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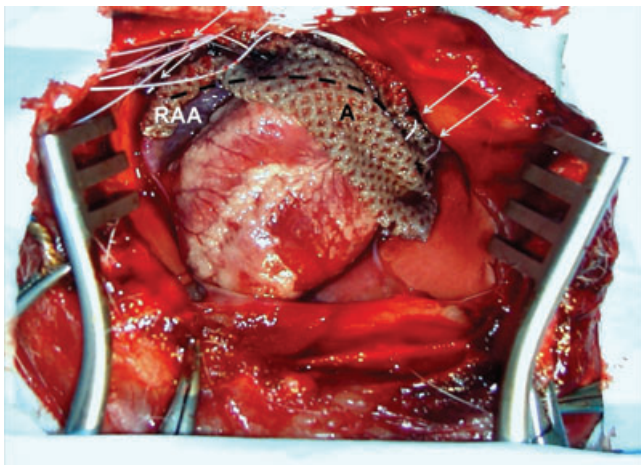


Figure 1. Open-chest preparation of the intact rabbit heart
The nylon network (A) supports four unipolar electrodes (arrows) to record electrograms along the atria (from the high-lateral right atrium to the high-lateral left atrium). Abbreviation: RAA, right atrial appendage.

veins (PVs), near the area of insertion in the LA, for recording of electrograms and pacing. Atrial epicardial activation was recorded during normal sinus rhythm, and the conduction time between RA and LA and between RA and PVs measured in baseline conditions and during vagal stimulation. The recorded signals were amplified, filtered at 0.5–300 Hz (Neurolog, Digitimer, UK) and digitized (PowerLab, ADInstruments; Fig. 2).

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Bipolar electrical stimulation was performed with a Teflon-coated silver wire electrode (0.1 mm diameter), positioned with a micromanipulator (WPI, M330), and with a coaxial electrode adapted to the PVs for the assessment of ERPs and induction of AF. An eight-channel, programmable stimulator (Master8, AMPI, Israel) was used for pacing. As a measure of local refractoriness, ERPs were assessed in each animal at four different sites [RA, right atrial appendage (RAA), left atrial appendage (LAA) and LA]. Effective refractory periods of the PVs were also measured in 10 animals. In stable conditions, after all the surgical procedures and under

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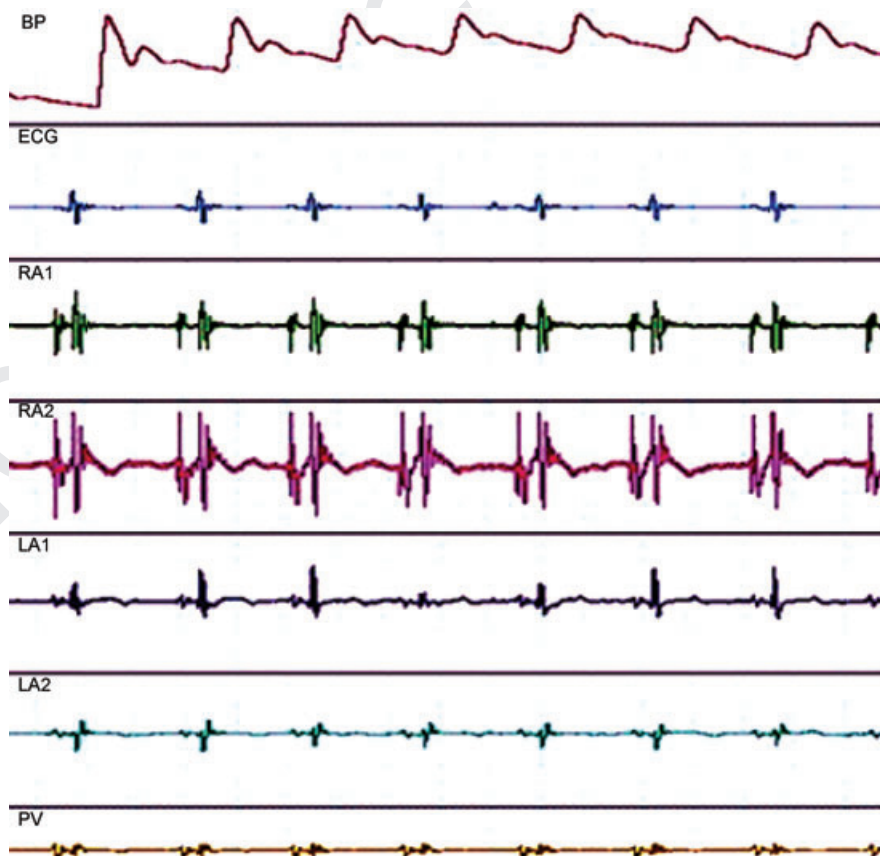


Figure 2. Continuous recording of blood pressure (BP), ECG, atrial and left pulmonary vein epicardial electrograms during sinus rhythm
Abbreviations: RA1, high-lateral right atrium; RA2, right atrial appendage; LA1, left atrial appendage; LA2, high-lateral left atrium; and PV, pulmonary veins.

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neuromuscular blockade, heart rate ranged between 175 and 222 beats min⁻¹ (195 ± 29 beats min⁻¹, *n* = 22), the systolic BP between 60 and 90 mmHg (82 ± 15 mmHg, *n* = 22), and the P wave between 38 and 60 ms (53 ± 8 ms, *n* = 22). Vagal stimulation at the cervical level for 5 s elicited a pronounced sinus bradycardia, with occurrence of systemic hypotension (<60 mmHg) in all animals.

Atrial and PV stimulation was performed with impulses of 2 ms duration at twice the diastolic threshold. A programmed electrical stimulation using a single premature stimulus was delivered, while pacing continuously at a basic drive cycle length of 300 ms (or 20 ms below the R–R interval in six rabbits with heart rate >200 beats min⁻¹). A premature beat was introduced in late diastole, beginning at a coupling interval of 100 ms less than the basic cycle length. The coupling interval of the premature stimulation was decreased by 10 ms steps until the ERP was reached. The ERP was defined as the longest S1–S2 interval that failed to initiate a propagation response, and measured in baseline conditions and during vagal stimulation. Dispersion of atrial refractoriness was calculated as the difference between the longest and the shortest ERP at the stimulation sites. The AF cycle length was measured in all sites with online calipers at a paper speed of 100 mm s⁻¹ by averaging five consecutive cycles before the termination of AF.

The concept of vulnerability to AF induction was evaluated with bursts (50 Hz, 10 s; supraliminal intensity), delivered with a bipolar electrode placed at the right and left atrial appendages, alone or combined with vagal stimulation. Atrial fibrillation was defined as a fast atrial rhythm characterized by a fluctuating beat-to-beat cycle length, polarity, configuration and amplitude of the

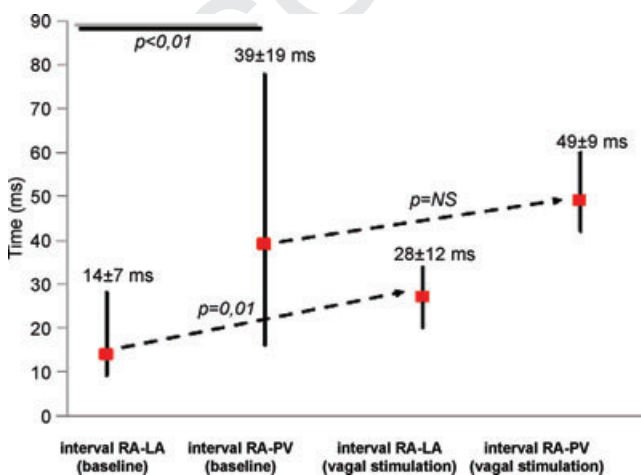


Figure 3. Atrial conduction times
Atrial conduction times between the high-lateral right atrium (RA) and the high-lateral left atrium (LA) and between the RA and left pulmonary veins (PV) near the area of insertion in the LA in baseline conditions and during right cervical vagal stimulation.

Table 1. Effective refractory periods (ERPs) measured during baseline conditions and during vagal stimulation

	Baseline ERP (ms)	ERP during vagal stimulation (ms)	% Δ	P value
Right atrium	86 ± 17	60 ± 20	-33 ± 20%	<0.01
Right atrial appendage	79 ± 18	60 ± 22	-21 ± 33%	<0.05
Left atrial appendage	105 ± 24*	76 ± 25	-20 ± 17%	<0.05
Left atrium	102 ± 27*	76 ± 23	-26 ± 15%	<0.01
Pulmonary veins	65 ± 40	38 ± 28	-31 ± 36%	<0.01

Definition: % Δ, percentage of variation of the average ERPs. **P* < 0.01 compared with high-lateral RA, RAA and PVs in baseline conditions.

recorded atrial electrograms and lasting more than five cycles (15).

Statistical analysis

Results are shown as means ± s.d. Categorical variables are expressed as frequencies and percentages. Normality of the distributions of the continuous variables was analysed with the Kolmogorov–Smirnov test. Student’s unpaired *t* test was used to assess all paired data in the same group. Comparisons between groups were made using Student’s unpaired *t* test or non-parametric analysis with the Mann–Whitney *U* test, as appropriate. The χ² test, with Yates correction, was used to evaluate the differences in categorical variables. All statistical tests were two-sided, and a probability value <0.05 was required for statistical significance. Data were analysed using GraphPad Instat, version 3.05 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Effects of vagal stimulation on electrophysiological parameters of the atria

The conduction time from the RA to the LA in baseline conditions was 14 ± 7 ms (10–30 ms), which increased to 28 ± 10 ms (20–35 ms) on stimulation (20 Hz, 0.2 ms, 150–200 μA) of the right vagus nerve (*n* = 22, *P* < 0.05; Fig. 3). In baseline conditions, during normal sinus rhythm, there was a significant delay in conduction between the RA and the PVs, slightly influenced by vagal stimulation (Fig. 3).

Table 1 shows the ERPs obtained in baseline conditions and during vagal activation. In baseline conditions, there was a significant increase of atrial refractoriness from the RA, RAA and PVs to the LA and LAA. Vagal stimulation produced a reproducible reduction in the atrial ERPs assessed in all sites.

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Table 2. Mean atrial fibrillation cycle lengths for atria and pulmonary veins measured in control conditions and following vagal stimulation

	High-lateral right atrium	Right atrial appendage	High-lateral left atrium	Left atrial appendage	Proximal segment of pulmonary veins
Baseline AFCL (ms)	154 ± 35	170 ± 23	173 ± 16	171 ± 16	152 ± 25
AFCL during vagal stimulation (ms)	83 ± 34*	77 ± 21**	86 ± 35**	110 ± 48§	71 ± 17**

Definition: AFCL, atrial fibrillation cycle length. * $P = 0.002$, ** $P < 0.0001$, § $P < 0.05$ for the comparison between control conditions and following vagal stimulation. Measurements obtained at different sites in the same conditions were not significantly different from each other.

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The dispersion of ERPs based only on atrial measurements was significantly lower than the dispersion of ERPs calculated with atrial and PV refractoriness values (33 ± 14 versus 64 ± 25 ms, $P < 0.05$). Compared with baseline conditions, vagal stimulation did not significantly affect the dispersion of refractoriness (33 ± 14 versus 38 ± 17 ms for atrial sites, n.s.; and 64 ± 25 versus 67 ± 28 ms for all sites, n.s.).

Effects of vagal stimulation on atrial fibrillation inducibility

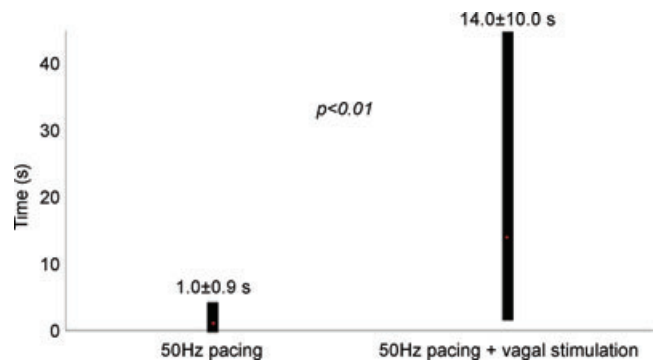
The inducibility and the duration of AF were significantly increased by vagal stimulation (Fig. 4). Atrial fibrillation was induced in 50% of the rabbits with rapid atrial bursts delivered to the RAA or PVs, but could not be induced by LAA high-rate pacing alone. During vagal activation, AF was consistently induced in 100, 60 and 20% of the rabbits by pacing the RAA, PVs and LAA, respectively (RAA versus LAA, $P < 0.05$). The mean AF cycle length measured in baseline conditions and during vagal stimulation is represented in Table 2. The cycle lengths during AF induced by 50 Hz pacing were reduced significantly by vagal stimulation in all atrial sites and PVs. On three occasions, AF was initiated by a single extrastimulus combined with vagal stimulation during ERP measurements. Pulmonary vein tachycardia (cycle length 80–90 ms) was induced by high-rate PV pacing combined with vagal stimulation in three animals (Fig. 5). The duration of the inducible AF changed from 1.0 ± 0.8 s with 50 Hz rapid stimulation alone to 14.0 ± 10.0 s in response to 50 Hz rapid stimulation combined with vagal stimulation ($P < 0.01$; Fig. 4). Atrial fibrillation lasted > 10 s in 45.5% of the rabbits (only during vagal stimulation) and terminated immediately after the cessation of vagal stimulation in seven of those 10 cases. These animals had similar interatrial and atrial to PV activation times. However, they showed the shortest ERPs in the RA, with higher dispersion of atrial refractoriness, at baseline and also during vagal stimulation (Table 3).

Discussion

The present experimental *in vivo* model allowed investigation of the effects of vagus nerve stimulation on cardiac electrophysiological properties and on the vulnerability to AF. We have characterized the influence of direct acute stimulation of the right cervical vagal trunk, the effect of which on heart rate is much larger than for identical left vagal stimulation (Loeb *et al.* 1981), on the electrical conduction and refractoriness properties of the atria and PVs. We have also confirmed a role of vagal activity in the vulnerability to the initiation of AF and its duration.

Main findings

This model shows a significant activation delay from the atria to the PVs during sinus rhythm. In fact, the mean conduction times from the RA to the PVs were about three times greater than the RA to LA interval measured in baseline conditions (Fig. 3). Also, a significant increase of baseline atrial ERPs was observed from the RA to the LA, suggesting heterogeneity of the electrophysiological properties of the atria. In addition, ERPs of the PV were significantly lower than those measured in the LA and LAA. In this preparation, vagal activity was important

**Figure 4. Duration of inducible atrial fibrillation episodes**

Duration of the inducible atrial fibrillation episodes with rapid atrial stimulation alone (50 Hz pacing) and in response to rapid atrial stimulation combined with vagal activation (50 Hz pacing + vagal stimulation).

for obtaining significant reproducible changes in atrial conduction times, atrial and PV ERPs, frequency of AF induction, mean AF cycle length and duration of the induced AF. Moreover, we demonstrated significant differences in atrial dispersion of refractoriness and RA ERPs in the group of animals with AF lasting > 10 s.

Animal models and mechanisms of atrial fibrillation

Various animal models have been developed to explore the mechanisms underlying AF. The classic hypothesis of multiple circuit re-entry with variable spatial orientation, focal activity and single rotors with fibrillatory conduction, which remains the framework for our understanding of AF, was established based on experimental studies in animals. However, the development of models that allow electrophysiological studies and a contribution to the comprehension of the conditions associated with the generation of arrhythmias remains a major challenge. In dogs, micro-re-entry, abnormal automaticity and triggered activity have been

observed as possible mechanisms for the occurrence of atrial arrhythmias (Friedman *et al.* 1996; Hocini *et al.* 2002). Hayashi *et al.* (1998) used efferent cervical vagal stimulation to induce AF by bursts of atrial pacing in anaesthetized open-chested dogs and evaluated the efficacy of class Ic and class III antiarrhythmic drugs to terminate AF. Other investigators have used versions of this model to explore the ability of different antiarrhythmics to terminate induced AF (Goldberger & Pavelec, 1986; David *et al.* 1990). Nevertheless, data regarding the use of experimental models with preserved autonomic innervation to assess the fundamental aspects of the electrophysiological properties of the atria and PVs underlying the vulnerability to development of AF are still limited. Abnormal atrial electrophysiology and higher vagal reflex activity may play an important role in the genesis of AF (Chen *et al.* 1999). In fact, it has been accepted that the ANS contributes as a modulator of the initiation, perpetuation, ventricular response rate and termination of AF, but its precise role remains controversial (Olshansky, 2005; Vos *et al.* 2008). The

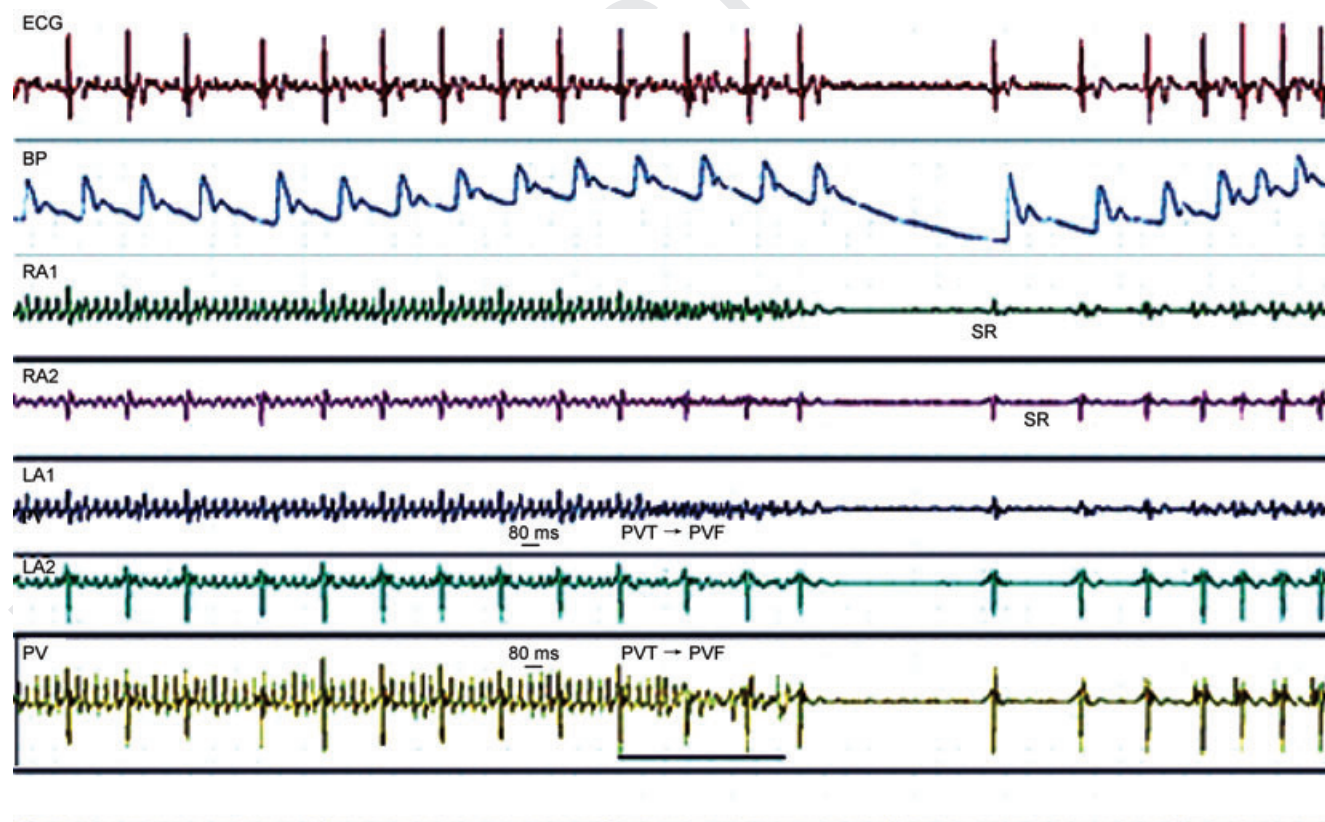


Figure 5. Example of a recording with pulmonary vein tachycardia (PVT) induced by stimulation of the pulmonary veins (50 Hz, 10 s) combined with vagal activation, showing the transition to pulmonary vein fibrillation (PVF) followed by termination of the arrhythmia

Abbreviations: BP, blood pressure; ECG, electrocardiogram; LA1, left atrial appendage; LA2, high-lateral left atrium; PV, pulmonary veins; PVF, pulmonary veins fibrillation; PVT, pulmonary veins tachycardia; RA1, high-lateral right atrium; RA2, right atrial appendage; and SR, sinus rhythm.

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Table 3. Characteristics, atrial refractoriness and atrial conduction times in the rabbits with atrial fibrillation lasting <10 s and those with atrial fibrillation lasting >10 s

Parameter	Group AF <10 s	Group AF >10 s	P value
P wave duration (ms)	35 ± 9	50 ± 14	n.s. (0.08)
Baseline heart rate (beats min ⁻¹)	196 ± 13	208 ± 28	n.s.
Baseline systolic BP (mmHg)	78 ± 11	75 ± 15	n.s.
Weight (kg)	4,4 ± 0,3	4,3 ± 0,4	n.s.
Atrial dispersion of ERP baseline (ms)	22 ± 15	42 ± 5	0.03
Atrial + PVs dispersion of ERP baseline	73 ± 38	60 ± 26	n.s.
Atrial dispersion of ERP vagal	28 ± 15	48 ± 11	0.03
Atrial + PVs dispersion of ERP vagal	72 ± 40	63 ± 13	n.s.
RA ERP baseline (ms)	97 ± 12	70 ± 10	<0.05
RA ERP vagal	80 ± 20	40 ± 10	<0.05
RAA ERP baseline	67 ± 15	80 ± 11	n.s.
RAA ERP vagal	53 ± 15	64 ± 27	n.s.
LAA ERP baseline	102 ± 21	107 ± 6	n.s.
LAA ERP vagal	75 ± 24	88 ± 19	n.s.
LA ERP baseline	105 ± 35	100 ± 27	n.s.
LA ERP vagal	80 ± 56	73 ± 16	n.s.
PVs ERP baseline	75 ± 51	58 ± 38	n.s.
PVs ERP vagal	41 ± 33	34 ± 30	n.s.
Interval RA–LA baseline (ms)	10 ± 7	17 ± 4	n.s.
Interval RA–LA vagal (ms)	26 ± 9	31 ± 16	n.s.
Interval RA–PVs baseline (ms)	55 ± 50	40 ± 20	n.s.
Interval RA–PVs vagal (ms)	58 ± 20	51 ± 8	n.s.

Definitions: AF, atrial fibrillation; BP, blood pressure; ERP, effective refractory period; PVs, left pulmonary veins; and vagal, during cervical vagal nerve stimulation. Atrial + PV dispersion includes ERP measurements from high-lateral right (RA) and left atrium (LA), right (RAA) and left atrial appendage (LAA) and PVs.

importance of the ANS in AF is supported by animal experiments and recent clinical studies showing that vagal denervation enhances the efficacy of circumferential PV ablation in preventing AF recurrence (Chen & Tan, 2007).

Decreased atrial ERP and its spatial dispersion heterogeneity have been accepted to promote AF re-initiation and to provide a substrate for the re-entry of multiple wavelets to enhance the ability of the disorder to sustain itself (Nattel *et al.* 2000). In previous studies, sympathetic stimulation was much less effective than vagal stimulation in promoting AF and heterogeneity in atrial refractoriness (Liu & Nattel, 1997; Chen *et al.* 1999). Vagus nerve stimulation has been associated with atrial ERP shortening and facilitation of AF inducibility in dogs (Arora *et al.* 2008).

Our present data, using the intact rabbit heart model, support the importance of vagal activity in determining significant acute changes of atrial refractoriness and its contribution to the induction and cycle length of AF. The calculated dispersion of atrial refractoriness has not been significantly modified by vagal stimulation. This may explain the inability to sustain AF despite maintenance of vagal activation. In fact, reproducible induction of AF during vagal stimulation was obtained in all animals with a significant increase in the duration of the episodes,

but the longest duration of AF was 45 s, lasting >10 s only in 45.5% of the rabbits. Interestingly, those animals with longer AF duration showed higher dispersion of atrial refractoriness and shortest RA ERPs, despite similar conduction activation times. This emphasizes the role of refractoriness properties as important markers of vulnerability to AF. We were able to obtain conditions that decrease atrial wavelength, the product of ERP and conduction velocity, by significantly decreasing the ERPs and the conduction velocity of the atrial tissue, which permits multiple wavelets and promotes AF.

Effects of vagal stimulation on conduction times, refractoriness and vulnerability to atrial fibrillation

Electrophysiological abnormalities behind the genesis of AF include conduction disturbances, shortening of the ERP and increase in atrial dispersion of refractoriness (Fuster *et al.* 2001). Vagal tone may influence the refractoriness and conduction velocity in the atrial tissue, contributing to the vulnerability to the occurrence of AF (Chen *et al.* 2007). However, the assessment of autonomic fluctuations before the onset of paroxysmal AF, measured with heart rate variability, has shown conflicting results (Chen *et al.* 2006).

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In the present study, the electrophysiological characterization of the recognized effects of vagal stimulation in conduction and refractoriness properties of the atria and PVs was clearly shown. Oh *et al.* (2006), using a canine model, demonstrated that the effects of vagal stimulation on atrial ERPs and AF inducibility were significantly changed immediately after epicardial fat pad ablation, suggesting that it is possible to achieve temporary suppression of the vagal tone in the atria. Recently, in a study with dogs, sustained AF could be induced easily during vagal nerve stimulation *in vivo* (Lemola *et al.* 2008). Zhang *et al.* (2009) also used burst stimulation of the atria and right cervical vagal stimulation to induce AF in dogs. Their study suggested that strong vagal stimulation facilitates AF inducibility. In our rabbit model, we have shown that vagal activation was able to decrease the atrial and PV cycle length during AF and significantly increase the duration of the induced AF episodes (Table 2 and Fig. 4). Vagal stimulation significantly increased the interatrial activation times, without changes in the conduction velocity between atrial and PV activation (Fig. 3). This suggests non-uniformity of vagal responses, with evidence of regional differences in conduction delay, and supports the concept that the presence of anisotropic conduction properties and slow conduction at the area of the PV–LA junction may contribute to promote re-entry formation and thus play a role as a substrate for the maintenance of AF (Kumagai *et al.* 2004; Atienza & Jalife, 2007). Also, the vagal effect on the degree of ERP reduction was slightly higher in the RA, LA and PVs.

Vagal activity seemed to influence the termination of AF directly, since in 70% of those animals with AF duration > 10 s the arrhythmia stopped immediately after cessation of vagal stimulation. Therefore, despite the inability to demonstrate a significant impact on the dispersion of the ERP, vagal activation decreased the conduction velocity in the atria and evoked reductions of the ERP, contributing to the induction, duration and termination of AF.

Limitations of the study

Although programmed electrical stimulation with a single premature stimulus has been widely used for the study of atrial refractoriness properties, we believe that further studies, using different electrophysiological techniques, may be needed to confirm these findings, because the present method may be of relatively low resolution. Also, the low tendency of the rabbit atria to fibrillate and maintain the arrhythmia owing to its small size might contribute to the inability to sustain AF in this preparation. Although brief episodes of AF in the rabbit reflect an evidence of arrhythmic vulnerability, the precise mechanisms underlying AF maintenance may be different

in humans. Further experimental studies using more accurate methods may be needed to elucidate the impact of autonomic innervation on the dynamics of recurrent sustained AF episodes.

Conclusions

The acute stimulation of parasympathetic activity exerts a reduction of atrial conduction velocity and decreases ERPs in multiple atrial sites and in the PVs. This experimental preparation also displays the impact of vagal stimulation in enhancing the propensity for the inducibility of AF, demonstrating an important role in the vulnerability to the occurrence of AF and contributing to the duration of AF episodes, particularly in those animals with higher dispersion of refractoriness. Our data suggest that this experimental vagal stimulation model may provide a useful method for assessment of the influence of the ANS in the pathophysiology of AF.

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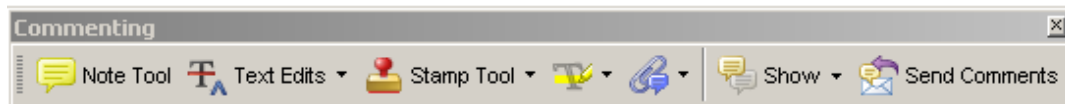
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Q13	Author: please check all the tables very carefully. I have made some style changes.	
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Q21	Author: is the wording of the table heading OK now?	
Q22	Author: please provide units for all the parameters in column 1 of Table 3.	
Q23	Author: is “the intact rabbit heart model” OK now?	
Q24	Chen <i>et al.</i> 2007 is not listed in the reference list. Please check.	
Q25	Author: is “the vagal effect on the degree of ERP reduction” OK now?	
Q26	Author: please provide page numbers for Camm (1997).	
Q27	Chen <i>et al.</i> 2000 is not cited in the text. Please check.	
Q28	Elvan <i>et al.</i> 1995 is not cited in the text. Please check.	
Q29	Author: is the title of the paper by Fuster et al. correct now?	
Q30	Wang <i>et al.</i> 2005 is not cited in the text. Please check.	

USING E-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required Software

Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: <http://www.adobe.com/products/acrobat/readstep2.html>

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:



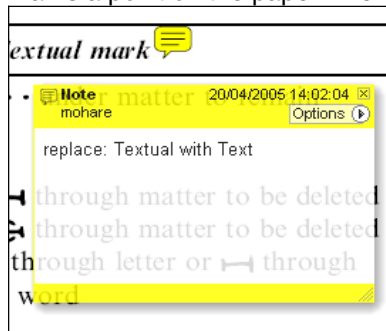
If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the “Documents” category under “Edit – Preferences”, please select the category ‘Documents’ and change the setting “PDF/A mode:” to “Never”.



Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.

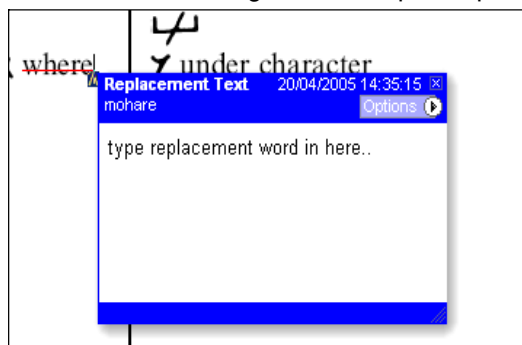


How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.

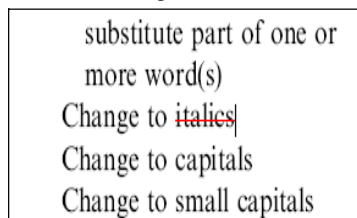


How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

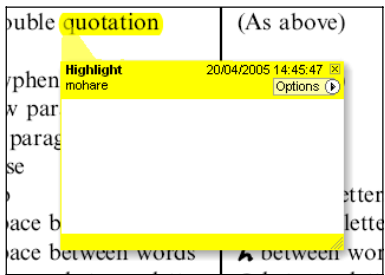
Approved tool — For approving a proof and that no corrections at all are required.



- How to use it:**
1. Click on the Stamp Tool in the toolbar
 2. Select the Approved rubber stamp from the 'standard business' selection
 3. Click on the text where you want to rubber stamp to appear (usually first page)

Highlight tool — For highlighting selection that should be changed to bold or italic.

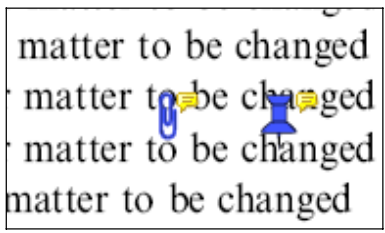
Highlights text in yellow and opens up a text box.



- How to use it:**
1. Select Highlighter Tool from the commenting toolbar
 2. Highlight the desired text
 3. Add a note detailing the required change

Attach File Tool — For inserting large amounts of text or replacement figures as a files.

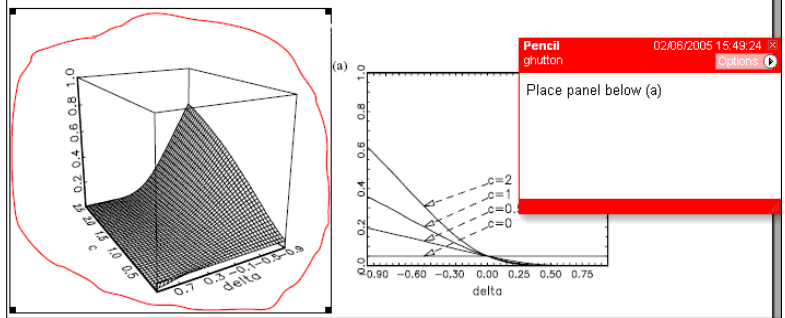
Inserts symbol and speech bubble where a file has been inserted.



- How to use it:**
1. Click on paperclip icon in the commenting toolbar
 2. Click where you want to insert the attachment
 3. Select the saved file from your PC/network
 4. Select appearance of icon (paperclip, graph, attachment or tag) and close

Pencil tool — For circling parts of figures or making freeform marks

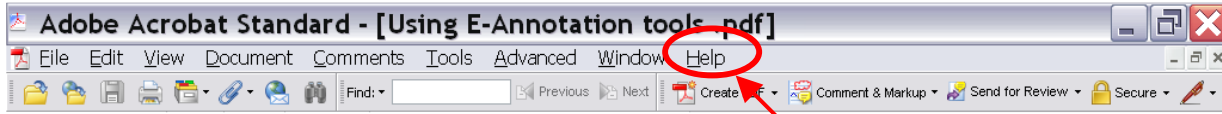
Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.



- How to use it:**
1. Select Tools > Drawing Markups > Pencil Tool
 2. Draw with the cursor
 3. Multiple pieces of pencil annotation can be grouped together
 4. Once finished, move the cursor over the shape until an arrowhead appears and right click
 5. Select Open Pop-Up Note and type in a details of required change
 6. Click the X in the top right hand corner of the note box to close.

Help

For further information on how to annotate proofs click on the Help button to activate a list of instructions:



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MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	••• under matter to remain	ⓓ
Insert in text the matter indicated in the margin	⋈	New matter followed by ⋈ or ⋈ⓓ
Delete	/ through single character, rule or underline or ⎯⎯⎯ through all characters to be deleted	Ⓞ or Ⓞⓓ
Substitute character or substitute part of one or more word(s)	/ through letter or ⎯⎯⎯ through characters	new character / or new characters /
Change to italics	— under matter to be changed	↵
Change to capitals	≡≡ under matter to be changed	≡≡
Change to small capitals	== under matter to be changed	==
Change to bold type	~ under matter to be changed	~
Change to bold italic	≈ under matter to be changed	≈
Change to lower case	Encircle matter to be changed	⊖
Change italic to upright type	(As above)	⊕
Change bold to non-bold type	(As above)	⊖
Insert 'superior' character	/ through character or ⋈ where required	γ or γ under character e.g. γ̇ or γ̈
Insert 'inferior' character	(As above)	⋈ over character e.g. ⋈̇
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	γ̇ or γ̈ and/or γ̇ or γ̈
Insert double quotation marks	(As above)	γ̇ or γ̈ and/or γ̇ or γ̈
Insert hyphen	(As above)	⊖
Start new paragraph	┌	┌
No new paragraph	┐	┐
Transpose	└	└
Close up	linking ○ characters	○
Insert or substitute space between characters or words	/ through character or ⋈ where required	γ̇
Reduce space between characters or words		↑