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Intermittent Left Cervical Vagal Nerve Stimulation Damages the Stellate Ganglia and Reduces Ventricular Rate During Sustained Atrial Fibrillation in Ambulatory Dogs

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Abstract

Background—The effects of intermittent open loop vagal nerve stimulation (VNS) on ventricular rate (VR) during atrial fibrillation (AF) remain unclear.

Objective—To test the hypothesis that VNS damages the stellate ganglion (SG) and improves VR control during persistent AF.

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Conflict of Interest: Drs Peng-Sheng Chen and Shien-Fong Lin have equity interest in Arrhythmotech, LLC. Indiana University has filed patent application related to the findings of this study.

Methods—We performed left cervical VNS in ambulatory dogs while simultaneously recording the left SG nerve activity (SGNA) and vagal nerve activity. Tyrosine hydroxylase (TH) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining were used to assess neuronal cell death in SG.

Results—We induced persistent AF by atrial pacing in 6 dogs, followed by intermittent VNS with short ON-time (14 s) and long OFF-time (66 s). The integrated SGNA (iSGNA) and VR during AF were 4.84 mV-s [95% confidence interval, CI, 3.08 to 6.60] and 142 bpm [CI, 116 to 168], respectively. VNS reduced iSGNA and VR, respectively, during AF to 3.74 mV-s [CI, 2.27 to 5.20; p=0.021] and 115 bpm [CI, 96 to 134; p=0.016] during 66-s OFF-time, and to 4.07 mV-s [CI, 2.42 to 5.72; p=0.037] and 114 bpm [CI, 83 to 146; p=0.039] during 3-min OFF-time. VNS increased the frequencies of prolonged (>3 s) pauses during AF. TH staining showed large confluent areas of damage in the left SG, characterized by pyknotic nuclei, reduced TH staining, increased percentage of TH-negative ganglion cells and positive TUNEL staining. Occasional TUNEL-positive ganglion cells were also observed in the right SG.

Conclusions—VNS damaged the SG, leading to reduced SGNA and better rate control during persistent AF.

Keywords

Vagal nerve stimulation; Autonomic nervous system; Atrial fibrillation

Introduction

It is generally accepted that electrical vagal nerve stimulation (VNS) activates the parasympathetic components of the vagal nerve to achieve therapeutic effects, such as controlling the ventricular rate (VR) during atrial fibrillation (AF).[1, 2] However, if parasympathetic activation is responsible for VR control, then the therapeutic effects of the VNS should be limited only to the time when VNS is turned on (ON-time). When VNS is turned off (OFF-time), ventricular conduction should accelerate, leading to a loss of therapeutic benefit. It is therefore necessary to place a sensing electrode in the heart, so the VNS can be delivered in response to an increased VR (closed-loop VNS).[1, 3] However, in addition to parasympathetic nerves, the cervical and thoracic vagal nerves also contain significant sympathetic components.[4, 5] Because of the direct connection between the stellate ganglion (SG) and vagal nerves,[6] stimulation of the sympathetic component in the vagal nerve may retrogradely activate the ganglion cells in the SG at high rates.[7] In the central nervous system, excessive stimulation by neurotransmitters or electrical activation may cause excitotoxicity due to intracellular calcium accumulation and cell death.[8–10] It is possible that intermittent high rate electrical stimulation during the VNS ON-time is sufficient in causing excitotoxicity in the SG, resulting in SG damage and reduced SG nerve activity (SGNA) during the VNS OFF-time. Persistent reduction of the SGNA may then result in beneficial therapeutic effects, such as better rate control during sustained AF. The purpose of the present study was to perform left cervical VNS in ambulatory dogs in sinus rhythm to test if intermittent VNS can reduce left SGNA. We then performed VNS in ambulatory dogs with persistent AF to test the hypothesis that intermittent VNS with a brief

ON-time and a long OFF-time is effective in controlling the VR by reducing the left SGNA through SG damage.

Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and the Methodist Research institute, Indianapolis, Indiana. A detailed Method section is included in an Online Supplement.

Continuous Ambulatory Autonomic Nerve Recordings

We first completed a pilot study (Group 1) using 3 dogs (See Online Supplement for Methods, Results and Schematics of research protocol in Supplemental Figure 1). We found that VNS with 1.5 mA, 14-s ON, 66-s OFF provided the most effective results of SGNA and VR reduction during VNS OFF-time (Online Supplement Figure 2 and Table 1). Based on those results, we designed an experiment that included 6 mongrel dogs (Figure 1). All dogs had a Cyberonics Demipulse neurostimulator (Cyberonics Inc, Houston, TX) implanted to the left cervical vagal nerve. A Data Sciences International (DSI; St Paul, MN) radiotransmitter D70EEE was implanted to record nerve activity from the left SG (LSG), the left thoracic vagal nerve, and the left ventricle. VNS (14-s ON-time, 66-s OFF-time, 10 Hz, 0.5 ms pulse width) was used in the study.[11] Starting in week 4, high rate atrial pacing was performed to induce sustained AF. After sustained AF was documented, we continued to rapidly pace the atria between Monday and Wednesday to help maintain sustained AF. We performed DSI recordings only during the weekends when there was minimal traffic in the large animal research center. The rapid atrial pacing was restarted on Mondays while the VNS output was adjusted. At the end of protocol, bilateral SG, cervical and thoracic vagal nerves were harvested for histological analysis. The data were analyzed with a custom written software which selected the R waves and calculated the RR intervals automatically. All selections were then confirmed by manual examination. Integrated nerve activities and VR were determined for 2 min at the beginning, 20-min past and 40-min past the hour from each hour between 6 AM and 1 PM. RR-intervals were averaged over 1-min windows for a 24 hour period to construct the RR-intervals distribution curve.

Statistical Analysis

Data were expressed as mean and 95% confidence interval (CI). Statistical comparison of variables during baseline, AF and VNS was analyzed using paired *t*-test. Analyses of variance with Bonferroni post hoc test were used to compare the results of immunostaining of the LSG. Paired *t*-test for pairwise comparison was performed to compare the RR-intervals between different stages of experiments. Correlation coefficient between percent TUNEL-positive non-ganglion cells and ganglion cells were calculated accounting for the correlation of data from the same dog. Bootstrap method was used calculate the CI of the correlation coefficient. The statistics were computed using the PASW Statistics (version 18; SPSS Inc, Chicago, IL) and SAS 9.2 (SAS Inc, Cary, NC). A two-sided $P \leq 0.05$ was considered as statistically significant.

Results

Effects of VNS on SGNA and VR During AF

Figure 2 illustrates nerve activities and VR of a typical Group 2 dog at baseline sinus rhythm (A), baseline AF before VNS (B), VNS 1.5 mA 14-s ON 66-s OFF (C) and VNS 1.5 mA 14-s ON 3-min OFF (D). Note that the reduction of SGNA after 14-s of VNS resulted in reduced VR during the VNS OFF-time. For all dogs studied, the integrated SGNA (iSGNA) and VR significantly increased during AF when compared with baseline. Both VNS 66-s OFF-time and 3-min OFF-time significantly reduced iSGNA and VR when compared with during AF. When compared with AF, the VR during VNS withdrawal were significantly reduced while iSGNA were comparable. There were no statistically significant differences of iSGNA among 66-s OFF, 3-min OFF and VNS withdrawal. The VR during baseline AF and week 13 of the study were 142 bpm [CI 116 to 168] and 106 bpm [CI 86 to 126; p=0.007], respectively. The iSGNA were 4.84 mV-s [CI 3.08 to 6.60] and 2.40 mV-s [CI 1.52 to 3.28; p=0.019], respectively. The integrated vagal nerve activity (iVNA) was not significantly different in each time points. Table 1 summarizes the results.

We analyzed all RR-intervals over a 24-hr period (a weekend day with least amount of artifacts during that experimental period) and plotted the average distribution all 6 dogs studied (Figure 3). The RR-intervals were 0.77 s [CI, 0.60 to 0.93] during baseline sinus rhythm, 0.46 s [CI, 0.34 to 0.58] during AF, 0.53 s [CI, 0.40 to 0.64] during VNS 14-s ON/66-s OFF, 0.59 s [CI, 0.42 to 0.76] during VNS 14-s ON/3-min OFF and 0.64 s [CI, 0.50 to 0.77] during VNS withdrawal. As shown in the figure, the curve shifted to the left (red line) and the base of the curve narrowed during sustained AF. VNS 14-s ON/66-s OFF (blue line), VNS 14-s ON/3-min OFF (purple line) and VNS withdrawal (pink line) gradually moved the curves back towards the baseline sinus rhythm (black line). Significant differences were found between red and black curves (−0.30 s, [CI −0.53 to −0.08, p=0.0178]) and between blue and black curves (−0.24, [CI −0.44 to −0.05, p=0.0252]). However, there were no significant differences of RR-intervals between purple and black curves (−0.17 s, [CI −0.41−0.06, p=0.1176]) or between pink and black curves (−0.13, [CI −0.33 to 0.08, p=0.1673]). These data indicate that VNS progressively lengthened the RR-intervals during persistent AF. These changes were consistent in all dogs studied. These effects were not reversible by 2 weeks of VNS withdrawal.

Prolonged Pauses During AF

Prolonged (>3 s) pauses (asterisks, Figure 4A) were observed with increased frequency during VNS as compared with baseline AF. The average number of prolonged pauses over a 24-hr period was 1 [CI, 0 to 2] during baseline sinus rhythm, 17 [CI, 6 to 29; p=0.035 vs. baseline] during sustained AF, 63 [CI, 7 to 118] during VNS 14-s ON/66-s OFF, 142 [CI, −14 to 296] during VNS 14-s ON/3-min OFF, and 213 [CI, −95 to 520] during VNS withdrawal. Typically there was a SGNA surge and tachycardia followed by acute SGNA withdrawal and long pause (Figures 4A and 4B). For all dogs studied, the iSGNA during the pause (0.84 mV-s [CI, 0.65 to 1.03]) was much lower than not during the pause (7.35 mV-s [CI, 4.80 to 9.90; p=0.001]). In addition, there was a significant circadian distribution of the long pauses with most of the episodes occurring in the early morning (Supplemental Figure

3). The iVNA during the period before pauses was 1.93 mV-s [CI 0.39 to 3.46], while during the pause it was 2.01 mV-s [CI 0.15 to 3.36; p=0.311]. These data do not suggest a surge of vagal tone as the cause of pauses.

Effect of VNS on Left Ventricular (LV) Function

Supplemental Table 2 shows echocardiographic parameters of LV function and the blood NT-proBNP levels. AF dogs demonstrated both systolic dysfunction and LV dilatation as indicated by reduction of LV ejection fraction (LVEF) and fractional shortening (FS) and increased LV end systolic diameter (LVESD) and LV end diastolic diameter (LVEDD). The NT-proBNP levels increased significantly during AF. VNS did not significantly change the NT-proBNP levels or the LV function. Two-week VNS withdrawal did not significantly change the LVEF, either.

Left Cervical VNS Damages the LSG

LSG from 7 dogs (1 Group 1 and 6 Group 2) were available for analyses. The remaining 2 were not successfully harvested due to technical difficulties associated with extensive scar formation. In 6 of the 7 dogs, large confluent areas of damage were visible under low power view (Figures 5A and 5B). The damaged areas accounted for 40% [CI, 33% to 47%] of the LSG. Within the damaged region, 32% [CI, 14% to 49%] of the ganglion cells stained negative for tyrosine hydroxylase (TH). In a 7th dog, the damaged cells were distributed at multiple regions. The overall percentage of the TH-negative cells in all 7 specimens was 18% [CI, 11% to 25%]. Red arrows in Figure 5A mark the boundary between damaged region (DAM) and normal region (NL). The damaged region has reduced intensity of TH staining (Figure 5A) and increased fibrosis (blue in Figure 5B). Figure 5C shows the high power view of the TH staining at the junction between damaged and normal regions. Note that there were a large number of TH-negative cells (black arrows) in Figure 5C, more in the damaged than in the normal regions. Most of the ganglion cells in the damaged region appeared small, had pyknotic nuclei and stained negatively or weakly for TH. Larger, round, normal ganglion cells are found in the normal region. Figure 5D shows increased fibrosis (blue) in the damaged region. In addition to the present study, we retrospectively identified tissue blocks of LSG from a previous study in which VNS was performed without LSG recordings or rapid atrial pacing.[12] Large confluent damaged regions were found in those LSGs. The damaged region occupied 57% [CI, 36% to 78%] of the LSG and the percentage of TH-negative ganglion cells was 17% [CI, 11% to 23%] in the damaged region.

Absence of Damages in SG from Dogs Without VNS

Figures 6A shows LSG from a normal dog. Figure 6B shows LSG from a dog with AF and LSG recordings, but no VNS.[13] In spite of direct LSG recordings, the latter group of dogs did not have large confluent SG damages. No large and confluent damages were found in RSG of the present study (Figure 6C). Figure 6D shows the LSG from a Group 2 dog of the present study, showing a large damaged region (DAM). The percent TH-negative ganglion cells in all groups was shown in Figure 6E. Only LSG in 2 VNS groups had percent of TH-negative cells of >16%. Statistically significant differences of the percentage of TH-negative ganglion cells were found between the damaged region of the LSG in the present study and non-VNS groups.

The slides from the same LSG shown in Figure 5A were then double stained for TH and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). As shown in confocal immunofluorescent images in Figure 7, the ganglion cells in normal region mostly stained positive for TH (Red) and none stained for TUNEL (Green). TH-negative ganglion cells (arrows) were also TUNEL-negative. In contrast, cells in the damaged regions stained negative for TH, confirming results in Figure 5, but positive for TUNEL. Note that multiple ganglion cells and small endothelial or Schwann cells in the damaged region stained positive for TUNEL. All 7 VNS dogs had TUNEL-positive ganglion cells, but no TUNEL-positive ganglion cells were found in 5 normal control LSG. We measured TUNEL-positive ganglion and non-ganglion cells of all 12 LSGs using images taken with high power (20X) objectives. There were 5 images analyzed per dog except for 1 normal dog that had 4 images due to small sample size. In VNS group (N=7), 19.50% [CI 9.78 to 29.22] of ganglion cells and 16.18% [CI 4.02 to 28.35] of non-ganglion cells were TUNEL-positive. The correlation coefficient between % TUNEL-positive ganglion cells and non-ganglion cells was 0.48 [CI, 0.16 to 0.64]. In contrast, there were no TUNEL-positive ganglion cells and rare TUNEL-positive non-ganglion cells (0.39%, [CI 0.10 to 0.68]) in the normal LSG (N=5).

We performed TUNEL staining in 8 RSG. In spite of the absence of apparent RSG damage by TH staining, rare TUNEL-positive cells were found in 4 of 8 RSGs. The number of TUNEL-positive cells was low, averaging 1±3 cells (range 0 to 13 cells) per image. The TUNEL-positive cells accounted for 2.16±0.05% (range 0–17%) of the ganglion cells. Percent TUNEL-positive cells in RSG was significantly less than that in the LSG ($p=0.0007$).

Sections of cervical and thoracic vagal nerves were examined in 9 dogs. The cervical vagal nerves have less TH-staining than the thoracic vagal nerves (Online supplement Figure 4A). In addition, TUNEL-positive cells were found in 7 of 9 cervical vagal nerves and 8 of 9 thoracic vagal nerves. Among them, 3 cervical and 3 thoracic vagal nerves had TUNEL-positive stains in both neuronal and non-neuronal cells in the same section (Online supplement Figure 4B).

Discussion

A major finding of the present study is that open-loop VNS with short (14-s) ON-time and long (66-s or 3-min) OFF-time significantly reduced SGNA and VR during the VNS OFF-time in dogs with sustained AF. We also found that VNS caused significant SG damage. These findings suggest that open-loop left cervical VNS may be effective in control VR during chronic AF by damaging the SG.

Histological Changes Induced by VNS

The LSG showed large and confluent regions of damage. The cell death was confirmed by TUNEL staining. It is possible that these changes were secondary to either sustained AF or prolonged irritation caused by recording electrodes. Therefore, we retrospectively stained the LSG of dogs that underwent prolonged rapid atrial pacing and SGNA recording.[13] No damage was seen in those LSGs. We also stained LSG from dogs with continuous left cervical VNS but no SGNA recordings.[12] Because no thoracotomy was performed in the

latter study, the damages found in the LSG of the latter study could not be due to prolonged contact with the recording electrodes. These findings further support the conclusion that VNS may cause LSG damage.

Possible Mechanisms for LSG Damage

Vagal nerves have significant sympathetic components.[4, 5] In dogs, these sympathetic nerve fibers were distributed mostly in the periphery of the vagal nerve, close to the VNS electrodes. Previous studies showed that VNS may have antiadrenergic effects in acute studies[14] and suppress SGNA during chronic experiments.[15] Because of the direct connection between LSG and vagal nerves,[6] stimulation of the sympathetic component in the vagal nerve may retrogradely activate the ganglion cells in the LSG at high rates[7] and abruptly terminate SGNA.[12] However, chronic intermittent high rate excitation my cause neuronal cell death (excitotoxicity)[9] due to intracellular calcium accumulation.[10] Excitotoxicity can also be demonstrated *in vitro*, where 12–14 hours of electrical stimulation can cause cell death accompanied by increased percentage of TUNEL-positive cells.[16] The LSG changes in the present study, including the pyknotic and dense nuclei and positive TUNEL staining are consistent with excitotoxicity. There are some TUNEL-positive non-ganglion cells in normal LSG, consistent with physiological cell death. The percentage of TUNEL-positive cells in the LSG of VNS dogs greatly exceeded that in the LSG of normal dogs, suggesting that most of the TUNEL-positivity in the VNS group was not only due to physiological cell death. While excitotoxicity is a possible mechanism of SG damage caused by VNS, we did not measure the glutamate or other neurotransmitters in the excised ganglion. Therefore, whether or not excitotoxicity underlies the mechanism of cell death in the SG remains to be determined by future studies.

Rate Control of Chronic AF

We found that VNS reduced the average VR in dogs with persistent AF. Profound bradycardia and AV block have also been reported in patients with refractory epilepsy receiving VNS therapy.[17, 18] While VNS did not completely normalize the LVEF, the mean LVEF was maintained at 50% or higher throughout the study. In comparison, dogs with 3 months of AF and intact atrioventricular node were expected to have much lower LVEF (around 30%).[19] These data suggest that VNS might have prevented the progression of tachycardiomyopathy. However, we also found that there was an increased incidence of prolonged pauses during VNS. Because the SGNA during the pause was lower than that without pause, the prolonged RR-interval might have occurred as a consequence of SGNA suppression. If VNS was used in patients with chronic AF, symptomatic bradycardia should be considered as one of the anticipated side effects of VNS.

VNS for Other Types of Heart Diseases

VNS is known to reduce T wave alternans and improve sympathovagal balance in patients with drug-refractory partial-onset seizures.[20] Chronic VNS can prevent ventricular fibrillation and sudden cardiac death in conscious dogs with a healed myocardial infarction.[21] Left cardiac sympathetic denervation (LCSD) is beneficial in managing patients with congenital or acquired cardiac arrhythmias.[22–24] These studies indicate that

LSG is an important arrhythmogenic structure and that LSG ablation may be effective in arrhythmia control. Our present study shows that intermittent left VNS can remodel the LSG and reduce sympathetic outflow. These effects may be useful in managing cardiac arrhythmia.

Limitations

Thoracoscopic sympathectomy is used to treat severe palmar hyperhidrosis, but the beneficial effects were offset by the postoperative complications such as compensatory sweating and neurologic complications.[25] Such observations suggest a reversible process that might impact the clinical benefit of using VNS for AF rate control. We did not measure the effective refractory periods or AF inducibility before and after VNS protocols. We also did not measure the VNS threshold needed to reduce the sinus rate during the study. Therefore, it is unclear if the atrial electrophysiological parameters or VNS threshold changed during the study. We did not perform right VNS to test if that will also help control the VR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AF	atrial fibrillation
CI	confidence interval
DAM	damaged region
EF	ejection fraction
FS	fractional shortening
HF	heart failure
iSGNA	integrated stellate ganglion nerve activity
iVNA	integrated vagal nerve activity
IVS	interventricular septum thickness

LCSD	left cardiac sympathetic denervation
LSG	left stellate ganglion
LV	left ventricular
LVEDD	left ventricular end diastolic diameter
LVESD	left ventricular end systolic diameter
LVPW	left ventricular posterior wall thickness
NL	normal region
RSG	right stellate ganglion
SGNA	stellate ganglion nerve activity
TH	tyrosine hydroxylase
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
VNA	vagal nerve activity
VNS	vagal nerve stimulation

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Clinical Perspectives

We discovered that the chronic left cervical vagal nerve stimulation (VNS) in ambulatory dogs caused large and confluent damages of the left stellate ganglia and smaller amount of damages in the right stellate ganglia. These changes were associated with reduced stellate ganglion nerve activity and better rate control during persistent AF. The mechanisms by which VNS causes stellate ganglia damage are unclear. However, because cervical vagal nerves had significant sympathetic components, it is possible that VNS had caused rapid retrograde activation of the stellate ganglia, excitotoxicity and cell death. These findings suggest that VNS might be helpful in rate control of chronic atrial fibrillation. Our data also suggest that VNS might have additional protective effects against tachycardiomyopathy as the left ventricular function did not deteriorate during chronic untreated AF. However, the latter hypothesis has not been directly tested in this study. VNS is commonly used in humans to suppress drug refractory epilepsy. Since the device is already approved for clinical use, translating these findings to arrhythmia care can be done with prospective clinical trials.

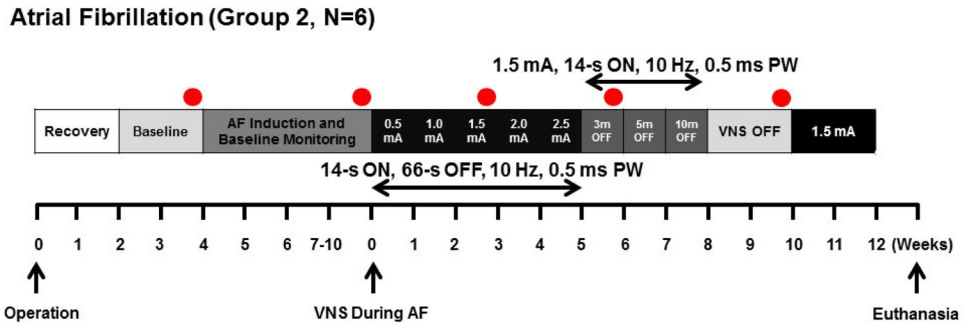

Figure 1.

Schematic of the study protocol (Group 2), persistent AF with VNS (n=6). After baseline recording, high rate atrial pacing was used to induce persistent AF between the times marked by the first two red dots. Once induced, persistent AF was maintained by rapid atrial pacing from Monday to Wednesday each week. The VNS output was upward adjusted each Monday until 2.5 mA was reached. Echocardiography and blood collection were performed at the times indicated by red dots. PW, pulse width.

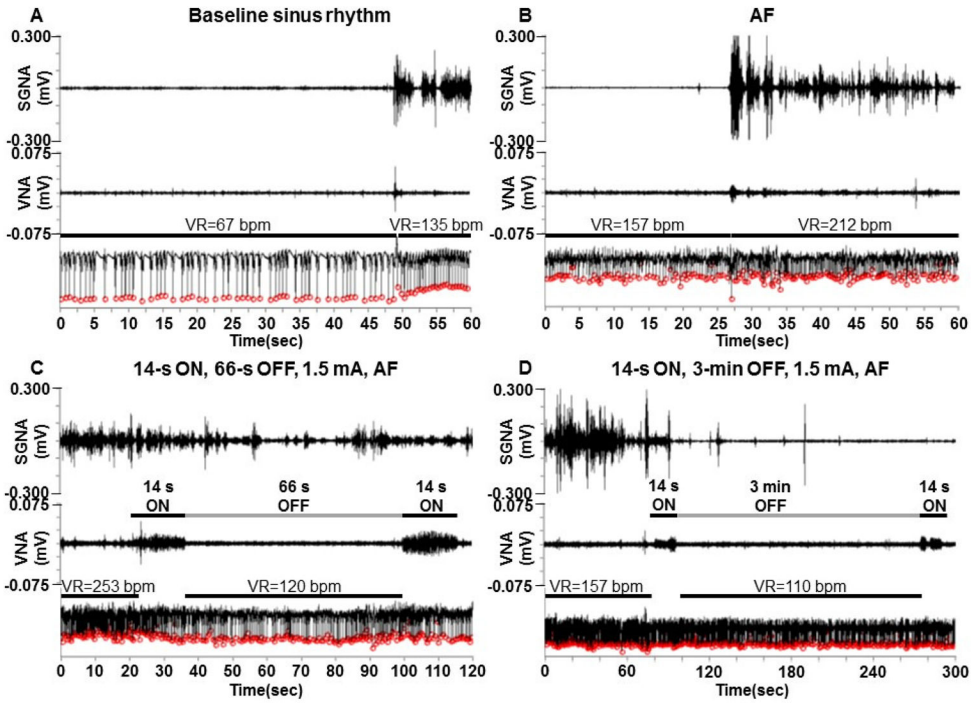

Figure 2.

RR intervals and nerve activities. A shows abrupt onset of SGNA caused sinus tachycardia at baseline. B shows VR acceleration during persistent AF. C and D were examples obtained during VNS with 66-s OFF-time and 3-min OFF-time, respectively, when output was adjusted to 1.5 mA. There was increased vagal nerve activity during 14-s VNS. The VNS was followed by reduced SGNA and VR during the OFF-time. AF, atrial fibrillation; SGNA, stellate ganglion nerve activity; VNA, vagal nerve activity; VR, ventricular rate; bpm, beats per minute; mV, millivolt.

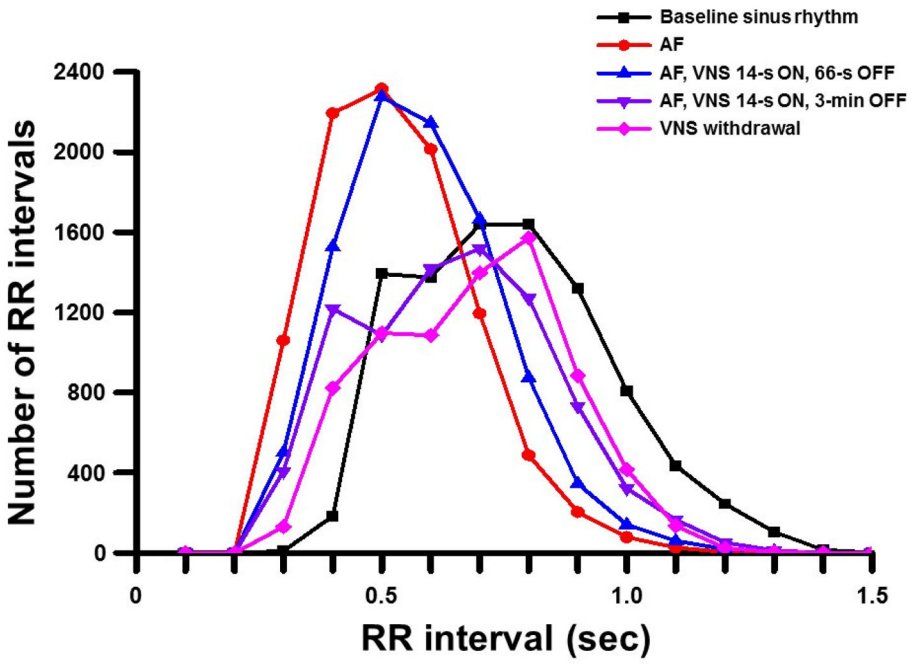

Figure 3.

RR-interval distribution of all 6 dogs studied. During baseline sinus rhythm (black line), the RR-intervals were widely distributed. However, during AF (red line), the peak increased and the range reduced, indicating overall shortened RR-interval and reduced ranges of the RR-interval. VNS (blue and purple lines) and VNS withdrawal (pink) shifted the curves to the right, similar to that observed in baseline sinus rhythm.

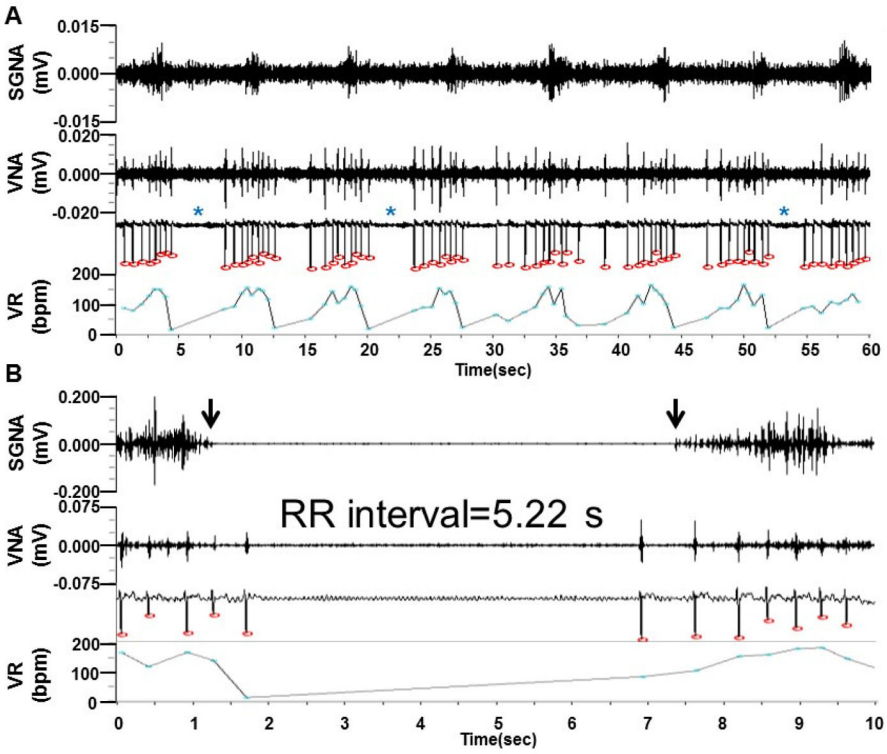

Figure 4.

Long pauses induced by VNS in AF. A shows intermittent SGNA associated with VR acceleration during AF. When SGNA was quiescent, the RR-interval lengthened. Three episodes of long pause (>3 s) were observed (asterisks). B. The longest pause (5.22 s) found in this study. Note that there were no SGNA between the arrows, suggesting that reduced SGNA is a cause of the pause. SGNA, stellate ganglion nerve activity; VNA, vagal nerve activity; VR, ventricular rate; mV, millivolt.

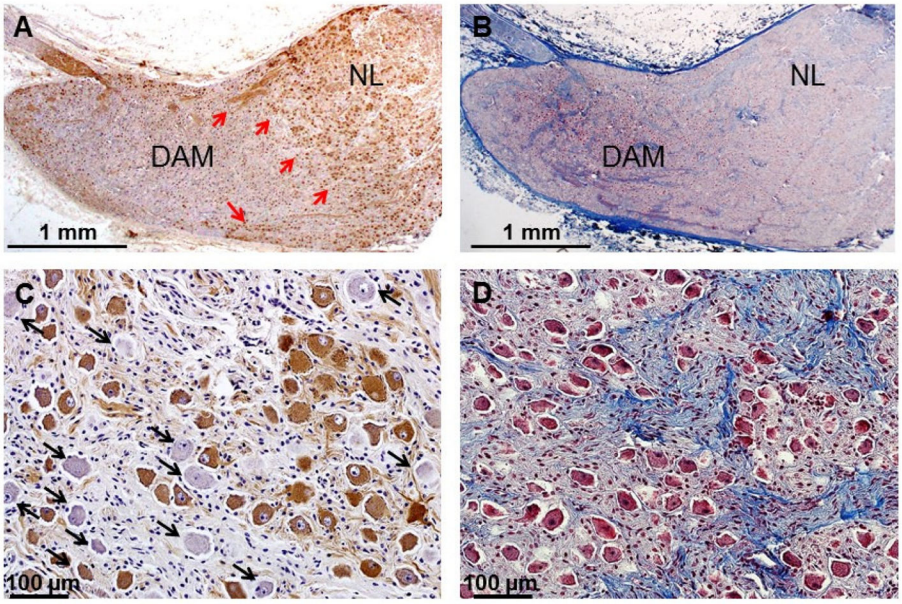

Figure 5.

Histology of the LSG in a dog from Protocol 2. A. Tyrosine hydroxylase (TH) immunostaining of LSG at low magnification shows the presence of both a damaged region (DAM) and normal region (NL) in the same LSG. B shows Masson's trichrome staining of the same LSG. The damaged area has increased fibrosis (blue). C shows a high magnification view of the TH staining at the junction between normal and damaged regions. Arrows point to ganglion cells that did not stain for tyrosine hydroxylase. Those cells were more frequently observed in damaged region than in normal region. D showed a high powered view of the damaged region with increased fibrosis (blue). A and B were taken with 4X objective lens, with total magnification of 40X. The horizontal bars are 1.0 mm in length. C and D were taken with 20X objective lens, with total magnification of 200X. The horizontal bars are 0.1 mm in length.

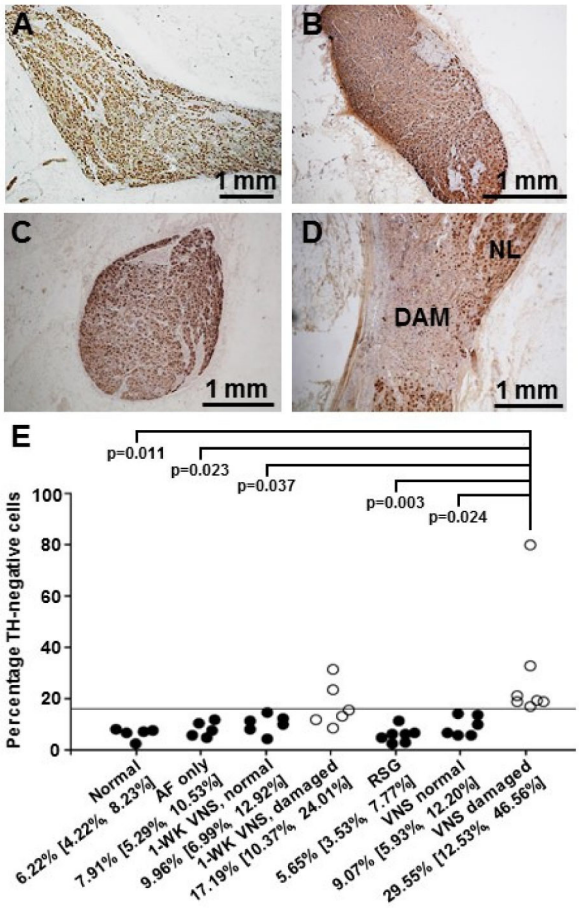

Figure 6.

Tyrosine hydroxylase (TH) staining of the SG in different groups of dogs. The LSG of a normal control dog (A), a dog with pacing-induced AF (B) from a previously published study[13] and the RSG from the present study (C) stained homogenously. There was no area of large and confluent damage. D shows the LSG from a Group 2 dog. There was evidence of nuclear pyknosis in ganglion cells and an increased number of TH-negative cells within the damaged area (DAM), whereas TH-negative cells in the normal area (NL) were comparable to the other five groups. E shows the percentage of TH-negative cells in each group of the SG. All fields with >16% TH-negative cells came from the damaged regions in the LSG of the two VNS groups.

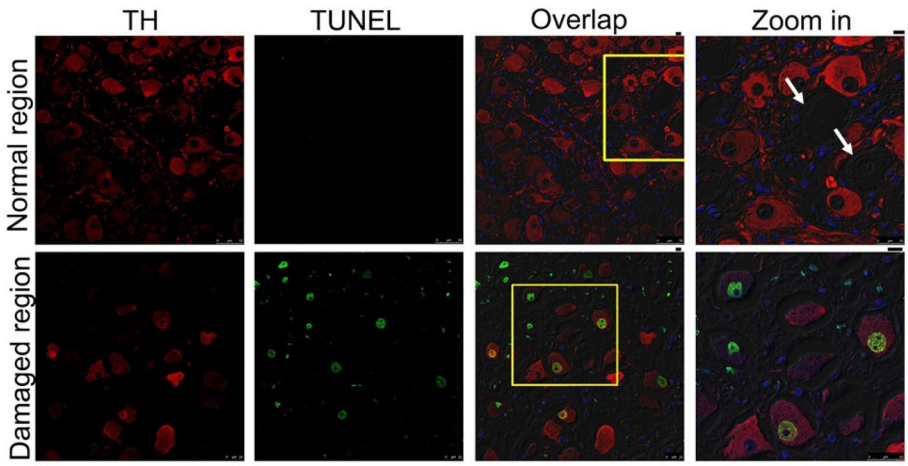

Figure 7.

Confocal images of TH and TUNEL double staining of a VNS LSG (same one as shown in Figure 5A). Green shows positive TUNEL stain, red indicates the positive TH stain and blue is the DAPI stain of the nuclei. The ganglion cells of normal region stained positive for TH and negative for TUNEL, while that of the damaged region stained negative for TH and positive for TUNEL. Right panels show high power view of the yellow boxed regions. Bar represents 10 μm. Arrows point to TH-negative ganglion cells in the normal region.

Table 1

Nerve activities and VR

Parameters	Baseline	AF	VNS 66-s OFF	VNS 3-min OFF	VNS withdrawal
iSGNA (mV-s)	2.89±1.17	4.84±2.19*	3.74±1.83†	4.07±2.06†	4.09±1.73
iVNA (mV-s)	1.10±0.54	1.52±1.21	1.15±0.72	1.54±1.00	1.58±1.17
VR (bpm)	86±20	142±33*	115±24†	114±39†	115±23†

Data were expressed as mean±standard deviation. Data were analyzed with paired t-test. iSGNA, integrated stellate nerve activity; iVNA, integrated vagal nerve activity; VR, ventricular rate; bpm, beats per minute.

*p<0.05 vs. Baseline,

†p<0.05 vs. AF.