***Online Supplement***

## Methods

*Optical Mapping Technique*

Briefly, after a bolus injection of 5 to 10 ml Di-4-ANEPPS (10 mg/mL), a CCD camera (SciMeasure, Decatur, GA) directed toward the left atrial appendage (LAA) recorded fluorescence changes from an area of ~3 $cm^2$ at 500-1000 frames/sec to obtain 5-second movies (80x80 pixels). Movies of the LAA, together with bipolar electrograms of the LAA, left atrial roof (LA roof) and right atrial appendage (RAA) made possible the characterization of the atrial dominant frequency (DF) distribution. DF maps were obtained for each optical movie after applying a Fast Fourier Transformation of the fluorescence signal recorded at each pixel. To analyze rotor dynamics, we also constructed phase movies after a phase analysis according to Gray et al.[1] but modified as to be based upon Hilbert transformation as in Warren et al.[2]

*Mechanisms of breakthroughs: Post-pacing interval and AF inducibility*

To further investigate the mechanisms of the breakthrough waves during AF and determine whether they were related to triggered or reentrant mechanisms, we recorded LAA electrograms as well as optical movies during burst pacing at various basic cycle lengths (BCLs): 200, 175, 150, 125, 100, 90, 80, 70, 60, 50 ms or until 2/1 conduction block or AF initiation ensued. The post-pacing interval between the last paced and the first spontaneously occurring LAA electrogram was measured under conditions of stretch (intraatrial pressure, 12 cm $H_2O$), stretch + isoproterenol 0.03 μM (ISO) and stretch+ISO+caffeine 5 mM (CA). Also, the first focal discharge activation pattern as

seen in optical movies was analyzed as described in the Methods section of the main paper.

*Computer Model*

The temporal values of the transmembrane potential were calculated numerically by solving the discrete standard reaction-diffusion equation, assuming a 2x2 cm isotropic tissue ($\Delta h$=0.1 mm and $\Delta t$=50 μsec), Neuman boundary conditions[3] and incorporating human atria ionic kinetics,[4] including the $I_{K,ACh}$ formulation of Kneller et al. for 1μmol/L acetylcholine.[5] We also implemented a commonly accepted IV relationship of stretch-activated channels as described by Kamkin et al. in human atrial cells.[6] We used a moderate stretch level of 4 μm.

*Numerical Protocol*

Two types of stimulations were conducted: 1) S1-S2 cross-field stimulation to induce reentry, followed by an extra stimulus of 4*4 nodes to mimic the effects of the spontaneous activity at varying distances from the rotor core; 2) S1-S2 protocol followed by several pulses mimicking the effects of spontaneous EADs or DADs discharges on rotor frequency and dynamics.

## Results

*Post-pacing interval and AF inducibility*

We determined the post-pacing interval of first breakthrough appearance using a 5-second burst of stimuli during stretch alone, and in the presence of stretch +ISO and

stretch+ISO+CA. The postpacing interval could not be reproducibly measured in the condition of stretch+ISO+CA because of occurrences of only 3 post-pacing extra-beats or AF initiation. The post-pacing interval was found to be positively correlated to the basic cycle length during stretch and stretch +ISO. Online table 1 illustrates AF vulnerability results under the 3 experimental conditions tested. Clearly, the conditions of stretch and stretch +ISO corresponded to a high yield of AF initiation (respectively 75 and 100%). In the online supplement figure (OSF) 1A, we have plotted the postpacing interval values at various BCLs under those 2 conditions. The rate-dependency of the postpacing interval, strongly suggests a delayed afterdepolarization mechanism. Careful analysis of all post-pacing extra-beats revealed that most of them occurred outside of the LAA field-of-view while only a few of them manifested as LAA breakthrough waves. In panel B, we illustrate an example of AF initiation by post-pacing breakthroughs under condition of stretch +ISO. After pacing at 100 ms, 2 breakthroughs appeared on the LAA; they were closely coupled to the previous paced impulse, and corresponded to beats 1 and 2 of the single pixel recordings presented in the upper portion of panel B.

*DF changes and AF termination in experimental groups 3 (ryanodine 10 μM: RYA) and 4 (caffeine 5 mM: CA)*

During perfusion of RYA alone, AF terminated in 5 out of 8 cases. OSF 2 depicts the optical DFmap at the LAA (lower panel) and the electrical DF values (upper panel) at the LA roof before and during RYA perfusion in one representative example. Both upper and lower panels clearly indicate that before terminating after RYA perfusion, AF frequency of activation sharply decreased. OSF 3 demonstrates that in the 5

experiments that terminated, LA roof DF decreased significantly after RYA perfusion while it remained unchanged in the 3 experiments that did not terminate. This observation is further exemplified in OSF 4 that shows 2 representative examples of the electrograms DF time-course at 3 atrial locations (LAA, RAA and LA roof) before and after perfusion of RYA. The comparison between the non-termination case (left panel) and the termination case (right panel) clearly indicate that, after RYA perfusion, the roof DF sharply decreased in the latter while it was unchanged in the former. As also presented in the left panel, we gradually increased RYA concentration to 40 μM during the AF episode that did not terminate, without additional frequency changes or AF termination. In figure OSF 5 frequency values during SRAF, after CA perfusion and immediately before AF termination are presented (left panel: average DF values; right panel: representative bipolar electrograms). In 5 animals, after an initial increase LA roof frequency progressively decreased before AF termination.

## References


1 Gray RA, Pertsov AM, Jalife J. Spatial and temporal organization during cardiac fibrillation. Nature 1998; 392:75-78

2 Warren M, Guha PK, Berenfeld O, et al. Blockade of the inward rectifying potassium current terminates ventricular fibrillation in the guinea pig heart. J Cardiovasc Electrophysiol 2003; 14:621-631

3 Pandit SV, Berenfeld O, Anumonwo JM, et al. Ionic determinants of functional reentry in a 2-D model of human atrial cells during simulated chronic atrial fibrillation. Biophys J 2005; 88:3806-3821

4 Courtemanche M, Ramirez RJ, Nattel S. Ionic targets for drug therapy and atrial fibrillation-induced electrical remodeling: insights from a mathematical model. Cardiovasc Res 1999; 42:477-489

5 Kneller J, Zou R, Vigmond EJ, et al. Cholinergic atrial fibrillation in a computer model of a two-dimensional sheet of canine atrial cells with realistic ionic properties. Circ Res 2002; 90:E73-87

6 Kamkin A, Kiseleva I, Wagner KD, et al. Characterization of stretch-activated ion currents in isolated atrial myocytes from human hearts. Pflugers Arch 2003; 446:339-346

## Figure Legends

**Online supplement figure 1. A,** Post-pacing interval-BCL relationship in the presence of stretch (12 cm $H_2O$), stretch (12 cm $H_2O$) +ISO. **B, *upper,*** representative single-pixel recording (asterisk) showing AF initiation after 5-second pacing at BCL 100 ms. **Lower,** activation maps corresponding to the last paced impulse (P) and to 2 centrifugal spontaneous focal discharges that initiated AF.

**Online supplement figure 2.** AF termination during perfusion of ryanodine 10 μM (Group 3). Electrogram DFs at the LA roof ***(Upper)*** and DFmaps at the LAA ***(Lower)*** before and during perfusion of ryanodine 10 μM. During the perfusion of ryanodine 10 μM, DF at the LAA and LA roof progressively decreased before AF termination.

**Online supplement figure 3.** DFmax at the LA roof, LAA and RAA before and during perfusion of ryanodine 10 μM (Group 3) in 5 animals in which AF terminated ***(left)*** and in 3 animals in which AF did not terminate ***(right)***.

**Online supplement figure 4.** Representative examples of a termination case and a non-termination case of SRAF during perfusion of ryanodine 10μM (Group 3). While DF significantly decreased at all atrial locations before AF termination ***(right)***, DF at the LA roof did not change significantly in an animal in which AF did not terminate ***(left)***.

**Online supplement figure 5.** DFmax at the LA roof, LAA and RAA during SRAF, CA 10 minutes and before AF termination in the Group 4 ***(left).*** Electrogram DF at the LA roof before AF termination in the presence of CA ***(right).***

## Supplemental Online Movies Legends

**Supplemental movie 1**

Representative phase movie during SRAF in the presence of ACS. Rotor drifting was increased due to an increased number of breakthroughs. Each color corresponds to a different phase of the action potential as illustrated in Figure 2.

**Supplemental movie 2**

Representative phase movie during SRAF in the presence of ACS+RYA. A stable and stationary rotor sometimes undergoing wavebreak formation was observed. Each color corresponds to a different phase of the action potential as illustrated in Figure 4A.

**Supplemental movie 3**

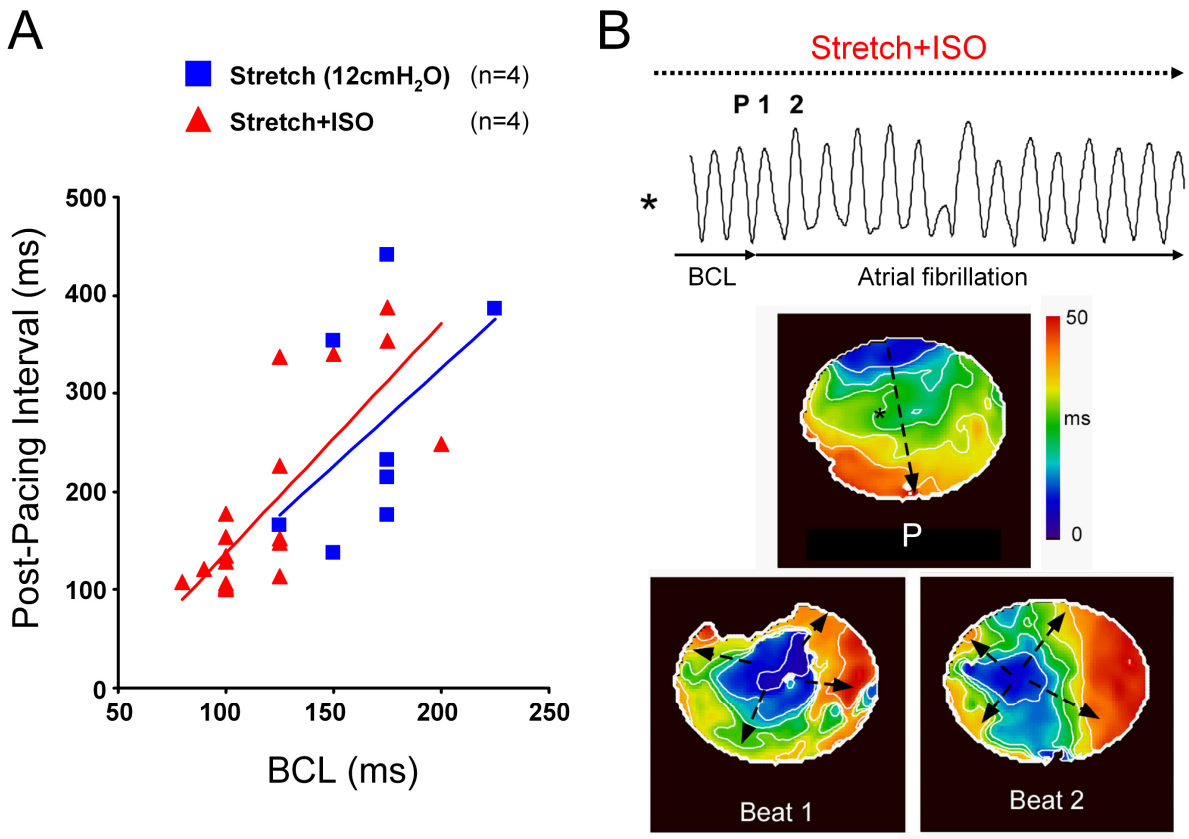
Representative phase movie during SRAF in the presence of ACS+CA. A stable and stationary rotor was observed. Each color corresponds to a different phase of the action potential as illustrated in Figure 4B

Online Table I: Incidence of NS-AF/S-AF elicited by rapid (5 sec) stimulation

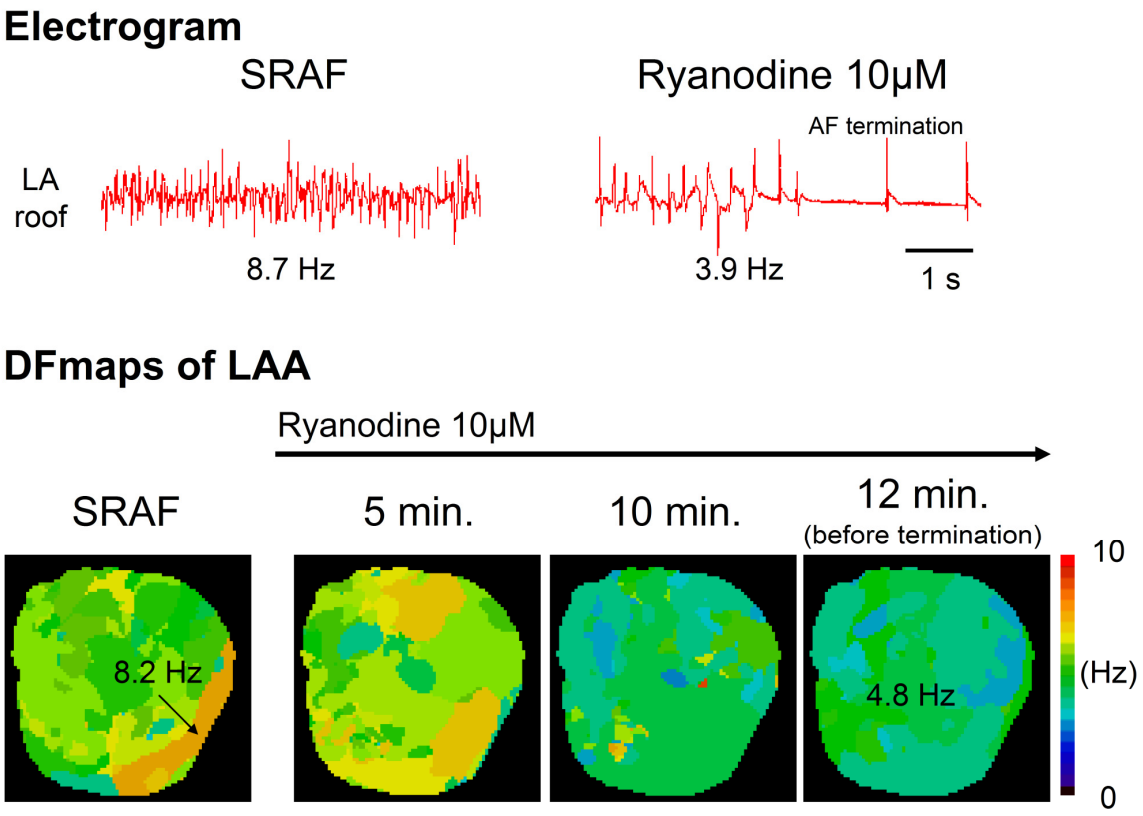
| | | NS-AF (<5min.) | S-AF (>5min.) | Vulnerability |
|---|---|---|---|---|
| Stretch (12 $cmH_2O$) | (n=4) | 0 | 3 | 75% (3/4) |
| Stretch (12 $cmH_2O$)+ISO | (n=4) | 0 | 4 | 100% (4/4) |
| Stretch (12 $cmH_2O$)+ISO+CA | (n=4) | 1 | 1 | 50% (2/4) |

NS-AF; Non-sustained atrial fibrillation, S-AF; Sustained atrial fibrillation
ISO; Isoproterenol 0.03μM, CA; Caffeine 5 mM

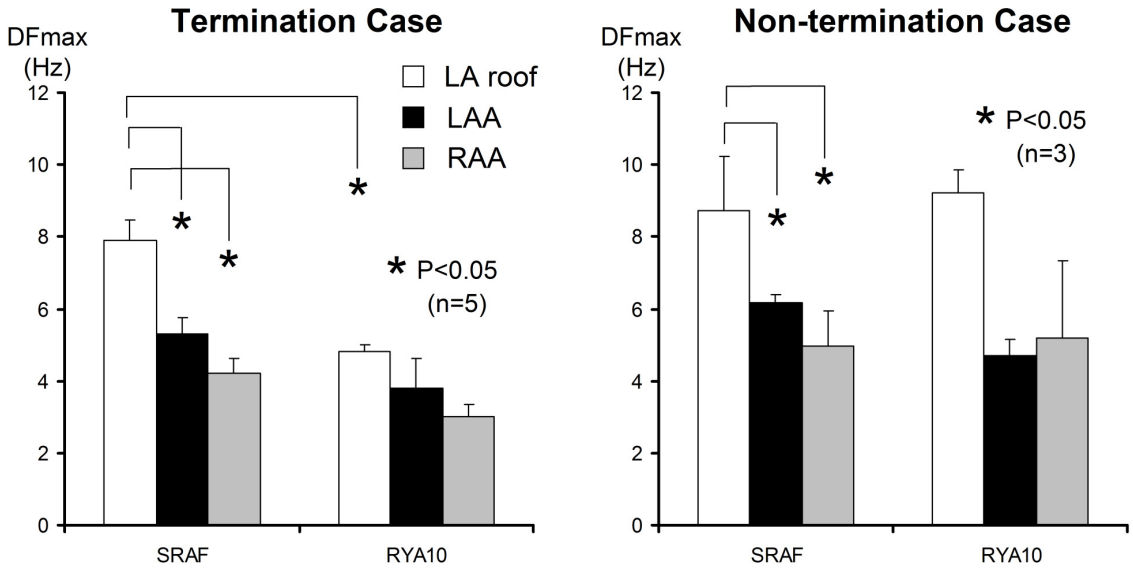
**Online supplement figure 1**



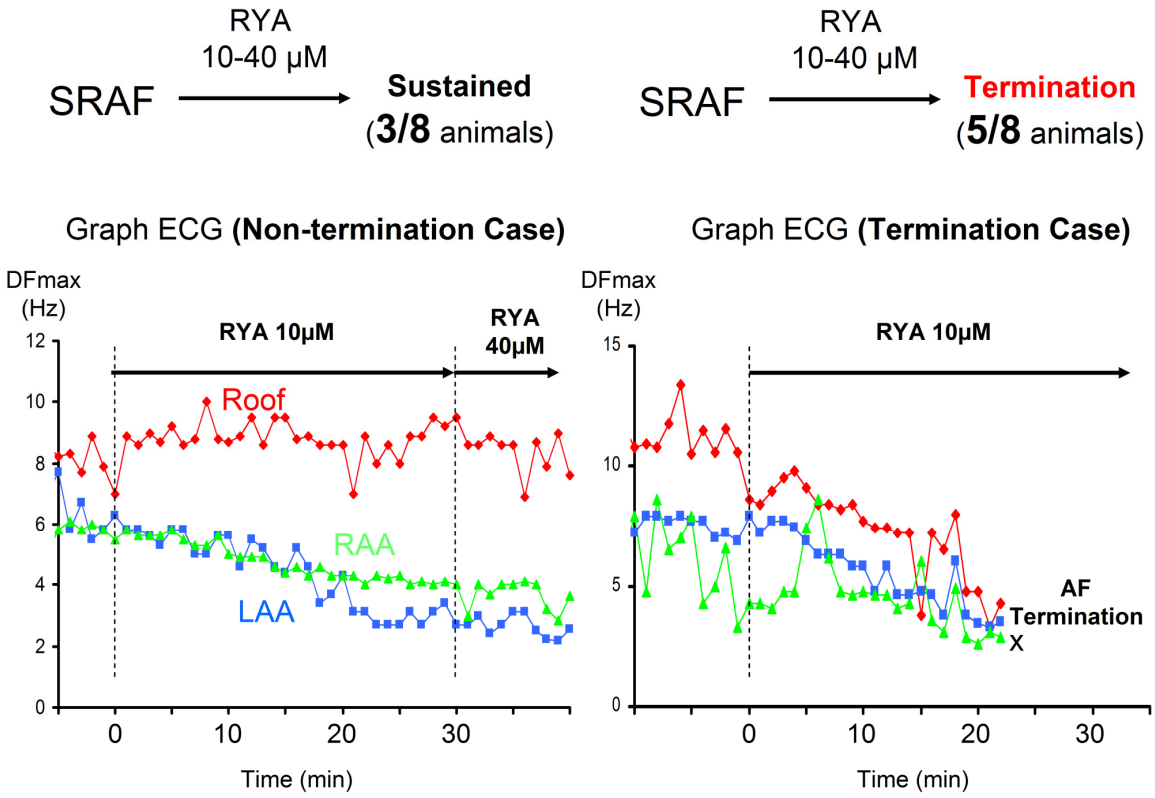
**Online supplement figure 2**




**Online supplement figure 3**



## Online supplement figure 4




## Online supplement figure 5