Bilateral atrial ganglionated plexus involvement in atrial responses to left-sided plexus stimulation in canines

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Aims
Given the clinical interest concerning ‘reflex vagal’ responses to identify left atrial (LA) targets for ablative therapy of atrial fibrillation, we investigated whether vagal and bilateral atrial neural pathways may be involved in chronotropic and atrial repolarization responses to LA ganglionated plexus (GP) stimulation.

Methods and results
Unipolar electrograms were recorded from 191 right atrial (RA) and LA sites in anaesthetized canines prior to and during electrical stimulation of the right vagus nerve (VgN), left VgN, or LAGP at baseline and following (i) bilateral VgN decentralization, and radiofrequency ablation of (ii) periaortic/superior vena cava (Ao/SVC) and (iii) RAGP in 14 animals (anterograde group), and in the reverse order in 7 (retrograde). Repolarization changes were also measured in similar preparations during Ao/SVC (n = 8) and RAGP stimulation (n = 23). Sinus cycle length (SCL) prolongation, and RA and LA repolarization changes (affected atrial surface area) were induced during LAGP stimulation. SCL prolongation and RA repolarization changes were unaffected by VgN decentralization but reduced following Ao/SVC and RAGP ablation in the anterograde group. In the retrograde group, chronotropic and RA repolarization changes were reduced following RAGP and abolished following Ao/SVC ablation. In contrast, LA repolarization responses to LAGP stimulation were reduced following VgN decentralization and each subsequent ablation step, with small residual responses after completing the anterograde protocol. Ao/SVC and RAGP stimulation exerted predominant influences in adjacent regions as well as demonstrating LA extensions.

Conclusion
Vagal as well as bilateral atrial neural pathways are involved in mediating chronotropic and LA repolarization responses to LAGP stimulation.

Keywords
Atrial fibrillation • Intrinsic cardiac nervous system • Left atrial ganglionated plexus • Radiofrequency ablation • Neurocardiology • Autonomic nervous system • Atrial repolarization • Atrial mapping

1. Introduction
The intrinsic cardiac nervous system has been described as a network of interconnected neuronal aggregates termed ganglionated plexuses1,2 exerting widespread, redundant neural influences on the atria and ventricles.3 The right atrial ganglonated plexus (RAGP) and the one located at the junction between the inferior vena cava and inferior left atrium (IVC-LA) exert predominant parasympathetic regulation of sinus node and AV node functions, respectively.4–8 The GP existing at the base of the aortic root and superior vena cava (Ao/SVC) has been implicated as a significant autonomic neural pathway in the canine heart2,10, in fact, this GP has been described as a ‘headstation’ receiving most of the vagal inputs extending to the atria, sinus node, and AV node.10 With regard to left-sided atrial GPs, little information is available concerning the corridors responsible for mediating their parasympathetic influences on the atria.8,11 Yet, such information is of paramount importance since radiofrequency (RF) catheter ablation of left-sided nerves and GPs has been proposed as adjunct therapy to pulmonary vein ablation in the treatment of paroxysmal atrial fibrillation.12–14 In several such reports,12,13,15,17 chronotropic and dromotropic ‘vagal responses’ elicited by high frequency stimulation applied to left-sided atrial loci were employed to identify putative neural targets for ablation.

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We have recently reported that, in anaesthetized canines, regional increases in the spatial heterogeneity of atrial repolarization properties identified by multi-electrode mapping are associated with the sites of origin of tachyarrhythmias induced by stimulating mediastinal nerve inputs to the intrinsic cardiac nervous system. Moreover, we have suggested that localizing such neurally induced repolarization changes might be useful to identify left atrial (LA) targets for ablative treatment of atrial tachyarrhythmias.

Using an experimental approach consisting of neural ablation and direct electrical activation, we investigated in anaesthetized canines the proposition that vagal and juxtag cardiac neural pathways may be involved in modulating LAGP-induced atrial rate and repolarization changes. Accordingly, we first investigated whether such responses to LAGP stimulation would be obfuscating following a stepwise protocol consisting of (i) bilateral vagus nerve (Vgn) decentralization, (ii) Ao/SVC GP ablation, and (iii) RAGP ablation. Secondly, we investigated whether LA repolarization changes occur in response to direct electrical stimulation of the Ao/SVC and RAGP as well as in response to LAGP stimulation.

2. Methods

2.1 Experimental procedures and procedures

Experiments were conducted in accordance with the guidelines of the Canadian Council for Animal Care (conforming to European Parliament Directive 2010/63/EU) and approved by the animal care Committee of the Sacré-Coeur Hospital Research Center. Adult mongrel dogs (25–35 kg, either sex) were anaesthetized with Na thiopental (25 mg/kg iv) followed by hourly α-chloralose (25 mg/kg iv). Depth of anaesthesia was monitored using a standard programmable stimulator (15 Hz, 0.5–1 mA, 1 ms).

At the end of the experiment, euthanasia was performed by iv injection of a saturated KCl solution under deep anaesthesia. Mechanical ventilation was maintained (Harvard Instruments, Millis, MA, USA) and blood O2 saturation was monitored by lingual pulse-oximetry (VetOx G2 Digital, Dolphin Medical, Hawthorne, CA, USA). Left femoral arterial and venous catheterization were done for blood pressure monitoring and fluid administration. Bilateral anterior thoracotomy was performed and a pericardial cradle consisting of (i) bilateral vagus nerve (Vgn) decentralization, (ii) Ao/SVC GP ablation, and (iii) RAGP ablation. In addition to electrical stimulation of the Vgns and LAGP (above), multiple atrial recordings were obtained from similar canine preparations studied in the intact state during electrical stimulation of the RAGP (23 animals), IVC-ILA (6 animals), and Ao/SVC (8 animals) GPs.

2.2 Signal processing: repolarization changes

Unipolar electrograms were amplified (0.05–450 Hz bandwidth) and converted to digital format at 1000 samples/s/channel. Spatial distribution of neural effects on repolarization (precisely: increased local heterogeneity of atrial repolarization) was assessed using isointegral mapping. The net area (integral) subtended by each unipolar electrogram with reference to isoelectric segments was measured at each electrode site in sinus rhythm recordings made just prior to (basal) and during neural stimulation. By algebraic subtraction of the integral value for the basal beat from the integral value measured during neural stimulation, area, difference maps were plotted indicating the atrial regions that were affected by neural stimulation and are not affected by concomitant atrial rate changes, i.e. not significantly different between recordings made during SCL prolongation and recordings made while keeping a constant rate by atrial pacing. As illustrated in Figure 1. Such atrial patterns of repolarization changes are caused by heterogeneous neural influences on atrial muscle and are not affected by concomitant atrial rate changes, i.e. not significantly different between recordings made during SCL prolongation and recordings made while keeping a constant rate by atrial pacing. Regional atrial repolarization maps can be presented as either selected examples in individual experiments (Figure 1) or cumulative incidence maps summarizing data from several animals (Figure 3). In the latter, the incidence of regional repolarization changes in response to neural stimulation was assessed by tallying, for each recording site, the number of preparations that presented changes ≥30 mV ms (corresponding to 2× SD in repeated unipolar recording measurements made under basal conditions). The atrial surface area (mm²) that was affected in response to neural stimulation was estimated from cumulative incidence maps.

2.3 Data analysis

Data are presented as mean ± SD. SCL prolongation and affected atrial surface areas (repolarization changes) induced in response to Vgn or LAGP stimulation were analysed by ANOVA for repeated measures
following subsequent ablation steps, after which comparisons were made by paired \( t \)-test analysis. Threshold for statistical significance was set at 0.05.

3. Results

3.1 Chronotropic responses

3.1.1 Vagus nerve stimulation

At baseline (intact nerves and GPs), right and left VgN stimulation resulted in significant sinus cycle length (CL) prolongations in all canines with CL increments of 1041 ± 1322 and 552 ± 451 ms, respectively (\( n = 21 \)), which were unaffected following bilateral VgN decentralization. Following Ao/SVC plexus ablation, the CL responses to right and left VgN stimulation were attenuated to CL prolongations of 503 ± 447 and 189 ± 161 ms, respectively. After RAGP ablation, CL prolongations in response to right and left VgN stimulation were markedly attenuated to 72 ± 79 and 46 ± 74 ms, respectively (\( P < 0.02 \)). In the retrograde group, the chronotropic responses to right and left VgN stimulation were maximally attenuated immediately after RAGP ablation to 111 ± 88 and 138 ± 155 ms, respectively (\( P < 0.05 \)). Thus, VgN effects on sinus CL appeared to be primarily mediated via the RAGP.

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**Figure 1** Mapping the spatial distribution of repolarization changes in response to nerve stimulation. Upper tracings. Lead II ECG recordings during the course of the experimental protocol. Pre AV-block: sinus rhythm prior to any intervention. Post AV-block: in the absence of ventricular pacing, atrial (A) and escape ventricular (V) complexes were dissociated. Lower tracing: atrial cycle length prolongation during right vagus nerve (VgN) stimulation performed in the absence of ventricular pacing. Lower left hand panel: multielectrode mapping grid. Right (RA) and left atrial (LA) surfaces are shown unfolded and viewed from a posterior projection. Epicardial plaque electrodes were positioned on (1) Bachmann’s bundle (BB, blue), (2) RA free wall (yellow) and appendage (RAA, green), (3) lateral LA wall and posterior wall between pulmonary veins (PV) (orange), and (4) LA appendage (LAA, pink). SVC, IVC, CSO indicate superior, inferior vena cava, and coronary sinus ostia. Lower right-hand panel: spatial distribution of atrial repolarization changes (mV ms) in response to right VgN stimulation. At each of the 191 electrode sites, unipolar wave form changes between basal state (pre-stimulation) and neural stimulation were calculated as the difference in the electrogram integrals between the two conditions (differences of 10 mV ms between isolines). In this example, marked neural effects (dark blue) were identified in the RA free wall, superior LA and BB. Selected unipolar recordings during basal state (dark tracing) and neural stimulation (red tracing) illustrate increased repolarization changes in the RA free wall (blue), whereas recordings from LA and RAA (red to green) remained unchanged. Atrial surface encompassing marked positive changes lies within the gold line.
RAGP ablation, CL prolongation was still induced in 5 of 19 animals of the anterograde and retrograde groups, it is noteworthy that, after Ao/SVC plexus ablation. Combining RAGP ablation, physiologically significant CL prolongations were reduced to only three of the seven animals and chronotropic responses, whereas Ao/SVC and RAGP ablation produced stepwise reductions in the number of responding animals, suggesting that although the LAGP influences on the RAFW occur predominantly via the RAGP, connections to the RAFW can occur independently from the RAGP in some animals.

3.2 Repolarization changes

3.2.1 Vagus nerve stimulation

Repolarization changes were induced throughout both atria in response to right or left VgN stimulation (Figure 3A and B). The total biatrial surface areas thus affected (right VgN: 2081 ± 539, left: 2022 ± 292 mm²) were significantly modified during the anterograde ablation protocol, with stepwise reductions of the responses to right VgN stimulation following Ao/SVC and RAGP ablation (Figure 4A: left hand diagram) and reduction of the response to left VgN stimulation following RAGP ablation (middle diagram), suggesting the involvement of juxtacardiac neural pathways via the GPs in mediating right and left VgN influences on atrial muscle.

3.2.2 LAGP stimulation

In the intact state, no repolarization change was identified by epicardial mapping in response to LAGP stimulation in 3 of 14 animals, and 2 other animals did not contribute repolarization change data because of failure to induce stable atrioventricular block. In the remaining nine animals (the anterograde group), repolarization changes were induced in the superior left atrium, Bachmann’s bundle and superior RA wall (Figure 3C). The total bilateral atrial surface area that displayed repolarization changes in response to LAGP stimulation (1706 ± 747 mm²) was significantly modified during the anterograde ablation protocol, with stepwise reductions following VgN decentralization, Ao/SVC, and RAGP ablation (Figure 4A, right-hand diagram), indicating that (in contrast to chronotropic responses) vagal afferents as well as juxtacardiac GP connections contributed to such changes.

When RA regional changes were considered, it was found that the RA surface areas that were affected by LAGP stimulation were significantly reduced following RAGP ablation (Figure 4B, RAFW). Such reduction occurred in the majority of the preparations (illustrated in Figure 5, upper panel: animal d11, and cumulative maps shown in Figure 6, upper diagrams); however, the lower panel of Figure 5 is from one of the two animals (d10) in which RA surface area displaying repolarization changes persisted throughout the entire ablation protocol, again suggesting that although the LAGP influences on the RAFW occur predominantly via the RAGP, connections to the RAFW can occur independently from the RAGP in some animals.

In the left atrium, repolarization responses to LAGP stimulation were identified at all stages of the anterograde ablation sequence, displaying reductions after vagal nerve decentralization and Ao/SVC plexus ablation but not following RAGP ablation (Figure 4B: left atrium excluding Bachmann’s bundle, P = 0.055; see also Figure 6: upper diagrams). Likewise, stepwise reductions occurred in the Bachmann bundle responses following VgN decentralization and Ao/SVC ablation (Figure 4B: Bachmann’s bundle, P < 0.05; and Figure 6).

In the retrograde ablation protocol, the RA free wall area displaying repolarization changes in response to LAGP stimulation was significantly reduced after RAGP ablation (from 272 ± 118 mm² in the intact state to 109 ± 138 mm², P < 0.01) and completely abolished following Ao/SVC plexus ablation (6 ± 10 mm²). Likewise, the area of repolarization changes identified in Bachmann’s bundle was reduced in a stepwise fashion (intact: 252 ± 269 mm², RAGP ablation: 145 ± 153 mm²; Ao/SVC ablation: 43 ± 48 mm²). In contrast, the LA areas of repolarization changes remained unchanged throughout the retrograde ablation protocol (intact: 654 ± 317 mm², RAGP ablation: 638 ± 322 mm²).
Ao/SVC ablation: 481 ± 261 mm²). Combining the three regions, step-wise reductions were identified from 1564 ± 589 mm² in the intact state to 1026 ± 138 mm² following RAGP ablation (P = 0.09) and to 543 ± 266 mm² following Ao/SVC plexus ablation (P < 0.002).

Altogether, the data show that juxtacardiac neural pathways occurring via the Ao/SVC and RAGPs are involved in mediating bilateral atrial repolarization responses to LAGP stimulation.

3.2.3 Direct stimulation of the Ao/SVC, IVC-ILA, and RAGP in the intact state

When the Ao/SVC plexus was stimulated directly, repolarization changes occurred in all animals in the superior dorsal atrial wall in the Ao/SVC region (Figure 3D, blue colour code), extending in ≥50% of the animals (green colour code) to Bachmann’s bundle and to the superior right and LA walls. In response to IVC-ILA plexus stimulation (Figure 3E), repolarization changes were identified in ~50% of the animals in the inferior left and superior right and LA walls (green colour code). In response to RAGP stimulation (Figure 3F), marked changes were induced in all animals in the RA wall (including the sinus node and subsidiary pacemaker region); changes were also identified in ~50% of the animals in localized regions of the superior LA wall and Bachmann’s bundle. The data confirm the Ao/SVC’s and RAGP’s predominant influences in adjacent regions in all animals as well as demonstrating LA extensions in ≥50% of the experiments.

4. Discussion

4.1 Major findings

First, chronotropic and RA repolarization responses to LAGP stimulation were essentially unaffected following bilateral VgN decentralization, suggesting that connections with the central nervous system (presumably vagal afferents) were not involved in generating such RA responses in this model and that they may have been mediated via intra- and/or juxta-cardiac nerve connections involving the Ao/SVC and RAGP. Accordingly, Butler et al. 24 reported that chronotropic responses to RAGP stimulation persisted in the majority of animals following bilateral VgN and stellate ganglion decentralization.

Secondly, mapping of repolarization changes indicates that intrinsic cardiac neural connections exist from LAGP to the right atrium (including sinus node and subsidiary pacemaker complex) and that they occurred via either (i) the Ao/SVC plexus (Figure 6, lower diagrams: connection 1) since repolarization changes were abolished in 5/12 animals.
following Ao/SVC ablation in the anterograde protocol, or (ii) via alternative nerve connections to RAGP (connection 2) since changes were abolished in five additional animals following RAGP ablation. Data also suggest the existence of independent connections from LAGP to sinus node (connection 3) since chronotropic responses persisted following RAGP ablation in the two remaining animals. Moreover, the retrograde ablation protocol provided evidence for projections from Ao/SVC plexus to sinus node (connection 4) because of the three responsive animals following RAGP ablation.

Thirdly, in contrast to chronotropic and RA repolarization responses, LA and Bachmann’s bundle repolarization responses to LAGP stimulation were clearly affected (albeit not completely abolished) following bilateral VgN decentralization (Figure 6, lower diagrams: connection 5). LA repolarization changes were further affected following Ao/SVC (connection 6) and RAGP plexus ablation (connection 7), supporting the notion that RAGP (as well as each of the other atrial GPs) may exert widespread (bilateral atrial) rather than localized influences.3,8 Accordingly, changes frequently occurred in the superior left atrium and in Bachmann’s bundle during direct Ao/SVC and RAGP stimulation (Figure 3). Unsurprisingly, however, the Ao/SVC’s and RAGP’s influences were most consistently identified in the immediately adjacent atrial tissues.

### Table 1 Atrial cycle length changes in response to LAGP stimulation (ms)

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Intact</th>
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<th>Ao/SVC ablation</th>
<th>RAGP ablation</th>
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<td>(A) Anterograde ablation protocol (n = 12)</td>
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<td>d10</td>
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<td>Incidence</td>
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<td>12/12</td>
<td>7/12</td>
<td>2/12</td>
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</table>

Data (cycle length) analysed by ANOVA for repeated measures: A, P = 0.23. Ao/SVC, LAGP, RAGP, periaortic/superior vena cava, left atrial, right atrial ganglionated plexus. Incidence of cycle length changes ≥ 13 ms.

### Table 2 Atrial cycle length changes in response to LAGP stimulation (ms)

<table>
<thead>
<tr>
<th>Animal ID</th>
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<th>RAGP ablation</th>
<th>Ao/SVC ablation</th>
<th>Decentralization</th>
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<td>(B) Retrograde ablation protocol (n = 7)</td>
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<td>3</td>
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<td>–1</td>
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<td>0 ± 2*</td>
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</table>

Data (cycle length) analysed by ANOVA for repeated measures: B, P < 0.001. Ao/SVC, LAGP, RAGP, periaortic/superior vena cava, left atrial, right atrial ganglionated plexus. Incidence of cycle length changes ≥ 13 ms.

*P < 0.05 vs. preceding stimulation by paired t-test.
Figure 5 Repolarization changes in response to LAGP stimulation in selected cases illustrating abolition (A) or persistence (B) of residual right atrial changes at completion of the anterograde ablation protocol. Mapping grid and colour-coded representation of repolarization changes (mV ms) are as in Figure 1: slight to marked neural effects—light to dark blue, no change—red to green. White arrows indicate the presumed location of the sinus node area, as reported from baseline sinus rhythm activation mapping (data not shown). (A) Animal d11 exhibiting stepwise reductions of neurally induced changes until total suppression of right atrial changes following RAGP ablation (right-hand map: disappearance of dark blue shading). (B) Animal d10, exhibiting persistence of left and right atrial repolarization changes (persisting dark blue).

Figure 6 Cumulative incidence maps illustrating modifications of the atrial distribution of repolarization changes in response to LAGP stimulation during the anterograde ablation protocol (upper diagrams) in relation to putative connections to the central nervous system (CNS) and among ganglionated plexuses (lower diagrams). Upper: following each ablation step, number of animals displaying repolarization changes (maximum of nine animals: dark blue) decreased at specific sites. Note persisting effects in the Bachmann bundle (BB) region in two animals after VgN decentralization. The two animals with persistent effects after RAGP ablation are indicated by a pale yellow mark in the RA free wall. Ao/SVC, RAGP: periaortic/superior vena cava and right atrial ganglionated plexuses. Lower: putative connection pathways between LAGP and sinus node (SN) as well as RA and LA muscle on the basis of disappearance of repolarization changes following stepwise ablations. Each connection is illustrated by a given arrow type (importance proportional to thickness) referenced by circled numbers. See Section 4.1 for explanation.
In contrast, after completion of the ablation protocol, spatially limited LA changes persisted in a small number of animals in response to LAGP stimulation (Figure 6: connection 8). This pointed to a paucity of local LA projections from the LAGP per se and to a need for cooperation among several GPs to exert full-fledged physiological influences on the left atrium.

4.2 Intrinsic cardiac nerve connections

Such findings are in agreement with previous reports that the main neural pathways between the left vagosympathetic trunk and the sinus node transit via the LAGP and RAGP sequentially before proceeding to the sinus node, suggesting that the RAGP may serve as an integration centre for bilateral parasympathetic influences on the sinus node. However, our previously reported data suggest that chronotropic effects can be elicited, albeit less consistently, in response to pharmacological activation of neurons in GPs other than the RAGP. Moreover, residual chronotropic responses to VgN stimulation persist following RAGP ablation or local hexamethonium injection. Accordingly, Hou et al. and Mick et al. also recognized the existence of connections from the vagosympathetic nerves and LAGP to the sinus node that bypass the RAGP. Herein, we expanded on such previous findings by showing the possible involvement of the Ao/SVC plexus in neural projections from LAGP to sinus node. The present study represents, to the best of our knowledge, the first description as it relates to a possible role of the Ao/SVC plexus in mediating chronotropic neural influences arising from the LAGP, thus filling a gap concerning the role of the peri-aortic/SVC GP as previously pointed out by Hou et al. and Mick et al.

Potential confounding factors inherent to this model include the following: thioptental-α-chloralose anaesthesia, an open-chest state, and a fall in arterial blood pressure during the brief period of asystole that occurred while interrupting support ventricular pacing in the presence of AV block to record electrograms without contamination of atrial repolarization by ventricular complexes. However, such factors were shared between all conditions under which repolarization changes were compared.

4.3 Clinical implications

In several studies investigating atrial denervation as adjunct therapy to pulmonary vein ablation for treatment of atrial fibrillation, neurally active target sites were identified as the ones from which ‘vagal reflex responses’ could be induced in response to high frequency stimulation. Our first major finding suggests that such chronotropic (and, by extension, dromotropic) responses may in effect be mediated via intracardiac and juxtacardiac (mediastinal) nerve connections rather than vagal afferents.

We have previously reported that (i) regional atrial repolarization changes may be induced in response to left-sided juxtacardiac nerve stimulation without inducing concomitant chronotropic responses and that (ii) such repolarization changes were associated with the sites of origin of neurally induced atrial tachyarrhythmias. Herein, stepwise reduction in the LA areas of LAGP-induced repolarization changes during the anterograde protocol emphasizes the participation of central and intracardiac connections in forming a neural arrhythmia substrate. Owing to the redundancy and complexity of the cardiac neuronal hierarchy, focal ablation of individual left-sided nerves or GPs may not produce the desired effect on the neural substrate of vagal paroxysmal atrial fibrillation. Inasmuch as our findings may be relevant to the clinical situation, it may be necessary to consider a more global approach to cardiac denervation that may include targeting RA GPs in addition to left-sided ones. In patients with vagal paroxysmal atrial fibrillation, ablation of right-sided GPs induced a depression of heart rate variability (denervation) that lasted for 6 months while recovering by 12 months (suggesting reinervation), with ~95% protection against atrial fibrillation recurrence at 6 months but still over 80% protection at 12 months. However, we do recognize that the experiments were performed in healthy canines and that, moreover, the preparations were not subjected to a pulmonary vein isolation procedure, which might per se affect neurally mediated responses.

Conclusion

Right-sided GPs and their intrinsic cardiac and juxtacardiac neural connections may be involved in mediating left-sided as well as right-sided atrial repolarization changes in response to LA GP stimulation.

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Conflict of interest: none declared.

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