Time-dependent cervical vagus nerve stimulation and frequency-dependent right atrial pacing mediates induction of atrial fibrillation

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Original Investigation

206

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ABSTRACT

Objective: This study aimed to investigate the effects of right cervical vagus trunk simulation (RVTS) and/or right atrial pacing (RAP) on the induction of atrial fibrillation (AF).

Methods: Twenty-four healthy adult dogs were randomly divided into four groups: RAP groups comprising RAP500 (RAP with 500 beats/min) and RAP1000 (RAP with 1000 beats/min) and RVTS groups comprising RVTS and RAP500+RVTS. All dogs underwent 12-h intermittent RAP and/or RVTS once every 2 h. The AF induction rate, AF duration, atrial effective refractory period (ERP), and dispersion of ERP (dERP) were compared after every 2 h of RAP or/and RVTS.

Results: All groups had successful AF induction. The RAP1000 group had the highest AF induction rate and the longest AF duration. The RAP1000 group also had a shortened ERP in comparison to the other groups as well as the maximum dERP. Compared to the RAP500 group, RAP500+RVTS had an increased capacity to induce AF as measured by the AF induction rates, AF duration, ERP, and dERP.

Conclusion: Increased tension in the vagus nerve and the intrinsic cardiac autonomic nervous system plays an important role in AF induction through different potential mechanisms. Interventions involving the vagus nerve and/or intrinsic cardiac autonomic nervous system can be a future potential therapy for AF (Anatol J Cardiol 2018; 20: 206-12)

Keywords: vagus trunk, right atrial pacing, atrial fibrillation, intrinsic cardiac autonomic nervous system

Introduction

Atrial fibrillation (AF) is a common multifaceted tachyarrhythmia causing an increased rate of morbidity, disability (1), and mortality in affected patients (1, 2). For many years, the prevailing mechanism for AF has been considered to be “multiple reentrant circuits,” which is supported by computer modeling by Moe et al. (3) and involves the surgical maze procedure. Subsequently, research in the late 1990s demonstrated that pulmonary veins (PVs) are the most common trigger site for AF (4), which resulted in PV isolation (PVI) via radiofrequency (RF) ablation to become a gold standard treatment for paroxysmal AF (5).

Clinical and nonclinical studies revealed that the autonomic nervous system (ANS) is also an imperative component in AF initiation and progression. Lemola et al. (6) demonstrated that intact PVs are not required for the maintenance of experimental vagal AF and ganglioneuron plexi ablation may suppress the vagal response and prevent AF, indicating the importance of the ANS in the pathogenesis of AF. Although the association between cardiac innervation of the ANS from the brain and AF induction was well established during the last century (7), a majority of recent studies have focused on the roles of the ANS in terms of the mechanism (8-13) and/or treatment of AF (7, 14-18). Many theories have been proposed to explain the roles of the cardiac autonomic nervous system (CANS) in arrhythmia initiation and progression such as...
The Third Fat Pad,” (8) “Integration Center,” (19, 20) “Octopus” hypothesis, (9) “Little brain,” (10) and “Autonomic remodeling” (12); however, the roles of the CANS, including its upstream regulation during AF induction, remain inconclusive.

In the present study, we tested our hypothesis that increased tension in either the vagus trunk or the intrinsic CANS plays an important role in AF induction using a canine model. Our findings shed a light on future intervention involving the extrinsic CANS and/or intrinsic CANS in AF therapy.

Methods

Ethical consideration
The procedures involving animals were reviewed, approved, and supervised by the Ethics Committee of our institute.

Animal procedures
Twenty-four dogs were randomly divided into four groups: rapid right atrial pacing (RAP) groups comprising RAP_{500} (RAP with 500 beats/min of stimulation) (n=6) and RAP_{1000} (RAP with 1000 beats/min of stimulation) (n=6) and the right cervical vagus trunk stimulation (RVTS) groups comprising RVTS (n=6) and RVTS+RAP_{500} (n=6). All animal studies were reviewed and performed in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (21). All 24 healthy adult dogs weighing 15–20 kg were anesthetized with 20 mg/kg sodium pentobarbital by intraperitoneal injection and ventilated with room air by a positive-pressure respirator (Halowell EMC, Pittsfield, MA, US); the dogs also received additional doses (50–60 mg) administered hourly to maintain an adequate level of anesthesia. The core body temperature was maintained at 36.5±1.5 °C. The his bundle electrogram was recorded from a quadripolar electrode catheter introduced via the femoral artery and positioned in the aortic root. The blood pressure and standard lead II electrocardiogram were continuously monitored.

The thoracic cavity was accessed via a two-sided thoracotomy at the fourth intercostal space (22). The base of the left superior pulmonary vein (LSPV) and left inferior pulmonary vein (LIPV) were dissected from the visceral pleura, and a multielectrode catheter was sutured to the visceral pleura in order to record or pace PVs. Similar electrode catheters were sutured to the left and right atria to record atrial electrograms and perform RAP. All recordings were displayed on a computer-based Lab System (LEAD-7000 EP CONTROL, Sichuan Jinjiang Electronic Science and Technology Co., Ltd.), and the stimulation was suspended every 2 h to measure AF induction and ERP in different sites. In 18 dogs, atrial pacing at a cycle length of 300 ms (2x-diastolic threshold) was performed at the multi-electrode catheter in the RA. Stimulation with progressively higher intensities was applied at the RVT until AF was induced; no AF was induced at 8 V in six dogs. AF was defined as irregular atrial rates faster than 500 beats/min with a duration of >5 s, associated with irregular atrio-ventricular conduction (9). Speed suppression or electrical cardioversion was used to terminate AF if the duration lasted >10 min. AF was induced five times with burst pacing in the atria or PVs to calculate the AF induction rate in the individual sites (S1-
S1 stimulation, 100 ms in cycle length, 2 ms in duration, and fourfold threshold current), which was defined as the relative ratio of the number of successful AF induction events to the total number of stimulations, expressed in percentage. The S1-S2 interval decreased from 200 ms to refractoriness initially in decrements (S1:S2=8:1, 1–40 V, 0.5 ms in duration). Moreover, dERP was calculated as the coefficient of variation [standard deviation (SD)/mean] of the ERP at all recording sites (Fig. 1) (23).

**Statistical analyses**

All data were expressed as mean±SD (24). The mean values of parameters in multiple groups were compared using two-way analysis of variance with Tukey post-hoc tests. A p value of <0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 7.0a (Mac Edition, GraphPad Software Inc, La Jolla, CA, USA).

**Results**

The RAP<sub>1000</sub> group shows increased AF induction

AF was induced with the various methods described above. The mean AF induction rate in each group at every time point was calculated (n=6). As shown in Figure 2 and Table 1, four methods used in this study were effective in inducing AF over time measured at four different anatomic sites—the LA, RA, LSPV, and LIPV. The RAP<sub>1000</sub> group had the highest AF induction rate compared with that of the RAP<sub>500</sub> (p<0.001), RVTS (p<0.0001), and RAP<sub>500</sub>+RVTS (p < 0.05) groups at various sites (Fig. 2a-2d).

Moreover, the RAP<sub>500</sub>+RVTS group had a higher induction rate compared with that of the RAP<sub>500</sub> group (p<0.05 or p<0.01 at different recording sites). These data indicate that the RAP<sub>1000</sub> group had the most effective influence on AF induction, and the RAP<sub>500</sub>+RVTS group had a relatively strong effect on AF induction, suggesting that vagus stimulation and intrinsic CANS activation likely play a synergistic role in the pathogenesis of AF.

**Table 1. AF induction rates at recording sites in groups at 12 h after the initial stimulation**

| RVTS  | RAP<sub>500</sub> | RAP<sub>500</sub>+RVTS | RAP<sub>1000</sub> | P value |
|-------|------------------|-------------------------|-------------------|---------|
| LA    | 33.33±10.33      | 83.33±8.165             | 96.67±8.165       | 100±0   | <0.0001 |
| RA    | 33.33±10.33      | 73.33±10.33             | 86.67±10.33       | 100±0   | <0.0001 |
| LIPV  | 40±0             | 86.67±10.33             | 93.33±10.33       | 100±0   | <0.0001 |
| LSPV  | 36.67±8.165      | 83.33±8.165             | 93.33±10.33       | 100±0   | <0.0001 |

LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP<sub>500</sub> - RAP with a frequency of 500 beats/min; RAP<sub>1000</sub> - RAP with a frequency of 1000 beats/min. Data are presented as mean±standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

**Table 2. AF duration (s) at recording sites in groups at 12 h after the initial stimulation**

| RVTS  | RAP<sub>500</sub> | RAP<sub>500</sub>+RVTS | RAP<sub>1000</sub> | P value |
|-------|------------------|-------------------------|-------------------|---------|
| LA    | 13.17±3.601      | 26±4.427                | 34.17±3.764       | 45.81±4.011 | <0.0001 |
| RA    | 13.67±4.179      | 27.17±4.75              | 35.83±2.317       | 44.5±1.871  | <0.0001 |
| LIPV  | 16.83±2.317      | 30.83±0.9832            | 38.67±1.751       | 48.17±0.4082 | <0.0001 |
| LSPV  | 16±3.406         | 29.83±0.7528            | 36.83±1.722       | 46.83±0.7528 | <0.0001 |

LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP<sub>500</sub> - RAP with a frequency of 500 beats/min; RAP<sub>1000</sub> - RAP with a frequency of 1000 beats/min. Data are presented as mean±standard deviation (SD). Statistical analyses were performed by two-way analysis of variance

**Figure 2.** The RAP<sub>1000</sub> group shows increased AF induction rate. AF was induced and recorded at the following positions—the left atrium (LA, Panel A), the right atrium (RA, Panel B), the left inferior pulmonary vein (LIPV, Panel C), and the left superior pulmonary vein (LSPV, Panel D). The RAP<sub>1000</sub> group showed the highest AF induction rate in comparison to other groups. Meanwhile, the RAP<sub>500</sub>+RVTS group had a higher AF induction rate compared with that of the RAP500 group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001, analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6)
The RAP group has longer AF duration at all recording sites

AF duration was also recorded in various groups at the same four anatomic sites. As illustrated in Figure 3 and Table 2, the RAP group showed a significantly longer AF duration recorded at all four sites in comparison to the RVTS, RAP_1000, and RAP_500+RVTS groups. It is worth noting that the RAP group had longer AF durations in comparison to the RAP group (p<0.05, p<0.01, or p<0.0001, according to different recording sites).

The RAP group has a shortened ERP

A shortened ERP is positively correlated with increased susceptibility of AF (25), and medications terminate AF by prolonging ERP (26); therefore, we investigated the effects of different stimulations on ERPs at various time points at the four anatomic sites. As shown in Figure 4 and Table 3, the RAP group showed the shortest ERP at all recording sites, highlighting the effects of atrial pacing and RVTS in shortening ERPs. Moreover, the RAP group had a shorter ERP compared with that of the RAP group (p<0.05 at all recording sites). We also observed

Table 3. AF ERP (ms) at recording sites in groups at 12 h after the initial stimulation

|        | RVTS       | RAP_500    | RAP_500+RVTS | RAP_1000  | P value |
|--------|------------|------------|--------------|-----------|---------|
| LA     | 128.3±2.582| 118.3±4.082| 105.8±2.041  | 88.33±7.146| <0.0001 |
| RA     | 125.8±2.041| 118.3±5.164| 106.7±2.582  | 81.33±6.022| <0.0001 |
| LIPV   | 121.7±4.082| 113.3±4.082| 103.3±5.164  | 76.67±5.317| <0.0001 |
| LSPV   | 121.7±4.082| 111.7±5.164| 95.83±8.01   | 75.33±5.317| <0.0001 |

Table 4. AF dERP at 12 h after the initial stimulation

|        | RVTS       | RAP_500    | RAP_500+RVTS | RAP_1000  | P value |
|--------|------------|------------|--------------|-----------|---------|
|        | 0.0285±0.008| 0.04±0.005 | 0.055±0.010  | 0.0735±0.004| <0.0001 |

dERP - dispersion of effective refractory period; LA - left atrium; RA - right atrium; LIPV - left inferior pulmonary vein; LSPV - left superior pulmonary vein; RVTS - right cervical vagus trunk stimulation; RAP - rapid atrial pacing; RAP_500 - RAP with a frequency of 500 beats/min; RAP_1000 - RAP with a frequency of 1000 beats/min. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed by two-way analysis of variance.
that the effects of stimuli in shortening ERP were not significant after 8 h (analyses not shown), indicating the importance of stimuli during the initial phases of AF.

The RAP_1000 group has increased dERP

Increased dERP has been reported to be well correlated with vulnerability of AF (24); therefore, we investigated the consequences of stimulation in dERP. As shown in Figure 5 and Table 4, the RAP_1000 group had the maximum dERP compared with that of the other groups, indicating the importance of rapid atrial stimulation in the pathogenesis of AF. The RAP_1000+RVTS group had a higher dERP compared with that of the RAP_500 group. It is worth noting that the peak effects in increasing dERP occurred at 8 h following the initial stimulation, and further effects were observed after the 8-h time point in all four groups (statistical analysis not shown), suggesting the significance of stimulation to the RA and RVT during the initial phases of AF.

Discussion

In the present study, we investigated the significance of stimulation to the RA and RVT in the pathogenesis of AF. Our findings indicated that (1) the RAP_1000 group had the highest AF induction and longest AF duration, (2) the rapid right atrial stimulation (the RAP_1000 group) and invigoration in the RA and RVT (RAP_500+RVTS) shortened the ERP, and (3) the RAP_1000 group had the most pronounced increase in dERP during the initial phases of AF. These data indicate a possible mechanism of RAP and/or RVTS in AF induction—RAP and/or RVTS mediates AF induction by decreasing the ERP during the entire event and increasing dERP during the initial phases.

ANS has been shown to have an important role in the initiation and maintenance of AF (17). Although previous studies have reported that sympathetic stimulation may be a trigger for AF (27), increasing evidence has indicated that the parasympathetic nervous system also has a significant role in initiating AF [reviewed in (28)]. Activation of either the extrinsic parasympa-

Figure 5. The RAP_1000 group shows the most prolonged dispersion of effective refractory period (dERP). AF induction and dERP measurement are described in the Methods section. The RAP_1000 group had the most prolonged dERP: **p<0.05; *** p<0.01; **** p<0.0001 analyzed by two-way analysis of variance with Tukey post-hoc tests (n=6).
stimulation of RAP may cause neural remodeling, AF with rapid atrial rates may require intervention of the intrinsic CANS.

**Study limitations**

The current study has certain potential pitfalls. First, we used short-term RAP to induce AF rather than chronic RAP, because the major objective of this study was to determine the effects of RVTS. Second, we did not consider the effects of sympathetic nerve stimulation. Lastly, we did not compare the current experimental system with established low-level vagosympathetic nerve stimulation and low-level transcutaneous stimulation models. Because this pilot study indicated the significance of the clinical application and future potential therapies for AF, we will conduct additional investigations based on these current results.

**Conclusion**

In conclusion, a high-tension state of the vagus trunk initiates AF by affecting the activity of the intrinsic CANS or by promoting acute electrical remodeling during short-term RAP, which is helpful in AF initiation and progression during the initial phase. In addition, the activation of the intrinsic CANS may enhance the acute electrical remodeling that depends on or cooperates with extrinsic CANS activity during RAP.

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**References**

1. Seet RC, Friedman PA, Rabinstein AA. Prolonged rhythm monitoring for the detection of occult paroxysmal atrial fibrillation in ischemic stroke of unknown cause. Circulation. 2011; 124: 477-86.
2. Jabre P, Jouven X, Adnet F, Thabut G, Bielinski SJ, Weston SA, et al. Atrial fibrillation and death after myocardial infarction: a community study. Circulation 2011; 123: 2094-100.
3. Moe GK, Rheinboldt WC, Abildskov JA. A computer model of atrial fibrillation. Am Heart J 1964; 67: 200-20.
4. Haïssaguerre M, Jais P, Shah DC, Takahashi A, Hocini M, Quiniou G, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998; 339: 659-66.
5. Cappato R, Calkins H, Chen SA, Davies W, Iesaka Y, Kalman J, et al. Updated worldwide survey on the methods, efficacy, and safety of catheter ablation for human atrial fibrillation. Circ Arrhythm Electrophysiol 2010; 3: 32-8.
6. Lemola K, Chartier D, Yeh YH, Dubuc M, Cartier R, Armour A, et al. Pulmonary vein region ablation in experimental vagal atrial fibrillation: role of pulmonary veins versus autonomic ganglia. Circulation 2008; 117: 470-7.
7. Yu M, Ting DT, Stott SL, Wittner BS, Ozsolak F, Paul S, et al. RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. Nature 2012; 487: 510-3.
8. Chiu CW, Eble JN, Zipes DP. Efferent vagal innervation of the canine atria and sinus and atrioventricular nodes. The third fat pad. Circulation 1997; 95: 2573-84.
9. Zhou J, Scherlag BJ, Edwards J, Jackman WM, Lazzara R, Po SS. Gradients of atrial refractoriness and inducibility of atrial fibrillation due to stimulation of ganglionated plexi. J Cardiovasc Electrophysiol 2007; 18: 83-90.
10. Armour JA. Potential clinical relevance of the ‘little brain’ on the mammalian heart. Exp Physiol 2008; 93: 165-76.
11. Choi EK, Shen MJ, Han S, Kim D, Huang S, Sayfo S, et al. Intrinsic cardiac nerve activity and paroxysmal atrial tachyarrhythmia in ambulatory dogs. Circulation 2010; 121: 2615-23.
12. Yu L, Scherlag BJ, Sha Y, Li S, Sharma T, Nakagawa H, et al. Interactions between atrial electrical remodeling and autonomic remodeling: how to break the vicious cycle. Heart rhythm 2012; 9: 804-9.
13. Stavrakis S, Nakagawa H, Po SS, Scherlag BJ, Lazzara R, Jackman WM. The role of the autonomic ganglia in atrial fibrillation. JACC Clin Electrophysiol 2015; 1: 1-13.
14. Scanavacca M, Pisani CF, Hachul D, Lara S, Hardy C, Darriex F, et al. Selective atrial vagal denervation guided by evoked vagal reflex to treat patients with paroxysmal atrial fibrillation. Circulation 2006; 114: 876-85.
15. Shen MJ, Shinohara T, Park HW, Frick K, Ice DS, Choi EK, et al. Continuous low-level vagus nerve stimulation reduces stellate ganglion nerve activity and paroxysmal atrial tachyarrhythmias in ambulatory canines. Circulation 2011; 123: 2204-12.
16. Sheng X, Scherlag BJ, Yu L, Li S, Ali R, Zhang Y, et al. Prevention and reversal of atrial fibrillation inducibility and autonomic remodeling by low-level vagosympathetic nerve stimulation. J Am Coll Cardiol 2011; 57: 563-71.
17. Chen PS, Chen LS, Fishbein MC, Lin SF, Nattel S. Role of the autonomic nervous system in atrial fibrillation: pathophysiology and therapy. Circ Res 2014; 114: 1500-15.
18. Huang JH, Lin YK, Hsieh MH, Chen SA, Chiu WC, Chen YJ. Modulation of Autonomic Nervous Activity in the Termination of Paroxysmal Atrial Fibrillation. Pacing Clin Electrophysiol 2017; 40: 401-8.
19. Zhou Q, Zhang L, Wang K, Xu X, Ji M, Zhang F, et al. Effect of interconnection between cervical vagus trunk, epicardial fat pad on sinus node function, and atrial fibrillation. Pacing Clin Electrophysiol 2014; 37: 356-63.
20. Hou Y, Scherlag BJ, Lin J, Zhang Y, Lu Z, Truong K, et al. Ganglionated plexi modulate extrinsic cardiac autonomic nerve input: effects on sinus rate, atrioventricular conduction, refractoriness, and inducibility of atrial fibrillation. J Am Coll Cardiol 2007; 50: 61-8.
21. In: th, editor. Guide for the Care and Use of Laboratory Animals. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC) 2011.
22. Po SS, Scherlag BJ, Yamanashi WS, Edwards J, Zhou J, Wu R, et al. Experimental model for paroxysmal atrial fibrillation arising at the
23. Lu Z, Scherlag BJ, Lin J, Niu G, Fung KM, Zhao L, et al. Atrial fibrillation begets atrial fibrillation: autonomic mechanism for atrial electrical remodeling induced by short-term rapid atrial pacing. Circ Arrhythm Electrophysiol 2008; 1: 184-92.

24. Soylu M, Demir AD, Ozdemir O, Soylu O, Topaloglu S, Kunt A, et al. Increased dispersion of refractoriness in patients with atrial fibrillation in the early postoperative period after coronary artery bypass grafting. J Cardiovasc Electrophysiol 2003; 14: 28-31.

25. Ravelli F, Allessie M. Effects of atrial dilatation on refractory period and vulnerability to atrial fibrillation in the isolated Langendorff-perfused rabbit heart. Circulation 1997; 96: 1686-95.

26. Hashimoto N, Yamashita T, Tsuruzoe N. Tertiapin, a selective IKACH blocker, terminates atrial fibrillation with selective atrial effective refractory period prolongation. Pharmacol Res 2006; 54: 136-41.

27. Coumel P. Paroxysmal atrial fibrillation: a disorder of autonomic tone? Eur Heart J 1994; 15 Suppl A: 9-16.

28. Carpenter A, Frontera A, Bond R, Duncan E, Thomas G. Vagal atrial fibrillation: What is it and should we treat it? Int J Cardiol 2015; 201: 415-21.

29. Patterson E, Po SS, Scherlag BJ, Lazzara R. Triggered firing in pulmonary veins initiated by in vitro autonomic nerve stimulation. Heart Rhythm 2005; 2: 624-31.

30. Kuyumcu MS, Ozek O, Cay S, Ozcan F, Bayraktar MF, Kara M, et al. The short-term impact of the catheter ablation on noninvasive autonomic nervous system parameters in patients with paroxysmal atrial fibrillation. Pacing Clin Electrophysiol 2017; 40: 1193-9.

31. Zimmermann M, Kalusche D. Fluctuation in autonomic tone is a major determinant of sustained atrial arrhythmias in patients with focal ectopy originating from the pulmonary veins. J Cardiovasc Electrophysiol 2001; 12: 285-91.

32. Spach MS, Heidlage JF, Dolber PC, Barr RC. Electrophysiological effects of remodeling cardiac gap junctions and cell size: experimental and model studies of normal cardiac growth. Circ Res 2000; 86: 302-11.

33. Liu L, Nattel S. Differing sympathetic and vagal effects on atrial fibrillation in dogs: role of refractoriness heterogeneity. Am J Physiol 1997; 273: H805-16.

34. Kneller J, Zou R, Vigmond EJ, Wang Z, Leon LJ, Nattel S. Cholinergic atrial fibrillation in a computer model of a two-dimensional sheet of canine atrial cells with realistic ionic properties. Circ Res 2002; 90: E73-87.

35. Stavrakis S, Humphrey MB, Scherlag BJ, Hu Y, Jackman WM, Nakagawa H, et al. Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. J Am Coll Cardiol 2015; 65: 867-75.

36. Yu L, Scherlag BJ, Li S, Sheng X, Lu Z, Nakagawa H, et al. Low-level vagosympathetic nerve stimulation inhibits atrial fibrillation inducibility: direct evidence by neural recordings from intrinsic cardiac ganglia. J Cardiovasc Electrophysiol 2011; 22: 455-63.

37. Li S, Scherlag BJ, Yu L, Sheng X, Zhang Y, Ali R, et al. Low-level vagosympathetic stimulation: a paradox and potential new modality for the treatment of focal atrial fibrillation. Circ Arrhythm Electrophysiol 2009; 2: 645-51.

38. Yuan Y, Jiang Z, Zhao Y, Tsai WC, Patel J, Chen LS, et al. Long-term intermittent high-amplitude subcutaneous nerve stimulation reduces sympathetic tone in ambulatory dogs. Heart Rhythm 2018; 15: 451-9.

39. Yu L, Scherlag BJ, Li S, Fan Y, Dyer J, Male S, et al. Low-level transcutaneous electrical stimulation of the auricular branch of the vagus nerve: a noninvasive approach to treat the initial phase of atrial fibrillation. Heart Rhythm 2013; 10: 428-35.