Surface and Intramural Reentrant Patterns during Atrial Fibrillation in the Sheep

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1. Introduction

Atrial fibrillation (AF) is to date the most common sustained cardiac arrhythmia in humans and is predicted to dramatically increase its prevalence in the near future [1]. The pulmonary veins (PVs) and the anatomical region surrounding their antrum into the left atrium were found to play an important role in the initiation and maintenance of the arrhythmia. High-resolution mapping data and Fourier power spectrum analysis with its dominant frequency (DF) recently published by our group support the hypothesis that acute AF in the structurally normal sheep heart [2, 3] and in some patients [4, 5] is not a totally random phenomenon and often presents organized drivers in the form of periodic surface re-entries or breakthroughs [6, 7]. Nevertheless, the dynamics of those surface patterns of activity, as well as their intramural components are still poorly understood.

Objective: To present data on AF waves from the surface of isolated sheep hearts and discuss the interpretation of their intramural patterns.

Methods: We used a combination of endocardial-epicardial optical mapping with phase and spectral analysis as well as computer simulation of the re-entrant activity in the myocardial wall.

Results: Analysis of the surfaces’ optical mapping data in the phase domain reveals that activation of the posterior left atrium (PLA) consisted of alternating patterns of breakthroughs and reentries. The patterns on the endocardial and epicardial PLA surface at any given moment of time of the AF could be either identical or not identical, and the activity in the thickness of the PLA wall is hypothesized to conform to either ectopic discharge or reentrant scroll waves, but a definite evidence for the presence of such mechanisms is currently lacking. A universal minimal-principle theory is shown in a computer model to result in a tendency of the axis of the scroll waves to align with the myocardial fibers inside the wall.

Conclusion: The tendency of filaments of scroll waves to align with myocardial fibers may contribute to the variety and intermittency of surface rotors seen in AF.

2. Methods

2.1 Isolated Hearts

All animal experiments were carried out according to the University of Michigan Committee on Use and Care of Animals and the National Institutes of Health guidelines. In three sets of experiments a total of 16 sheep (45–50 kg) were anesthetized with intra-venous bolus injection of propofol
(5–10 mg/kg). All hearts were excised and Langendorff-perfused with warm oxygenated Tyrode’s solution (pH 7.4; 95% O₂, 5% CO₂ and 36–38 °C). After perforation of the intra-atrial septum, we sealed all venous orifices except the inferior vena cava for controlling the level of intra-atrial pressure to 12 cm H₂O or higher [8]. AF, or stretch-related AF (SRAF) was induced by burst pacing at 10 Hz.

2.2 Optical Mapping

The optical set-up included up to three synchronized CCD cameras recording from the epicardial right atrial and left atrial appendage (RAA, LAA) and either the endocardial LAA or posterior left atrium (PLA). The latter camera is connected to either a flexible or rigid cardio-endoscope introduced in the left atrium by transseptal route as described previously [9, 10]. A bolus injection of 15 ml Di-4-ANEPPS (10 mg/mL), enabled recording fluorescence changes from an area of ~3 × 3 cm² (80 × 80 pixels) for each CCD at 500 frames/sec to obtain 5-second movies. To reduce motion artifacts, we added 10 μM blebbistatin to the perfusate. After fluorescence acquisition, the voltage time-series at each pixel was obtained by subtracting the time-constant fluorescence background level from the raw signal. Movies of the PLA, LAA and RAA were obtained together with bipolar electrograms of the LAA, left atrium-pulmonary vein (LA-PV) junction, RAA and coronary sinus (CS). The local activation rate of the recordings was determined in the frequency domain as the frequency with maximal power following fast Fourier transform (dominant frequency, DF) [11].

2.3 Analysis of Activation Patterns and Dynamics

Simultaneous endocardial and epicardial movies were analyzed wave-by-wave based on phase domain analysis according to Gray et al. [12] performed with the Hilbert transform [8, 13]. Briefly, each of the optically probed voltage time-series was first band-pass filtered to remove low frequency drifting, residual DC and high-frequency noise, and then every spectral component of that time-series was shifted by its corresponding quarter cycle via the Hilbert transform. Following, the instantaneous phase of the action potentials recorded at each pixel was obtained from the inverse tangent of the ratio of the transformed signal (quarter-cycle shifted) to the original signal (non-shifted). The reliance on optical mapping signals that contain less high frequency noise than electrical recordings, together with the motion reduction with blebbistatin, the use of the Hilbert transform instead of the single lagging return map approach [12] as well as the band-pass filtering, increased the robustness of our phase analysis against artifacts. Finally, the phase angle, with values between −π and π radians, is represented in the figures as a continuous color scheme to form a phase map, in which the continuous spatial phase change reflects the process of excitation, repolarization and recovery. The phase movies were analyzed with specific attention to wave patterns as follows.

i) Rotors, defined as a wave pivoting around a point in the phase map toward which all the phases (colors) converge (that is, a phase singularity, PS, point [8, 13]) for more than one rotation;
ii) breakthroughs (BTs): are defined as waves observed at least in part, for at least 12 ms (6 frames) and initiated within the field-of-view.

3. Results

3.1 Activation Patterns of Stretch-related AF

In the atria of five normal sheep hearts we identified on average about 1, 6 and 3 waves/sec of endo-epi identical reentries, breakthroughs and uniform wave spreading, respectively during SRAF [8]. In Figure 1 we demonstrate an alternation, respectively during SRAF in the PLA of five sheep hearts were found to have identical BT patterns on the endocardial and epicardial surfaces [8]. In panel A we demonstrate two snapshots of reentrant activity whose pivoting point is located similarly close to the left PVs both on the endocardial and epicardial surfaces. In this case, it’s easy to suggest that the hidden intramural activity adopts the form of a scroll wave whose filament is extended between the two surfaces in what is known as an I-shaped filament [14]. Fifteen percent of the activations mapped during SRAF in the PLA of five sheep hearts were found to have identical BT patterns on the endocardial and epicardial surfaces [8]. In panel B of Figure 2, an example of such simultaneous breakthrough activity on the epicardial and endocardial surface is shown. In this case, it is easy to conceive that a wave spreading
3.3 Non-identical Endo-epi Activation Patterns

As described above, in the normal sheep heart, SRAF was characterized by multiple centrifugal BT activations, wavebreaks and short-lived reentries, all suggesting interplay between reentrant and spontaneous focal discharge mechanisms [15]. The various patterns could further differ between the endocardial and epicardial surfaces, suggesting the existence of transmural propagation [16] and linked to increased stability of AF [17]. In Figure 3 we present and discuss data on such transmural dissociated excitation patterns. On the left side of panel A, the epicardial phase snapshot shows reentrant activity, and the simultaneous endocardial snapshot shows a breakthrough pattern. On the right side we present the same snapshots in a format that illustrates a possible scroll wave with a bent filament, also known as L-shaped filament, which may underlie such non-identical surfaces' activation patterns. Accordingly, one free-end of the filament resides in the epicardial surface while the other free-end induces wave, however, if the PLA wall is sufficiently thick, then an ectopic pacemaker should be less likely and an intramural scroll wave, whose filament is fully hidden on the mapped surfaces here, may have sufficient room to rotate and spread waves that appear as breakthroughs on the epicardial and endocardial surfaces.

radially outward from a discharge originating from an intramural ectopic focus underlies this surface activation. However, in this case the seemingly obvious suggested ectopic mechanisms may be not so certain after more careful examination: The volume of tissue surrounding the pacemaking cells is likely to exert an electrotonic load that tends to inhibit the transmembrane potential suprathreshold depolarization needed for the generation of a spreading wave. If the PLA wall at the breakthrough is very thin, then the load may not be fully inhibiting the ectopic-induced wave, however, if the PLA wall is sufficiently thick, then an ectopic pacemaker should be less likely and an intramural scroll wave, whose filament is fully hidden on the mapped surfaces here, may have sufficient room to rotate and spread waves that appear as breakthroughs on the epicardial and endocardial surfaces.

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of the filament is extending toward the outside of the fields mapped. It should be noted that the latter free-end of the filament must be at a myocardial boundary somewhere, and based on a topological theorem it cannot reside inside the tissue, even not at the boundary of a scar 3-D island [18]. The possibility of a focal discharge in this case is not conceivable. This example also highlights the importance of simultaneous dual surfaces mapping. In case only the breakthrough activity would have been mapped on a single surface, this would erroneously suggest that the origin of this AF wave is an ectopic discharge. In Panel B of Figure 3 we discuss yet another example of dissociated activity. On the left panel two counter-rotating re-entries are seen on the epicardial surface and two simultaneous breakthroughs are seen on the endocardial surface. On the right panel we show the combined phase snapshots with a hypothesized U-shaped filament connecting the two singularity points on the same surface. It is of course possible that the two reentries do not belong to the same scroll wave and are actually two separated L-shaped filaments as in panel A. This panel again highlights the importance of dual surface mapping to better characterize the patterns of excitation in relation to the possible ectopic or reentrant activity.

### 3.4 Breakthroughs and Scroll-waves in the PV Region

Overall, in our study of SRAF in five sheep isolated hearts we found 59% of the waves mapped in the PLA to be identical and 41% of the waves to be different (dissociated) between the endo-epicardial surfaces. Comparing the lifespan of endo-epicardial identical reentries (that is, I-filament scroll waves) in term of the number of rotations we found that while in normal hearts I-filament scroll waves never lasted more than three rotations, we found examples of I-filament scroll waves lasting six rotations or more in 12/16 movies from another model of persistent AF sheep hearts [19]. In a previous study of AF in six sheep hearts we found that the BTs during AF were the most common pattern of activation and they tended to cluster at the middle of the PLA, where the radius encircling about 75% of the BTs resided in the area between the PVs, and not including the PVs themselves [20]. Here, in SRAF in four other normal sheep hearts, there was no preference in spatial distribution of the density of the breakthroughs at any of either the left or right PVs, nor to any other non-PV region in the PLA. In yet four other normal sheep hearts during SRAF multiple rotor PSs formed at the PLA during each one second episode of AF. The ro- tors PS points were traced for the duration of the rotor and their location has been superimposed on the corresponding PLA background anatomical pictures of each of the four hearts. The trajectory of those PSs demonstrates that they are confined to the PLA with some small meandering and drifting mostly in the vicinity of the ostia and entral aspects of any of the PVs.

### 3.5 Dynamic Patterns of AF Waves

In yet another set of five sheep experiments we studied patterns of surface waves during persistent AF [21]. Calculation of the DF revealed that global DF max was consistently localized at the PLA with a statistically significant DF gradient from PLA to LAA and RAA (9.1 ± 1.0 vs. 7.9 ± 0.7 and 6.9 ± 0.9 Hz, respectively, p < 0.05) suggesting the driving role of the PLA [21]. To further establish the patterns of activation underlying the DF max values in the PLA we used

![Figure 3](image-url)
phase movies to compare the number of reentries and BTs. Figure 4A shows at 10 min intervals a significantly higher number of breakthroughs/cm²/2-sec compared to rotations/cm² in 5 separated hearts. Top, snapshots from a phase movie show a rotor appearing in the field of view of the LAA. The patterns of activation switch from breakthroughs (0-to-113 ms) to a meandering rotor (301-to-541 ms). Bottom, the DF_max is in the LAA when the rotor stays in the field of view and goes back to PLA when the rotor drifts outside the LAA. (Modified from Filgueiras-Rama et al., Circulation Arthyth Electrophys 2012)

3.6 A Universal Equilibration Dynamics of Intramural Reentries

Fiber organization inside the cardiac wall is complicated. Myocardial fibers are curved; fiber layers are organized in mainly transmural laminae; may be parallel or tilted with respect to the myocardial surface and alter their direction abruptly and discontinuously. All such factors may affect the configuration of the filament inside the myocardial wall and therefore require further investigation. As an initial approach to address the contribution of fiber bend and organization on scrolls we adopted the minimum-path principle leading to a geodesic hypothesis and provided with a universal dynamics for the equilibration of a filament inside the cardiac wall [22]. Figure 5A shows a simulated steady state scroll wave rotating in a medium with a twisted anisotropy as observed typically in the ventricular wall (see inset). Since the filament deviates significantly
from a straight line we use the inverse of the diffusion (i.e., the resistance) tensor of the model as a metric for the calculation of the filament's shape based on the minimal-path principle. Figure 5B illustrates the excellent agreement between the analytical calculation of the resistance geodesic and the filament of the simulated scroll wave. This brings us to a most important result: Given a uniform set of ionic properties of the myocardium, the configuration of the fibers determines the shape of the filament at equilibrium along the resistance geodesic [22]. Accordingly, the filament will constantly tend to conform to a trajectory whose total resistance (that is, the sum of all the intercellular resistances along its path) is the least among all the possible trajectories inside the myocardium. This filament equilibration property is universal in the sense that it holds for a wide range of ionic properties, as long as they are uniform across the tissue [22].

4. Conclusions

We used a combination of optical mapping and spectral analysis to study AF waves patterns on the surfaces of isolated sheep hearts and have found rapid sources that are located in the PLA and PV region. Analysis of the surfaces’ optical mapping data in the phase domain reveals that activation of the PLA and LAA consisted of alternating patterns of breakthroughs, reentries and relatively simple waves sweeping through the field of view. The patterns on the endocardial and epicardial PLA surf- face at any given moment of time of the AF could be altering between either identical or not identical, and the activity in the 3-D thickness of the PLA wall is hypothesized to conform to either ectopic discharge or scroll waves, but a definite evidence for the presence of such mechanisms is currently lacking. We further propose that the diffusion tensor of the myocardial wall is the metric that encompasses necessary information on the configuration of a filament of an ensuing scroll wave. Accordingly, the filaments tendency to align with fibers is universal in the sense that it does not depend on the ionic parameters of the action potential. This tendency to align with fibers is one way of explaining why sustained ro- tors, although hypothesized to drive many cases of AF, are not always observed on the surfaces. As the myocardial fibers run substantially parallel to such surfaces, epi- and endocardial rotors would be nothing but transient manifestations of intramural scrolls.

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