Lifetimes of epicardial rotors in panoramic optical maps of fibrillating swine ventricles

Matthew W. Kay, Gregory P. Walcott, James D. Gladden, Sharon B. Melnick, and Jack M. Rogers. Lifetime of epicardial rotors in panoramic optical maps of fibrillating swine ventricles. *Am J Physiol Heart Circ Physiol* 291: H1935–H1941, 2006. First published April 21, 2006; doi:10.1152/ajpheart.00276.2006.—During ventricular fibrillation (VF), electrical activation waves are fragmented, and the heart cannot contract in synchrony. It has been proposed that VF waves emanate from stable periodic sources (often called “mother rotors”). The objective of the present study was to determine if stable rotors are consistently present on the epicardial surface of hearts comparable in size to human hearts. Using new optical mapping technology, we imaged VF from nearly the entire ventricular surface of six isolated swine hearts. Using newly developed pattern analysis algorithms, we identified and tracked VF wave fronts and phase singularities (PS; the pivot point of a reentrant wave front). We introduce the notion of a compound rotor in which the rotor’s central PS can change and describe an algorithm for automatically identifying such patterns. This prevents rotor lifetimes from being inappropriately abbreviated by wave front fragmentation and collision events near the PS. We found that stable epicardial rotors were not consistently present during VF: only 1 of 17 VF episodes contained a compound rotor that lasted for the entire mapped interval of 4 s. However, shorter-lived rotors were common; 12.2 (SD 3.3) compound rotors with lifetime >200 ms were visible on the epicardium at any given instant. We conclude that epicardial mother rotors do not drive VF in this experimental model; if mother rotors do exist, they are intramural or septal. This paucity of persistent rotors suggests that individual rotors will eventually terminate by themselves and therefore that the continual formation of new rotors is critical for VF maintenance.

Some authors refer to these two basic mechanisms as type 2 and type 1 VF, respectively (30, 31).

Periodic wave sources in VF are generally thought to arise from reentry, which occurs when a wave circles back and reactivates tissue it has already passed through. For reentry to exist in the absence of an anatomical obstacle (i.e., a hole in the tissue or an inexcitable region), the pivoting end of the wave must contain a phase singularity (PS), sometimes called a wave break, which is a convergence point of tissue at all phases of the action potential (8, 28). Phase, in this context, is an angular variable that specifies a patch of tissue’s progression through the action potential. In two-dimensional mapping data, a wave front is an isoline of the phase value that corresponds to the upstroke. In three dimensions, a wave front is an isosurface, and the phase singularity is a one-dimensional filament snaking through the tissue (17, 28). The intersection of this filament with a surface such as the epicardium is the PS observed in two-dimensional mapping data. A PS has a chirality that is determined by the rotation direction of its wave front. A singular filament with a wave rotating about it is called a rotor. We refer to rotors whose central filament intersects the epicardium as epicardial rotors.

One step in substantiating the mother rotor hypothesis in large hearts is to determine if at least one stable rotor is consistently present during VF. Previous studies that mapped portions of the swine right ventricle (22, 23, 27), left ventricle (16, 21, 22, 27), or septum (10) were unable to document such a rotor, even though relatively long-lived rotors were sometimes observed (21). However, all of these studies mapped only a limited region, typically 20–40 cm², admitting the possibility that a mother rotor was present but located in an unmapped region.

The objective of the present study was to map from the entire epicardial surface of a large heart preparation to determine by direct observation if stable epicardial rotors are consistently present during VF. This was accomplished by using a newly developed optical mapping system that merges fluorescence data acquired from four high-speed video cameras into a single continuous data set (12) and newly developed algorithms that identify and track wave fronts and PSs as they move over the epicardium. In addition, we introduce the notion of a compound rotor in which the central PS can change and describe an algorithm for automatically identifying such patterns.

**METHODS**

**Animal preparation.** The use of experimental animals in this study was approved by the Institutional Animal Care and Use Committee at...

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the University of Alabama at Birmingham. We studied six isolated, perfused swine hearts. The animals were healthy mixed-breed pigs of either sex [weight 23 (SD 4) kg, range 21.0–30.2 kg]. Details of anesthesia, heart excision, and Langendorff perfusion have been described previously (18). These previous methods were slightly modified by placing a suction tube in each ventricle to remove perfusate, preventing it from running over the epicardium and distorting optical signals. The circulating volume of perfusate was 1.5 liters, and the flow rate was 200 ml/min. Fresh perfusate was continuously added to the system at 25 ml/min. When the perfusate reservoir exceeded a set level, a valve automatically opened to discard perfusate and restore the target volume.

Panoramic optical mapping. We used a newly developed optical mapping system that records electrical activity from nearly the entire ventricular epicardium (the tip of the apex is not well imaged and is generally obscured by dripping perfusate). Figure 1 shows a schematic of the system. The shape of the epicardium is acquired with the rotating geometry camera. Electrical activity is recorded by using a voltage-sensitive dye and the mapping cameras. The movies from the mapping cameras are merged into one dataset by mapping them onto the epicardial geometry. Details are as previously described (12) with the following exceptions.

1) The time required for an epicardial scan (1 rotation of the geometry camera) has been reduced from 9 min to 70 s.

2) We replaced the original dual-mapping cameras with four high-speed CCD cameras (iXon DV860, Andor Technology, South Windsor, CT). The cameras are positioned around the heart in 90° increments (128 × 64 pixels in each view). Final spatial resolution with this system is ~1.6 mm, and temporal sampling is 750 frames/s. The cameras are synchronized by using a computer-controlled PCI timing board (PCI-CTR05, Measurement Computing). Each mapping camera is fitted with a 2.2-mm, F number/1.0, 1/4-in. format video lens (Pentax, Golden, CO) and positioned ~10 cm from the heart. The cameras are mounted on a track so they can be backed out of the way of the geometry camera while it circles the heart and then be returned accurately to the mapping position.

3) Excitation light is now provided by 32 blue light-emitting diodes (LEDs, Luxeon V Star, 470 nm, Lumileds Lighting, LLC, San Jose, CA). Each LED dissipates 5 W with luminous flux of 48 lumens. Six LEDs are mounted on the front of each mapping camera, and two are mounted on each of four positional arms.

Study protocol. Hearts were immobilized with 2,3-butanedione monoxime (BDM, 20 mM) to eliminate contraction artifacts in the optical signals and stained with a 5–7 ml bolus of the voltage-sensitive dye di-4-ANEPPS (15 μM). The hearts were periodically restained during the study if signal amplitude decreased. After an ~30-min interval for the preparation to stabilize (12), the following procedure was repeated three times: 1) scan the epicardial geometry; 2) optically map 4 s of a paced rhythm; 3) induce VF by applying a 9-V battery to the right ventricle; 4) ~20 s after induction, optically map 4 s of VF; and 5) defibrillate with a minimum reliable-strength shock (5–20 J) delivered through external paddles. One induction resulted in a monomorphic tachycardia rather than VF; this episode was excluded from analysis. We therefore analyzed a total of 17 VF episodes. At the end of each experiment, all cameras were calibrated as previously described (12).

Geometric reconstruction and decimation. By using images collected by the geometry camera at 5° increments, the epicardial models were constructed with submillimeter resolution (12). However, because analysis of VF wave fronts requires only about 1- to 2-mm spatial resolution (1), we subsequently reduced the spatial resolution of each model to ~1.6 mm. In this procedure, 2,300 points are randomly scattered onto the surface of the high-resolution model. The points are then moved about the surface under the influence of an iterative mutual repulsion function to achieve approximately equal spacing (26). A new triangular mesh is then formed from this set of points using the alpha shapes suite of programs (6). Triangles in the new mesh were typically spaced by ~1.6 mm (centroid to centroid). This procedure occasionally produced topological defects in the output mesh (e.g., more than 2 triangles meeting along an edge). These defects were repaired with an interactive program. The model was further corrected so that the three vertexes for each triangle in the mesh were listed counterclockwise when viewed from the outside. This ensured that the normal vectors for each triangle pointed away from the center of the model.

Texture mapping of fluorescence data. Background-subtracted fluorescence data from the high-speed cameras were texture mapped onto the decimated epicardial models by using a modification of our previously described procedure (12). In the modified method, the vertexes of each triangle in the mesh are projected onto the image plane of the camera(s) that optimally map the triangle [by using previously described optimization rules (12)]. The fluorescence assigned to the triangle is a weighted average of fluorescence from each pixel that falls completely or partially within the triangle in the image plane. Pixel weights are determined by the percentage of the pixel that falls within the projected triangle. The fluorescence for triangles that are optimally mapped by more than one camera is a weighted average of the triangle data from each camera image, where weights are determined by the distance of the triangle to the edge of the heart (12).

In one typical geometry, the resolution of the mapping camera images was 1.42 (SD 0.05) mm (mean and SD). The triangles in the mesh were spaced by 1.61 (SD 0.16) mm (centroid to centroid). Triangles received contributions from 8.6 (SD 2.6) image plane pixels. Thus texture mapping imposed some spatial smoothing on the fluorescence data. After texture mapping, the signal for each triangle was temporally smoothed using a low-pass Butterworth filter (55-Hz pass band, 65-Hz stop band, 3-dB pass band loss, 10-dB stop band attenuation) and then normalized to the range 0:100. Spatial and temporal smoothing is useful to reduce high-frequency shot noise present in the optical recordings. Without smoothing, spurious signal

Fig. 1. Panoramic optical mapping system. The geometry camera is mounted on a rotating arm. Images are acquired every 5 degrees and used to reconstruct the epicardial geometry. The mapping cameras are track-mounted and can be positioned close to the heart for mapping or backed away for geometry scans. Excitation light is provided by 32 blue light-emitting diodes (LEDs). Emitted fluorescence is filtered by 590-nm lens-mounted long-pass filters.
fluctuations can cause temporary wavebreaks to appear in activation wave fronts and inappropriately increase the number of very short-lived PSs registered by our analysis algorithms. Filtering may obscure fine spatial and temporal details of the activation patterns but should not affect the macroscopic, long-lived patterns that are the primary focus of this study.

All optical recordings were mapped onto the geometric model acquired most recently before the mapping run. The atria and regions with poor signals acquired during pacing were clipped from the model by using an interactive program. Such regions typically included small fat-obscured areas at the atrioventricular groove, and the tip of the apex, which was poorly imaged by all mapping cameras and was also obscured by dripping perfusate.

Wave front and PS analysis. The resulting VF datasets were processed with newly developed algorithms that automatically identify PSs and wave fronts and construct data structures that describe wave front-wave front, PS-wave front, and PS-PS relationships (20). Wave front-wave front interactions are codified in a directed graph (2) in which the graph edges are wave fronts and the vertexes are wave front initiation, termination, or contact events (20, 24). Contact events include fragmentations, in which one parent wave front breaks up into two or more child wave fronts, and collisions, in which two or more parent wave fronts collide and coalesce to produce a single child wave front. We call this data structure a wave front graph. Figure 2 shows a simple example.

**Compound rotors.** In the traditional definition of a rotor, a wave front propagates about a single PS. The rotor terminates when its PS is annihilated by collision with a PS of opposite chirality or with a boundary (8). However, we commonly observed wave fragmentation or collision events near the PS at the tip of a rotating wave front. In such events, the original PS was annihilated, but a child wave front continued to rotate in the same direction about a new PS in very nearly the same position. Figure 3 shows an example of this phenomenon. In Fig. 3A, *wave front 1* is rotating counterclockwise about PS *i*. By Fig. 3B, *wave front 1* has fragmented into *wave fronts 2* and *3*. *Wave front 3* contains the original PS *i*, in addition to a new PS of opposite chirality (gray triangle). *Wave front 2* contains another new PS, PS *ii*, with the same chirality as PS *i*. By Fig. 3C, *wave front 3* has contracted, allowing the oppositely rotating PSs on either end to annihilate each other and terminate the wave front. One of the annihilated PSs is the original PS *i*. However, *wave front 2* now propagates about the new PS *ii* much as wave front 1 propagated about PS *i*. It can be argued that such a pattern constitutes reentry about a single organizing center even though the central PS changes.

We developed an algorithm to automatically detect these events. We create, for each VF episode, two new directed graphs (2) called singularity graphs. There is one such graph for each of the two chiralities. To generate a singularity graph, we first set the chirality-appropriate PSs to be the singularity graph’s nodes. We then iterate over all contact events in the VF episode’s wave front graph. The wave front graph for the activation pattern in Fig. 3, *A–C*, is shown in Fig. 3D. This graph contains one contact event, the fragmentation of *wave front 1* into *wave fronts 2* and *3*. For each contact, we identify the PSs that continue through the contact (e.g., PS *i*) and those that either begin or end at the contact. PS *ii* is an example of a beginning PS. We compute the spatial distance between each continuing PS and each beginning or ending PS. If this distance is no more than five triangle-to-triangle hops (~8 mm), a graph edge is added between the two corresponding PSs. The edges are directed from continuing-to-beginning or ending-to-continuing PSs. The singularity graph for the black singularities in Fig. 3, *A–C*, is shown in Fig. 3E. There are two nodes (*i* and *ii*) and a single edge directed from *i* to *ii*.

Although we have described the algorithm with a fragmentation event, it works in the same way for collisions. For example, if the flow of time is reversed in Fig. 3, *wave front 3* appears de novo on the epicardium and coalesces with *wave front 2* to form *wave front 1*. In this situation, PS *i* is again a continuing PS and PS *ii* is an ending PS. In the singularity graph, the arrow is directed from *ii* to *i*.

A singularity graph may contain multiple subgraphs, each of which is disconnected from all other subgraphs. In graph theory, each of these subgraphs is called a component of the full graph (2). In the limiting case of a VF episode in which no events such as the one depicted in Fig. 3, A–C, occurred, both singularity graphs (one for each chirality) would consist only of nodes with no connecting edges. Each node would therefore be its own component. The singularity graph in Fig. 3E is the opposite limiting case: all nodes are connected by edges, and the entire graph therefore consists of a single component spanning all PSs. We treat each singularity graph component as a compound rotor. A compound rotor contains one or more PSs, all of which have the same chirality. The number of PSs in the compound rotor is the number of nodes in the singularity graph component. Its lifetime spans the lifetimes of all of its constituent PSs.

**RESULTS**

We studied a total of 17 VF episodes in six hearts. Each episode was mapped for 4 s starting ~20 s after induction. Figure 4 shows a snapshot of a panoramically mapped VF episode. Figure 4, A and B, shows fluorescence, and Fig. 4, C...
and D, shows the wave fronts and PSs found by our algorithms. Supplemental movies S1\(^1\) and S2\(^2\) animate the entire episode.

Over all VF episodes, we observed 310,356 PSs. Most PSs were very short in duration: 65% had lifetimes $\leq 10$ ms and 99.6% were $< 200$ ms (PSs lasting longer than 200 ms have completed at least 1 cycle of reentry, but less than 2). Figure 5 shows a histogram of all PS lifetimes (note the log scale). Only one PS persisted for its entire mapped interval.

Combining PSs into compound rotors substantially prolonged lifetimes (Fig. 5). Over all VF episodes, there were 1.6 (SD 2.0) PSs per compound rotor. Seventy-nine percent of compound rotors had only one PS, and an additional 10% had only two. The compound rotor with the most PSs had 80.

Figure 6 shows the distribution of compound rotor lifetimes exceeding 200 ms averaged across episodes. Compound rotors lasting longer than 1 s were rare. The lifetime of the longest-lived compound rotor from each episode is listed in Table 1. One episode contained two compound rotors that persisted for the entire mapped interval (episode 11; the listed time is slightly less than 4.0 s because a few temporal samples were removed from either end of the episode as part of the filtering process). Supplemental movie S3\(^3\) animates this episode. None of the other episodes contained a compound rotor that persisted for the entire mapped interval.

Table 1 lists the mean number of compound rotors exceeding 200 ms that were present at one time in each episode. The mean over all episodes was 12.2 (SD 3.3). Figure 7 is an example of how the number of compound rotors fluctuates with time. We fit a straight line to these data for each episode (e.g., the dashed line in Fig. 7). The mean slope of the regression line over all episodes was $-0.3 \pm 1$ (SD 1.0). This number was not significantly different from 0 by $t$-test ($P > 0.2$); thus the

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\(^1\) Supplemental movie S1, available with the online version of this article, shows animation of fluorescence data from one VF episode (episode 6 from Table 1) texture-mapped onto a model of the epicardial geometry. Figure 4 in the main manuscript shows one frame from this sequence. Blue tissue is resting, and red is activated.

\(^2\) Supplemental movie S2, available with the online version of this article, shows wave fronts and PSs identified in the same VF episode shown in Supplemental Movie S1. Wave fronts are cyan. PSs are yellow or red, depending on chirality. The geometry is displayed with a Hammer map projection.

\(^3\) Supplemental movie S3, available with the online version of this article, shows a VF episode with persistent rotors (episode 11 from Table 1). The rotors are located at top right of the image. The format is the same as in Supplemental Movie S2.
number of compound rotors did not consistently increase or decrease during the mapped interval.

DISCUSSION

In the present study, we mapped epicardial activation patterns during VF in isolated swine hearts. We analyzed 4-s intervals starting ∼20 s after induction. This corresponds to Wiggers’ stage II VF, which is clinically relevant because it is the time during which implantable defibrillators deliver therapy. Our major finding is that stable epicardial rotors are not consistently present during VF in this experimental model.

Compound rotors. In the strictest sense, the sequence shown in Fig. 3 contains two counterclockwise rotors: one that rotates about PS i and terminates with the termination of wave front 3, and another that rotates about PS ii and is formed when wave front 1 fragments into wave fronts 2 and 3. We argue that sequences such as this should be regarded as a single rotating entity even though the central PS changes. We therefore introduced the notion of a compound rotor, which allows multiple PSs to be associated with a single rotor, and described an objective and automatic algorithm for identifying these entities. In essence, this algorithm allows small-scale fragmentation and collision events near rotor tips to be neglected when associating PSs and wave fronts with rotors. This guards against such events (which may be noise artifacts) from resulting in inappropriately abbreviated rotor lifetimes. The use of this relaxed definition of a rotor, which has the effect of registering rotor lifetimes that are longer than those for traditional single-PS rotors, is particularly important for the present study because it amplifies our major finding that individual rotor lifetimes are generally shorter than the duration of VF.

Rotor lifetimes. All 17 VF episodes contained compound rotors that lasted for hundreds of milliseconds, and each had at least one compound rotor that lasted for 1 s or more. However,

Table 1. Compound rotor parameters per episode

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<th>Animal</th>
<th>Episode</th>
<th>Maximum Life Time, s</th>
<th>No. of Rotors</th>
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<tr>
<td>1</td>
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<td>1.001 (SD 2.2)</td>
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<td>2</td>
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Maximum lifetime values are maximum compound rotor lifetimes; no. of rotors are mean (SD) no. of compound rotors present at any instant.

Fig. 5. Distribution of PS and compound rotor lifetimes. Data from all VF episodes are lumped. Note the log scale.

Fig. 6. Lifetime distribution for compound rotors that lasted longer than 200 ms. Bars are averages across episodes. Error bars are SDs. Inset: an expansion showing lifetimes exceeding 1.0 s.

Fig. 7. Temporal fluctuation in the no. of compound rotors (with lifetimes exceeding 200 ms) simultaneously present on the epicardium. Data are from episode 6 in Table 1. Dashed line is a linear regression line.
LIFETIMES OF ROTORS DURING VF

a compound rotor cannot drive the VF episode (i.e., be a mother rotor) unless it persists for the entire episode. Only 1 of the 17 VF episodes we analyzed contained compound rotors that lasted for the entire mapped interval. Previous epicardial mapping studies were unable to document mother rotors in either the left (16, 21, 22) or right (22, 23) ventricle. However, these studies could not exclude epicardial mother rotors because only limited epicardial regions were sampled. In the present study, we used a newly developed optical mapping system (12) that can identify and track VF wave fronts across nearly the entire epicardium. Thus we extend the previous results and exclude stable epicardial rotors (with the possible exception of a rotor at the very tip of the apex) as a requirement for VF maintenance in this experimental model.

The possibility still exists that VF in pig hearts is driven by a stable mother rotor whose filament does not intersect the epicardium and therefore cannot be observed directly with epicardial mapping. However, stable mother rotors were not observed in studies mapping limited nonepicardial regions, including the septum (10) and sections across the right (27) and left (21, 27) ventricular free walls. Further studies are needed to confirm or exclude the presence of stable nonepicardial mother rotors.

Despite the paucity of compound rotors that persisted for the entire mapped interval, shorter-lived rotors were common. We found that at any given instant, roughly a dozen (with lifetimes of at least 200 ms) were simultaneously present on the epicardium. These data suggest that the formation of new rotors is critical for VF maintenance because once started, rotors will eventually die out by themselves. This implies that rotor formation may be a more attractive target than rotor termination in the development of new anti-VF therapies.

Limitations. In the present study, we imaged VF activation patterns with high spatial (~1.6 mm) and temporal (750 frames/s) resolution over the great majority of the swine epicardial surface. However, patterns within the ventricular walls were not recorded. The study focused on VF relatively early after induction (Wiggers’ stage II). Activation patterns are known to change as VF proceeds (9, 29), so our findings may not apply to more prolonged VF. We mapped isolated perfused swine hearts that were exposed to BDM to eliminate motion artifacts in the optical recordings. Other species were not studied. We previously found that VF patterns in this preparation are slower and more regular than those in the same hearts before excision (18). Other authors have found that BDM converts VF to a stable tachycardia in isolated ventricular tissue from dogs (19) and pigs (13). In addition, Evans et al. (7) used BDM to stabilize reentry to create a model of monomorphic ventricular tachycardia in rabbit hearts (7). Thus, because of the slowing and stabilization effects of heart isolation and BDM on VF, we expect that rotor lifetimes are substantially shorter in intact hearts than they were in the present study.

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