Characterization of the Left Atrial Neural Network and its Impact on Autonomic Modification Procedures

Louisa C. Malcolme-Lawes, BSc, MRCP; Phang Boon Lim, PhD, MRCP; Ian Wright, BSc; Pipin Kojodjojo, PhD, MRCP; Michael Koa-Wing, MRCP, PhD; Shahnaz Jamil-Coley, MRCP; Hakim-Moulay Debbi, BSc, MSc; Darrel P. Francis, MD, FRCP; D. Wyn Davies, MD, FRCP, FHRS; Nicholas S. Peters, MD, FRCP, FHRS; Prapa Kanagaratnam, PhD, MRCP

Background—Left atrial (LA) ganglionated plexi (GP) are part of the intrinsic cardiac autonomic nervous system and implicated in the pathogenesis of atrial fibrillation. High frequency stimulation is used to identify GP sites in humans. The effect of ablation on neural pathways connecting GPs in humans is unknown.

Methods and Results—Thirty patients undergoing LA ablation with autonomic modification were recruited. In patients with persistent atrial fibrillation, endocardial continuous high frequency stimulation identified GP sites producing AV block. After right lower GP ablation (N=5), 2 of 15 sites remained positive, whereas after ablation of other GPs (N=5), leaving right lower GP intact, all 19 sites remained positive (right lower GP versus other GP, P<0.005), indicating that neural pathways between LAGPs and the AV node are via the right lower GP. In 20 patients with paroxysmal atrial fibrillation, synchronized high frequency stimulation identified sites initiating pulmonary vein (PV) ectopy. After PV isolation (N=8), no sites remained positive. After local GP ablation (N=9), 3 of 14 sites remained positive, suggesting neural connections to the PV were disrupted by both PV isolation and GP ablation. Heart rate variability indices reduced significantly after right upper GP ablation alone, suggesting that neural pathways from the LA to the SA node travel via the right upper GP.

Conclusions—We have demonstrated neural pathways connecting LA GPs with the PVs, AV node, and SA node. The effects of high frequency stimulation at GP sites can be prevented by ablating the GP site or the neural pathway. This further delineates the mechanism via which PV isolation prevents atrial fibrillation and highlights important caveats for autonomic modification end points. (Circ Arrhythm Electrophysiol. 2013;6:632-640.)

Key Words: ablation ■ atrial fibrillation ■ autonomic nervous system ■ ganglionated plexi ■ pulmonary vein isolation

A nimal and human studies have demonstrated that autonomic stimulation can trigger atrial fibrillation (AF) and promote atrial substrate changes that allow sustained periods of AF.1,2 In canine studies, epicardial ganglionated plexi (GP) ablation was able to abolish both AF induction and maintenance completely.3 Although clinical AF ablation has focused on pulmonary vein isolation (PVI), there is a high PV electric reconnection rate even in patients who remain symptom-free.4 This raises the possibility that ablation near the PVs may reduce ectopic triggering. Inadvertent modification of GP sites has been proposed as an explanation for both this and the superior outcomes from circumferential PV ablation compared with ostial segmental PVI alone.5

Editorial see p 458
Clinical Perspective on p 640

This has led to studies attempting to target GP sites specifically. The GPs are part of an epicardial neural network that comprises multiple ganglia with interconnecting neurons and axons, including afferent sensory fibers and sympathetic and parasympathetic efferents.6,7 Two techniques have been used to locate GP sites. Endocardial continuous high frequency stimulation (cont-HFS) at 10 V, 20 Hz for several seconds identifies GP sites that induce a vagal response of bradycardia or AV nodal block.8,9 This technique invariably leads to AF induction after high rates of local atrial capture. An alternative method, synchronized HFS (sync-HFS) involves current delivery during the local atrial refractory period avoiding AF induction and identifies GP sites that trigger ectopy (both PV and non-PV). Approximately 50% of GP sites that produce ectopic triggering by sync-HFS do not produce an AV nodal response by cont-HFS; however, 90% of sites with an AV nodal response will trigger ectopics.1 Canine studies suggest that the network has a common pathway into the AV node via the right lower (RL) GP (RLGP) and ablation here abolishes the AV nodal effects from other left atrial (LA) GP sites.7 Similarly, a common input to the SA node via the right upper (RU) GP (RUGP) exists in dogs, whereby ablation at the RUGP...
significantly attenuates sinus rate slowing in response to GP stimulation. These studies illustrate the complexities of the neural pathways that are still not well understood. Therefore, it may not be surprising that endocardial ablation of GP sites identified by the AV nodal response to cont-HFS has had mixed results for preventing AF in humans. Autonomic modification for AF may be more effective if ectopy-producing sites with connections to the PVs can be identified and targeted. We hypothesized that the response to GP stimulation could be abolished by ablation at the stimulation site or by transection of the neural pathway. We assessed the effect of ablation at each GP site (in particular the RLGP) on the AV nodal response to cont-HFS from nonablated GP sites. The neural inputs to the PVs were studied by assessing the ectopic response to sync-HFS after either GP ablation or PVI. Assessment of neural inputs to the SA node was performed using heart rate variability (HRV) as a measure of autonomic modulation of SA nodal activity.

Methods
Thirty patients undergoing clinically indicated AF ablation procedures were recruited and allocated to a study protocol, according to the flow chart in Figure 1A. Patients with persistent AF were allocated to the cont-HFS study, and patients with paroxysmal AF were allocated to the sync-HFS study. Within each group, patients were allocated at random to the different arms of the study. All antiarrhythmic agents were stopped 5 half lives before the procedure. All patients were in a fasted state. Conscious sedation (morphine and midazolam) or general anesthetic was used according to operator preference. Invasive arterial pressure monitoring was used in all patients. After transseptal puncture, heparin was administered to maintain an ACT (activated clotting time) >300. A 3-dimensional (3D) LA map was created using an electromagnetic navigation system (CARTO, Biosense Webster Inc). HFS was performed before ablation. The study was approved by St Mary’s Research Ethics Committee, and all patients signed a written informed consent.

Assessment of Neural Pathways From the Left Atrium to the AV Node
Ten patients with persistent AF were recruited. After completing the 3D LA map, the mapping catheter (3.5-mm irrigated tip) was placed at presumed anatomic GP location, with the duodecapolar catheter placed in adjacent PV. Cont-HFS was delivered endocardially using a Grass Stimulator (20 Hz, 10 ms pulse duration, 10 V). A positive response was defined as >50% prolongation in R wave to R wave (RR) interval compared with mean RR averaged over 10 beats before HFS (ie, RR interval ratio, longest post-HFS: mean pre-HFS>2). Positive HFS sites were marked on the 3D map (Figure 1B). Patients were allocated to 2 groups; the RLGP group and other GP group. The RLGP group had a cluster of radio frequency (RF) lesions placed over positive HFS sites at the RLGP followed by retesting of all previously positive unablated sites. The other GP group had a cluster of RF lesions placed over positive sites at the RUGP, LUGP, or LLGP followed by retesting of all previously positive unablated sites.

Figure 1. A, Flow chart demonstrating the different patient groups, the numbers of patients in each group, the type of high frequency stimulation (HFS) testing, and the intervention performed in each study arm. B, Electroanatomic map demonstrating location of sites that were tested using HFS. Design lines which were hand-drawn (purple lines) onto the anatomy to identify locations expected to be rich in ganglionated plexi (GP) sites based on previous publications. Not all locations within these areas had atrio-ventricular nodal effects or ectopy after HFS. Positive sites were tagged with an orange circle and negative with a purple circle. After locating the positive GP sites, ablation lesions were placed in clusters around the positive sites before retesting. AF indicates atrial fibrillation; LL, left lower; LU, left upper; PVI, pulmonary vein isolation; RLGP, right lower ganglionated plexi; and RU, right upper.
Assessment of Neural Pathways From Left Atrium to the Pulmonary Veins

A separate group of 20 patients with paroxysmal AF, attending in sinus rhythm, were recruited. Patients were allocated to control, PVI, or GP ablation group. Catheters were placed in the PV, coronary sinus, and high right atrium. PV connection was confirmed with a duodecapolar catheter. The map catheter was placed at the presumed anatomic GP location. Short bursts of HFS (12 V, 50 Hz, 10 ms pulse width), synchronized to the local atrial refractory period, were delivered through the map catheter. A positive site initiated PV ectopy (earliest activation seen within the PV) and was recorded distinctly from negative sites on the LA geometry.

Control Group
Reproducibility of PV ectopy initiation by sync-HFS was tested in 3 patients. Positive sites were identified and marked on the 3D geometry. After catheter relocation guided by the 3D map, the sites were retested with sync-HFS.

PVI Group
Sync-HFS was performed, and all sites producing PV ectopy were marked on the 3D map (Figure 2). PVI was performed using the Arctic Front Cryoballoon. During RF PVI procedures, there is a significant interoperator variation in the determination of the optimal sites for ablation. By using cryoballoon technology, we think we can reduce interoperator variation because the balloon can be only placed at 1 location. In brief, the 28-mm Arctic Front Balloon was inflated at the ostium of each PV in turn. Vein occlusion was confirmed with contrast injection, and two 5-minute freezes, aiming for temperature of −40°C, were applied to each vein. RF or Freezor Max was applied to any remaining PV sleeves. PV was confirmed using a duodecapolar circular catheter. Sync-HFS was repeated at marked positive sites after PVI.

GP Ablation Group
After completing the 3D map, sync-HFS was performed from the mapping catheter placed at presumed GP sites. Each positive site was marked on the electroanatomic map, followed by application of 4 to 5 minutes RF (25–30 W, irrigated at 17 mL/min, temperature limited to 50°C) in a cluster surrounding the GP site. If any further ectopy was induced, another 2 to 3 minutes of ablation was performed at this site followed by repeated testing. This process was repeated for all 4 PVs. The patient subsequently underwent PVI using the operator’s method of choice, and no further HFS testing was performed.

Assessment of Neural Pathways From Left Atrium to the Sinoatrial Node
In patients recruited for sync-HFS, multiple 40-second ECG recordings were taken at several time points during the procedure and exported for offline MatLab analysis to assess low frequency (LF) and high frequency (HF) parameters of HRV.12 This method has been previously validated by our group.13

PVI Group
Five 40-second, ectopy-free, ECG segments were exported at the following 5 time points: baseline, after sequential cryoballoon ablation of the left upper (LUPV), left lower (LLPV), RUPV, and finally the RLPV. PVs were ablated in the same order in all patients.

GP Ablation Group
Five 40-second, ectopy-free, ECG segments were exported before and after the ablation of each positive GP site. Patients remaining in AF after GP site stimulation were excluded from HRV analysis.

Statistics
All normal variables were expressed as mean and SD. Paired non-parametric data were compared using Wilcoxon signed-rank test. Paired, categorical variables were compared using McNemar test. Comparison of patient demographics across ≥2 groups was with χ2 test for categorical variables and Kruskal–Wallis test for continuous variables. Fisher exact test was used for small patient numbers. A significant result was defined as P<0.05.

A 2-level random intercept model was used to assess the difference in post- versus pre-RR interval ratio between the RLGP ablation group and the other GP group. Normality of within and between-patient residuals was assessed using normal Quantile–Quantile plots. Mutual independence between within and between-patient residuals was assessed using a scatter plot.

For the sync-HFS study, the measure used to compare the 3 groups, from a descriptive point of view only, was the difference in the number of sites initiating PV ectopy post- versus preablation. LF and HF measures of HRV underwent log transformation and were compared between time points using paired t tests.
Results

Left Atrium to AV Node

Ten patients were recruited for the cont-HFS study (Table 1). Twenty-eight of 40 GP sites tested were positive for AV nodal response. After ablation, 1 of 14 ablated GP sites remained positive during repeat HFS testing. This confirms the AV nodal response to cont-HFS can be abolished by ablation of the target GP site.

In the other GP group, all 19 unablated GP sites remained positive for AV nodal response after ablation of the target GP site. In the RLGP group, only 2 of 15 unablated sites remained positive after ablation of the RLGP. There was a statistically significant decrease in the delta RR interval ratio (postablation—preablation) after ablation in the RLGP group compared with the other group (P<0.005; Figure 3). These results demonstrate that ablation of the RLGP alone abolishes the AV nodal response to HFS produced by all LA GP sites, whereas ablation at other GP sites does not affect the AV nodal response of unablated GP sites.

The residuals of the mixed model are distributed according to the hypotheses: normal distributions of errors and independence of errors between the 2 levels, as shown in Appendix I in the online-only Data Supplement.

Left Atrium to PV

Twenty patients were recruited, and patient characteristics are detailed in Table 2. Patients were assigned 3 in the control group, 8 in the PVI group, and 9 in the GP ablation group. Forty-one of 123 sites tested were positive for PV ectopy.

Control Group

Eight of 17 sites tested were positive for PV ectopic response and recorded on the electroanatomic map. The catheter was moved away and back to the site of stimulation marked on the 3D map. All 8 sites remained positive for PV ectopy on retesting (Figure 4A).

PVI Group

Seventeen of 52 tested sites were positive for PV ectopy. Cryo-balloon ablation of all 4 PVs was subsequently performed. GP sites were not specifically targeted or avoided during ablation, and no ablation in addition to PVI was performed. The positive GP sites were restudied after PVI. Local atrial capture at all sites confirmed that the GP site had not been inadvertently ablated during PVI. Sync-HFS was repeated at the 17 marked positive sites, and none produced ectopy (Figure 4B).

GP Ablation Group

Sixteen of 54 tested GP sites initiated PV ectopy. In 2 patients, HFS produced AF that did not terminate; therefore, no retesting was possible. In the remaining patients, RF ablation was performed in a cluster around the positive site, and then the site was retested. Three of 14 sites were positive on retesting (Figure 4C). After additional RF ablation around the GP site, no further ectopy could be initiated on retesting. In 1 case, ablation at the LUGP site resulted in LUPV isolation; however, in all other cases, the local PV remained connected after the cluster ablation of the GP.

Left Atrium to SA Node

In 15 patients presenting in sinus rhythm recruited to assess the neural pathways from the LA to PVs (control group and patients remaining in AF after HFS were excluded), LF and HF HRV parameters were calculated at each procedural stage.

PVI Group

LF HRV parameters at the start of the procedure and after cryo-balloon ablation of each PV are shown in Figure 5, with a reduction in LF seen only after cryo-balloon ablation of the RUPV (P=0.03). Similarly, HF HRV parameters reduce only after RUPV ablation (P=0.02; Figure 5).

GP Ablation Group

LF parameters at the start of the procedure and after ablation of each identified GP site are shown in Figure 6. A reduction in LF HRV is seen only with ablation at the RUGP (P=0.02). Similarly, HF HRV parameters were also seen to reduce only after RUGP ablation (P=0.01; Figure 6).

Discussion

In this study, we investigated the feasibility of GP site identification and ablation using cont-HFS. We confirmed the presence of a LA neural network with a common entry to the AV.

Table 1: Patient Demographics for Continuous HFS Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Group Median (Range)</th>
<th>RLGP Group Median</th>
<th>Other GP Group Median</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58 (54–68)</td>
<td>56</td>
<td>62</td>
<td>0.371</td>
</tr>
<tr>
<td>Women/men</td>
<td>2/8</td>
<td>2/3</td>
<td>0/5</td>
<td>0.114</td>
</tr>
<tr>
<td>AF duration, y</td>
<td>3 (2–3.5)</td>
<td>3</td>
<td>3</td>
<td>0.491</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1 (1)</td>
<td>1</td>
<td>2</td>
<td>0.472</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>55 (50–60)</td>
<td>55</td>
<td>52</td>
<td>0.712</td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>44 (33–50)</td>
<td>42</td>
<td>46</td>
<td>0.140</td>
</tr>
<tr>
<td>CAD, %</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>0.292</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>70</td>
<td>100</td>
<td>40</td>
<td>0.167</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>TIA/CVA, %</td>
<td>20</td>
<td>0</td>
<td>40</td>
<td>0.444</td>
</tr>
<tr>
<td>General anesthetic, %</td>
<td>70</td>
<td>60</td>
<td>80</td>
<td>0.524</td>
</tr>
</tbody>
</table>

AAD indicates antiarrhythmic drug; AF, atrial fibrillation; CAD, coronary artery disease; CVA, cerebrovascular accident; GP, ganglionated plexi; HFS, high frequency stimulation; LA, left atrial; LVEF, left ventricular ejection fraction; RLGP, right lower ganglionated plexi; and TIA, transient ischemic attack.

*P value comparing RLGP and other GP group.
node via the RLGP. Therefore, if cont-HFS–induced AV nodal response is to be used to identify GP sites for ablation, the RLGP should be targeted last. We subsequently investigated the feasibility of GP site identification and ablation using sync-HFS. In patients in sinus rhythm, we demonstrated that sync-HFS can be used to identify GP sites producing PV ectopy. Ablation at these sites abolishes the PV ectopic response to stimulation. Antral cryoballoon ablation also prevents sync-HFS–induced PV ectopy, suggesting that neural inputs to the PVs are disrupted during PVI.

Further evidence for the existence of atrial neural networks in humans is demonstrated in this study because ablation at the RUGP alone produces significant reductions in the LF and HF oscillations of HRV, suggesting that the RUGP is the final common neural pathway to the SA node. Antral cryoballoon ablation at the RUGP also produces significant reductions in HRV parameters, whereas ablation of all other PVs does not, suggesting that neural inputs from the RUGP to the SA node may be transected during RUPV ablation. This implies that HRV changes after AF ablation do not reflect the extent of LA denervation, but instead reflect disruption of neural inputs to the SA node via the RUGP, precluding the use of HRV as an end point marker of autonomic ablation.

**Implication of Neural Networks for Adjunctive Autonomic Ablation**

Studies performing endocardial GP stimulation and ablation in patients with AF have provided mixed evidence regarding the role of adjunctive autonomic ablation. GP sites identified using cont-HFS before antral PVI or circumferential PV ablation were almost entirely absent on retesting after ablation.\(^9,14\) Similarly, during redo AF, cont-HFS testing at presumed GP

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**Table 2. Patient Demographics for Synchronized HFS Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Group=20, Median (Range)</th>
<th>Control Group=3, Median</th>
<th>PVI Group=8, Median</th>
<th>GP Ablation Group=9, Median</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63 (50–69)</td>
<td>52</td>
<td>61</td>
<td>57</td>
<td>0.712</td>
</tr>
<tr>
<td>Women/men</td>
<td>4/16</td>
<td>0/3</td>
<td>2/6</td>
<td>2/7</td>
<td>0.809</td>
</tr>
<tr>
<td>AF duration, y</td>
<td>4.0 (2.5–5.5)</td>
<td>3.3</td>
<td>4.3</td>
<td>4.5</td>
<td>0.776</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1 (1–2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.335</td>
</tr>
<tr>
<td>LVEF</td>
<td>60% (55–60)</td>
<td>60</td>
<td>57</td>
<td>60</td>
<td>0.812</td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>38 (4)</td>
<td>35</td>
<td>38</td>
<td>38</td>
<td>0.744</td>
</tr>
<tr>
<td>CAD, %</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.796</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>33</td>
<td>0.615</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>30</td>
<td>33</td>
<td>25</td>
<td>33</td>
<td>0.638</td>
</tr>
<tr>
<td>TIA/CVA, %</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.634</td>
</tr>
<tr>
<td>General anesthetic, %</td>
<td>40</td>
<td>33</td>
<td>50</td>
<td>33</td>
<td>0.809</td>
</tr>
</tbody>
</table>

AAD indicates antiarrhythmic drug; AF, atrial fibrillation; CAD, coronary artery disease; CVA, cerebrovascular accident; GP, ganglionated plexi; HFS, high frequency stimulation; LA, left atrial; LVEF, left ventricular ejection fraction; PV, pulmonary vein isolation; and TIA, transient ischemic attack.

\(^*\)\(^P\) value comparing distributions of variables across all 3 groups.
sites failed to demonstrate positive vagal responses. This led to the conclusion that vagal responses were eliminated by antral PVI ablation and did not recur, despite clinical recurrence of AF. The effect of GP ablation on AF inducibility in humans was investigated. Endocardial cont-HFS was used to identify GP sites, with subsequent endocardial and epicardial GP

![Figure 4](image)

Figure 4. A. Number of sites initiating pulmonary vein (PV) ectopy before and after catheter relocation in the control group. B. Number of sites initiating PV ectopy before and after PV isolation (PVI). C. Number of sites initiating PV ectopy before and after cluster ablation at identified ganglionated plexi (GP) sites. HFS indicates high frequency stimulation.

![Figure 5](image)

Figure 5. A. Low frequency (LF) parameters of heart rate variability (HRV) measured at baseline and after subsequent cryoablation of the left upper pulmonary vein (LUPV), left lower PV (LLPV), right upper PV (RUPV), and right lower PV (RLPV). A reduction in LF HRV is seen only after ablation of the RUPV. B. High frequency (HF) parameters of HRV measured at baseline and after subsequent cryoablation of the LUPV, LLPV, RUPV, and RLPV. A reduction in HF HRV is seen only after ablation of the RUPV.
Ablation performed until no further vagal responses could be elicited. However, AF remained inducible in 17 of 18 patients, from which it was concluded that GP ablation did not significantly affect the ability to induce and maintain AF in the catheter laboratory. The findings of both studies should be interpreted with caution in view of the findings of our study, demonstrating the interconnecting neural pathways between the LAGPs and the AV node. We found that ablation of the RLGP prevented the AV nodal response to stimulation from all other LAGP sites, suggesting that without an intact RLGP other active GPs may not be identified using the method of cont-HFS. Neither study reported performing RLGP ablation last in their methods; therefore, RLGP ablation may have prevented the identification of any remaining active GP sites in these studies.

Our findings may also have implications for outcome studies using cont-HFS to identify GP sites for adjunctive autonomic ablation to prevent AF. A comparative study of anatomic versus selective GP ablation (GP sites identified using cont-HFS in the latter group), found a marked difference in outcome favoring anatomically guided GP ablation (77.5% versus 42.5% patients free from AF at ≈1 year). These results suggested that the use of cont-HFS to identify GP sites did not improve outcome. It is interesting to note that anatomic GP ablation produces similar success rates to that currently quoted for circumferential PV ablation, which may prompt speculation that ablation of the non-GP areas encompassed by the circumferential PV ablation may not provide any additional outcome benefit. However, studies performing a direct, randomized comparison between the 2 methods would be required to confirm this. The investigators went on to demonstrate an improvement in outcome with anatomic GP ablation in addition to PVI versus PVI alone (73.5% versus 45.5%). However, again it is not clear from this study whether PVI provides additional benefit compared with GP ablation alone.

One study of patients with mixed paroxysmal and persistent AF described the use of sequential GP ablation guided by cont-HFS, performing RLGP ablation last, followed by PV antrum isolation. After a single procedure and a follow-up of 22 months, no AF or atrial tachycardia recurrence was seen in 36 of 42 (86%) patients. These results suggest that adjunctive autonomic ablation may be more successful if the RLGP is ablated last. However, we propose that the method of sync-HFS is able to identify and ablate GPs with connections to the local PV which can generate PV ectopy, likely to be responsible for AF initiation, without the need for PVI. Our study

Figure 6. A. Low frequency (LF) parameters of heart rate variability (HRV) measured before and after ablation of the right upper ganglionated plexi (RUGP) demonstrate a significant reduction after RUGP ablation. B. LF parameters of HRV measured before and after ablation of all other GP sites demonstrate no change in LF HRV after GP ablation. C. High frequency (HF) parameters of HRV measured before and after ablation of the RUGP demonstrate a significant reduction after RUGP ablation. D. HF parameters of HRV measured before and after ablation of all other GP sites demonstrate no change in HF HRV after GP ablation.
did not find any evidence of ectopic firing within the PVs after PVI; however, antral PV ablation using the large 28-mm cryoballoon may have transected neural inputs to the PVs in addition to ablating the myocardial sleeves. There are, however, reports in the literature of ongoing firing within isolated PVs, which can be terminated by ablation at the presumed local GP sites.20

Importantly, we found that sites targeted on an anatomic basis produced a positive response of only 63% of the time using continuous HFS and 33% of the time using synchronized HFS. Therefore localization and ablation of GP sites by anatomy alone, without functional testing, may result in unnecessary ablation. The use of functional testing in areas of the LA not traditionally expected to contain GP sites identify sites of additional ectopic triggers responsible for AF initiation. Ablation of all sites initiating ectopy may be a potential strategy to prevent paroxysmal AF at the neural trigger and possibly overcome the problem of PV reconnection and AF recurrence.

Limitations
The method of sync-HFS is only applicable to patients in normal sinus rhythm, and therefore the results of the second part of the study can only be applied to patients with paroxysmal AF. It is possible that patients with persistent AF may be cardiovascular to use this method; however, we did not attempt this during our study.

Furthermore, the effect of PVI was studied using only the 28-mm cryoballoon in our cohort. This result may not necessarily be extrapolated to patients undergoing conventional ostial PVI. The complication of phrenic nerve injury is less commonly observed with cryoballoon ablation than with conventional ostial RF ablation,17,21 which may have implications for other neural connections on the epicardial surface of the PVs. The effect of conventional ostial PVI on GP sites initiating PV ectopy remains to be elucidated.

Conclusions
We have demonstrated the atrial neural network of autonomic ganglia in patients undergoing ablation, in which the RLGP acts as the final common pathway for neural inputs to the AV node and the RUGP acts as the final common input to the SA node. An understanding of atrial neural networks is essential if endocardial stimulation is used to identify GP sites for the purpose of adjunctive autonomic ablation.

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References


**CLINICAL PERSPECTIVE**

It has been suggested that pulmonary vein isolation inadvertently damages upstream regulators, such as the atrial ganglionated plexi (GP), of the intrinsic cardiac autonomic nervous system. Animal studies indicate these may be potential targets for ablation to prevent atrial fibrillation. Continuous high frequency stimulation of GPs produces atrio-ventricular block and has been used to identify and ablate GPs in humans. However, animal studies have revealed a complex network of autonomic connections, which have not been investigated in humans. We found that the right lower GP is the final common pathway to the atrio-ventricular node and must remain intact for all other GPs to be identified and ablated. Heart rate variability has been suggested as an end point for autonomic modification. Using a novel intraprocedural, short-segment heart rate variability tool, we found that reduction in heart rate variability after atrial fibrillation ablation occurs only after ablation of the right upper GP and does not reflect inputs from any other left atrial GP, precluding its use as an end point for left atrial denervation. Furthermore, it would seem logical to target the parts of the network that trigger pulmonary vein ectopy rather than targeting GP sites that produce effects at the sinus node and atrio-ventricular node. We developed a technique to identify sites initiating ectopic triggers and found that the response could be abolished either by achieving pulmonary vein isolation or by targeted radio frequency ablation to the site. This raises the possibility of targeted autonomic denervation of culprit sites of atrial ectopy as an alternative strategy to pulmonary vein isolation to prevent atrial fibrillation in humans.
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Appendix 1: