maintained continuously for 4 weeks. Rabbits in sham control were operated with the identical surgery procedure but RAP.

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### 2.3. VNS

For VNS implantation in Group V, a pair of Teflon-coated stainless steel wires (UL1330; Triumph Cable Co, Ltd, Dongguan, China) was looped around the left vagal nerve in the neck. This was connected to the output terminals of the stimulator (BL-420S Data Acquisition and Analysis System; Chengdu TME Technology Co, Ltd) through the same subcutaneous tunnel with the pacing leads, which provides stimulation over a range of frequencies (0.1–100 Hz), strengths (1–10 V), and pulse widths (0.001–10 s). In the present study, the vagal nerve was stimulated with rectangular pulses of 40 ms duration at 1 Hz, 2 V.

### 2.4. ECG recordings

Electrocardiogram (ECG) was recorded before and after the pacing. During the period of pacing, ECG was measured every day to ensure that the pacemakers were working properly.

### 2.5. AF inducibility studies

At the end of the experiment, all rabbits were anesthetized and ventilated. The chest was opened with a midline sternotomy. A pericardial cradle was created at one LA site and one right atrium site. AF inducibility was assessed by single extrastimulus pacing and an atrial burst pacing protocol. Signals were sampled at 2 kHz and stored with the UnEmap system (University of Auckland, New Zealand). A total of 16 burst stimulations were performed for each animal, with each atrial site receiving eight burst pacings (4 for 6 s and 4 for 12 s) at a cycle length of 50 ms and a stimulus output of 0.5 V plus twice the diastolic threshold. AF was considered sustained if the induced episode lasted 30 min. If persistent AF was achieved at any point in the protocol, further testing was not performed, and the longest AF duration was taken as 1800 s.

### 2.6. Immunohistochemical studies

Tissues were obtained from LA at the end of AF inducibility study. The whole procedure was performed in cold conditions. Anti-growth-associated protein 43 (GAP43) antibody and anti-tyrosine hydroxylase (TH) antibody were used for immunohistochemical staining. Nerve densities were determined by a computer-assisted image analysis system (Image-Pro Plus 6.0). Each slide was examined under a microscope with 20× objectives, and three fields were selected randomly with the highest density of nerves. The computer automatically detected the stained nerves in these fields by the brown-stained color and then calculated the area occupied by the nerves in each field. The nerve density was the nerve area divided by the total area examined. The mean density of nerves in these three selected fields was used to represent the nerve density of that slide.

### 2.7. Statistical analysis

All offline measurements were made by investigators blinded to the treatment. Quantitative data were presented as mean±S.D. Statistical product and service solutions (SPSS) 22.0 software package (IBM SPSS Inc, Chicago, USA) was used for statistical analysis. The chi-square test was used to compare categorical variables between the two groups. The Student's *t* test was used to compare continuous variables between the two groups. A value of $P<.05$ was regarded as statistically significant.

## 3. Results

### 3.1. AF inducibility

Only rabbits in Group P developed sustained AF (5 of 10). There was no sustained AF in Group V. The incidence of sustained AF in Group V was significant lower than those in Group P ($P<.01$). VNS treatment resulted in a significant reduction in average AF duration from 1589±225 s in Group P to 305±54 s in Group V ($P<.001$).

### 3.2. Immunohistochemical studies

The nerves immunopositive to GAP43 and TH in the atrium of Group P were significantly higher than that of Group V (Figs. 1 and 2). In Group P, the nerve densities of GAP43 and TH are 6701±1385 $\mu m^2/mm^2$ and 3750±865 $\mu m^2/mm^2$, respectively. In comparison, VNS had reduced the nerve density of GAP43 (839±455 $\mu m^2/mm^2$, $P<.001$) and TH (494±285 $\mu m^2/mm^2$, $P<.001$).

## 4. Discussion

There is an association between abnormal autonomic innervation and AF in both animal models and humans. The abnormal autonomic innervation may be important in the mechanisms of AF [9,10]. Jayachandran et al. [11] used [C-11] hydroxyephedrine to label sympathetic nerve terminal in dogs with pacing-induced AF and documented heterogeneously increased atrial sympathetic innervation. The increased sympathetic nerve densities were later confirmed by immunohistochemical staining using antibody against TH in dogs with pacing-induced AF [12]. Atrial nerve sprouting and sympathetic hyperinnervation also occur after ventricular myocardial infraction and are associated with increased incidence and duration of AF [13]. Consistent with these results, atrial sympathetic nerve densities are also significantly increased in patients with chronic AF [14]. Olgin et al. [15] reported that sympathetic atrial denervation by phenol creates heterogeneous autonomic innervation, facilitating sustained AF. The present study extended their observations by documenting sympathetic hyperinnervation in the atrial using immunohistochemical techniques. GAP43, a protein expressed in the growth cones of sprouting axons [16], is a marker for nerve spouting. A robust increase of GAP43-positive nerves in rabbits of Group P suggests that nerve sprouting is responsible for the sympathetic hyperinnervation during pacing-induced AF.

One possible cause of nerve sprouting in this model is the electrical current, which has been used to induce nerve sprouting in the brain and in the kindling model of seizure disorder [17]. However, we do not have sufficient data from this study to test that hypothesis. It is also unclear whether neural remodeling is causally related to the pathogenesis of AF. Adrenergic stimulation in the electrically remodeled myocardium increases significant electrophysiological changes and may be proarrhythmic [18,19]. Sympathetic nerve sprouting and hyperinnervation may strengthen this interaction and contribute to the generation and maintenance of AF.

Although RAP resulted in an increase in AF duration, treatment with VNS prevented this change. This effect is consistent with the immunohistochemical finding of increased nerve sprouting and sympathetic hyperinnervation in the Group P that was markedly attenuated with VNS. It is highly desirable to develop a neuromodulation method targeted for sympathetic hyperinnervation in AF.

VNS can be used in the animal laboratory as a method to induce or maintain sustained AF [20]. Recently, Shen et al. [21] showed that, in ambulatory dogs, continuous left low-level VNS effectively suppresses left stellate ganglion nerve activity and significantly reduces the frequency of paroxysmal atrial tachyarrhythmia in paroxysmal AF dog model. Significant neural remodeling of left stellate ganglion was evident. Kusunose et al. [22] found that VNS improved LA function and volumes and suppressed LA fibrosis in the dog high-rate ventricular pacing model. The neural remodeling findings of atrium after VNS were never documented before. In the present study, we evaluated the effects of VNS treatment on AF inducibility in the rabbit AF model induced by RAP. Our findings show that