# Low-level vagosympathetic trunk stimulation inhibits atrial fibrillation in a rabbit model of obstructive sleep apnea

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**BACKGROUND** Atrial fibrillation (AF) is highly associated with obstructive sleep apnea (OSA) in which AF is triggered by hyperactivity of the cardiac autonomic nervous system. Previous studies showed that low-level vagosympathetic trunk stimulation (LLVS), at voltages not slowing sinus rate or AV conduction, inhibits AF by suppressing the cardiac autonomic nervous system.

**OBJECTIVE** The purpose of this study was to investigate whether LLVS delivered at the right vagosympathetic trunk suppresses AF in a rabbit model of OSA.

**METHODS** Eleven rabbits received a tracheostomy under general anesthesia. The endotracheal tube was clamped at end expiration for 1 minute to simulate OSA. Over a period of 4 hours, OSA was delivered every 6 minutes. Effective refractory period (ERP), blood pressure, intraesophageal pressure, and blood gases ($O_2$, $CO_2$, pH) were measured before and after each episode of OSA. AF duration and ERP were measured by programmed stimulation. Group 1 rabbits (n = 6) received LLVS (50% below that which slowed the sinus rate) in the first 3 hours. Group 2 rabbits (n = 5) only received OSA.

**RESULTS** Group 1 ERP began to lengthen progressively from the second hour compared to group 2. AF duration increased in the first hour for both groups but began to shorten progressively after the first hour in group 1 rabbits. Blood pH, $O_2$ or $CO_2$ level, intraesophageal pressure, and hypertensive response during OSA were not different between the 2 groups.

**CONCLUSION** LLVS is capable of suppressing ERP shortening and AF induced by OSA. LLVS may serve as a new therapeutic approach to treat OSA-induced AF.

**KEYWORDS** Obstructive sleep apnea; Atrial fibrillation; Autonomic nervous system

**ABBREVIATIONS AF** = atrial fibrillation; **ANS** = autonomic nervous system; **BP** = blood pressure; **ERP** = effective refractory period; **LLVS** = low-level vagosympathetic trunk stimulation; **OSA** = obstructive sleep apnea; **$Paco_2$** = partial pressure of carbon dioxide; **$Pao_2$** = partial pressure of oxygen



## Introduction

Sleep apnea, a severe form of sleep disordered breathing, is an important but overlooked risk factor for cardiovascular diseases and is associated with multiple morbidities.[1–4] Obstructive sleep apnea (OSA) is caused by obstruction of the upper airway, leading to marked reduction or even cessation of the airflow into the trachea, despite markedly increased efforts from the thoracic and abdominal muscles in an attempt to overcome the obstructed upper airway. Atrial fibrillation (AF) is highly associated with OSA.[5–12] OSA also has a substantially negative impact on the efficacy of AF therapy, such as cardioversion and ablation.[6,10]

Previous studies showed that low-level vagosympathetic trunk stimulation (LLVS), at voltages not slowing the sinus rate or AV conduction, inhibited AF inducibility and shortened AF duration by suppressing both the sympathetic and the parasympathetic components of the cardiac autonomic nervous system (ANS).[13,14] We sought to produce a rabbit model of OSA by clamping the endotracheal tube at end expiration every 6 minutes for 4 hours. Each apneic episode lasted for 1 minute, followed by 5 minutes of normal respiration. LLVS

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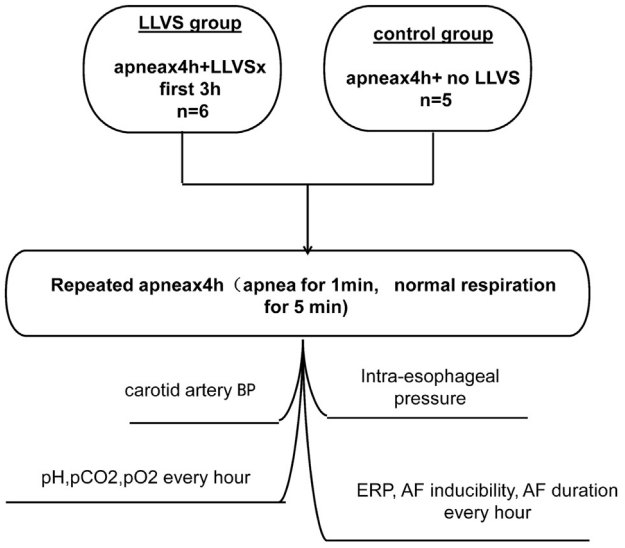


**Figure 1** Flow chart of the experimental design. AF = atrial fibrillation; BP = blood pressure; ERP = effective refractory period; LLVS = low-level vagosympathetic trunk stimulation.

was delivered at the right cervical vagosympathetic trunk in order to determine the suppression of AF.

## Methods

### Animal preparation

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center. Eleven adult male New Zealand white rabbits (weight 2.5–3.5 kg) were studied. Each animal was anesthetized with intramuscular injections of ketamine/xylazine (35 mg/5 mg/kg). Anesthesia was also maintained by intraperitoneal injection of Na-pentobarbital (25 mg) as needed. Standard surface ECG leads (I, II, III, aVR, aVL, and aVF) were continuously monitored. The left carotid artery was cannulated to continuously monitor arterial blood pressure (BP) and to obtain blood samples for blood gas and pH analyses. The right internal jugular vein was cannulated with a 4Fr electrode catheter inserted into the right atrium to record right atrial electrograms and to measure the effective refractory period (ERP). A tracheotomy was performed, followed by placement of a cuff endotracheal tube to secure a tight seal around the tube. Spontaneous breathing with room air was observed carefully to ensure the absence of airway obstruction in the baseline state. A tube connected to a pressure transducer was inserted into the esophagus to measure intra-esophageal pressure as a surrogate for intrathoracic pressure. The marginal ear vein was used for continuous saline infusion. Figure 1 illustrates the study design.

### Electrophysiologic study

All 11 rabbits received programmed stimulation at the high right atrium to determine the ERP and AF inducibility. Eight beats of S1–S1 stimuli (cycle length 220 ms) were followed by an extrastimulus (S2). The S1–S2 interval was progressively decreased by 10 ms and then 1 ms until ERP was reached at 10× the diastolic thresholds. AF was defined as irregular atrial beats >500 bpm lasting >5 seconds.

### Obstructive apnea model

OSA was induced by clamping the proximal end of the endotracheal cannula at the end expiration phase.[15,16] Apnea lasted 1 minute, and the rabbit was allowed to breathe spontaneously for 5 minutes (Figure 2). This apnea protocol repeated itself every 6 minutes for 4 hours. In the LLVS group, the right cervical vagosympathetic trunk was electrically stimulated (20 Hz, 0.1-ms square pulse width) for the first 3 hours at an intensity 50% below the voltage that slowed the sinus rate or AV conduction. LLVS was discontinued for the fourth hour to allow dissipation of its effects. In the control group, the right vagosympathetic trunk was isolated similar to the LLVS group but without the application of LLVS.

### Blood gas analysis

The arterial blood was drawn from the carotid arterial sheath through an anaerobic heparinized syringe. The pH value, partial pressure of carbon dioxide ($Paco_2$), and partial pressure of oxygen ($Pao_2$) were calculated using a VetScan i-STAT1 Analyzer (Abbott Point of Care Inc, Union City, CA). All the samples were analyzed within 5 minutes of their collection.

### Statistical analysis

All data are expressed as mean ± SD. The control and LLVS groups were compared using 2-way analysis of variance. Analysis of variance with *post hoc* Tukey test was used to compare continuous variables among different time points of apnea (Figures 3 and 4). A paired *t* test was used for comparisons of BP and blood gas values before and after apnea was induced (Figures 5 and 6). The Shapiro–Wilk test was used to formally assess the normality of the data. The null hypothesis for normality was not rejected for any of the data examined

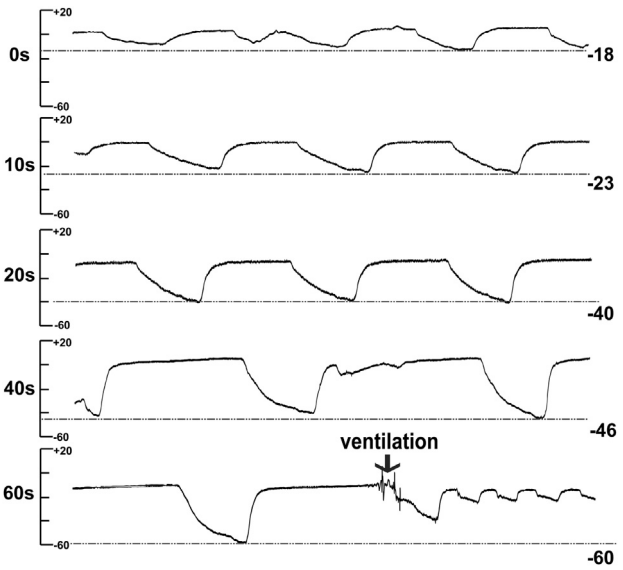


**Figure 2** Typical example of a progressive decrease of intraesophageal pressure from –7 to -60 mm Hg during a 1-minute obstructive sleep apnea episode.