Spatial Distribution of Phase Singularities in Ventricular Fibrillation

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**Background**—Multiple excitation wavelets are present during ventricular fibrillation (VF). The underlying wavelet organization of VF is unclear. Phase singularities (PSs)—locations of ambiguous activation state—underlie reentry and wavelet splitting and represent the sources of VF. Understanding the mechanisms of PS formation might be important in the development of effective therapies for sudden death.

**Methods and Results**—We performed voltage, phase, and PS mapping in fibrillating ventricles, applying an automated PS detection algorithm to optically recorded fibrillation signals. PS clustering was noted along epicardial vessels, ridges of endocardial trabeculae, and papillary muscle insertions. Microscopically, these locations correlated with areas of apposition of fibers with different angulations and intramural vessels. A total of 83.2% of PSs were formed at and meandered about these anatomic structures, which acted as stabilizers: PSs colocalizing at anatomic substrates had longer life spans than nonanatomic PS (82.46±60.8 versus 40.5±31.9 ms, P<0.01). The RV endocardium had a higher PS incidence than the epicardium (42.3±9.2 versus 23.5±11.6 PS/s, P<0.01). Autocorrelation showed that irregular behavior was spatially restricted to anatomic heterogeneities compared with other areas, which had nearly periodic behaviors. Simple spatial PS distributions underlay complex and variable activation patterns attributable to variable PS behaviors, life spans, and inter-PS interactions.

**Conclusions**—PSs occur in a nonrandom spatial distribution and colocalize with normal anatomic heterogeneities. Varying PS behaviors and life spans but stable PS spatial distributions cause ever-changing activation patterns that characterize VF. (**Circulation. 2003;108:354-359.**)

**Key Words:** fibrillation ■ arrhythmia ■ waves

*In this study we show a striking spatial colocalization of PSs with normal anatomic structures in fibrillating healthy hearts. These results suggest a critical role of anatomic structures in the maintenance of VF.*

**Methods**

**Isolated Swine Ventricle Preparations**

The experimental models have been previously described.9,10 For right ventricular (RV) studies, the RV wall was excised, perfused with Tyrode’s solution, and placed in a tissue bath. Optical mapping during VF was performed on the endocardial surface (n=12) as well as the epicardial surface (n=9). For left ventricular (LV, n=9) studies, we used a modified wedge preparation: A rim of tissue surrounding the left circumflex and the second obtuse marginal artery was excised and perfused, leaving an inverted L-shaped preparation that contained at least part of the posteromedial papillary muscle. The tissue was placed in the tissue bath with the transmural cut surface up, which was the mapped surface. In both ventricles, VF developed during tissue manipulation and persisted thereafter as long as perfusion was adequate. VF can persist in a stable fashion for hours in these models.9,10
developed automated PS tracking algorithm. This was compared (typically 200 frames), PSs were identified using our recently persisted. For cumulative PS display over long acquisition intervals was quantified as the number of frames for which individual PSs identified manually as sites where phase was ambiguous where all PSs in VF. For PS quantification and lifespan analysis, PSs were identified by wavebreaks (Figure 1). Phase portraits were generated by plotting the points where wavelet front and wavelet back meet are identified as wavebreak points (b). c, Simultaneous phase (θ) map, showing a point where all phases converge (PS), which is identified using the PS tracking algorithm (d) at the same location as the wavebreak point. e through i, Simultaneous cumulative displays of PS (top) and wavebreak (bottom) in 5 fibrillating Langendorff-perfused rabbit preparations, showing equal spatial distributions.

Rabbit Langendorff Preparation
New Zealand rabbits (∼3.5 kg, n=6) were obtained from a USDA-licensed commercial rabbit vendor in Southern California. The rabbits were anesthetized with sodium pentobarbital (60 mg/kg). The heart was removed, mounted in a Langendorff apparatus, and perfused with Tyrode’s solution with a pressure of ∼70 mm Hg. The heart was suspended in a vertical position from the perfusion catheter, and gauze was sutured to the apex to drain the venous efflux and mitigate motion. The anterolateral epicardial surface of the LV was mapped, with left anterior descending artery at the left edge of the mapping field. Pacing the RV at 5 times the diastolic threshold with decreasing cycle lengths (300 to 100 ms at 10-ms decrements) was performed until VF was induced.

Optical Mapping and Data Processing
The optical mapping system and spatiotemporal filtering methods have been described previously. The tissues were stained with 1 to 2 μmol/L di-4-ANEPPS. Light from a laser source (532 nm) was delivered to the tissue. The fluorescence was collected with a CCD camera. No mechanical uncouplers were used. Recorded during each acquisition were 2.3 to 11.5 seconds of data with a temporal resolution ranging from 255 to 420 frames per second. Phase mapping was performed to evaluate the location and evolution of PSs in VF. For PS quantification and lifespan analysis, PSs were identified manually as sites where phase was ambiguous where all phases converged. PSs were counted in each frame, and their lifespan was quantified as the number of frames for which individual PSs persisted. For cumulative PS display over long acquisition intervals (typically 200 frames), PSs were identified using our recently developed automated PS tracking algorithm. This was compared with a wavebreak tracking algorithm that initially identified the depolarizing front and repolarizing back of the wavelet by detecting adjacent pixels whose values cross the median in either direction. The points where wavelet front and wavelet back meet are identified as wavebreaks (Figure 1). Phase portraits were generated by plotting fluorescence F against F, where n is the frame number and r was chosen between 4 and 8 frames. To assess periodic behavior, voltage signals were processed by autocorrelation: An individual signal was correlated with itself with progressive imbedded time delays. In this analysis, correlation coefficients (r) are calculated for a range of delays ranging from 0 to 200 frames: Beyond a delay of zero (r=1), the delay corresponding with the most common cycle length of periodicity, if present, would yield the highest r. Additional delays would give variable r coefficients. In the presence of periodicity, subsequent peaks of high r coefficients will be present at delays corresponding with multiples of the cycle length of periodicity (see Figure 5). Data are presented as mean±SD. The proportions of anatomic versus nonanatomic PSs were compared using χ^2 tests, and PS lifespans were compared using t tests. P<0.05 was considered significant.

Histological Studies
After the mapping studies, 5-μm-thick transmural sections were cut parallel to the mapped surface from paraffin-embedded tissue blocks. The slides were stained routinely with trichrome stain.

Results
PS Localization
PSs and wavebreaks coincided spatially (Figure 1) and were not randomly distributed. Cumulative PS display during VF showed unequivocal alignment of PSs with certain anatomic structures. In the epicardium, PSs formed along the course of epicardial arteries, whereas in the endocardium, they formed along ridges of endocardial trabeculae. Figure 2 shows examples. Mapping of transmural surfaces revealed a non-random PS distribution. Superimposition of PS locations with low-power histological cuts of the mapped tissue allowed gross estimation of the PS histological determinants. PSs clustered at the intramural insertion of the papillary muscles, intramyocardial arteries, and between subepicardial bundles of myocyte fibers of different orientation (Figure 3). PSs were either generated at or attracted to anatomic structures. Once in a particular structure, they tended to meander within it until extinguished, either by reaching a tissue boundary or by wavelet collision. Figure 4 shows an example of a PS meandering on an epicardial artery (photograph shown in Figure 5). Movies of simultaneous voltage, phase, and PS mapping are available in the online Data Supplement.

Voltage Dynamics and Anatomy: Local Periodicity Versus Irregular Dynamics
Voltage and phase traces obtained from sites located in endocardial trabeculae ridges, the papillary muscle insertion, or epicardial arteries showed frequent instances of low amplitude and double potentials with voltages close to the mean value, interspersed with runs of fully developed poten-
These unstable voltage dynamics led to frequent indeterminate phases (close to the center in the phase portrait). Although this phenomenon was clearly associated with readily identifiable anatomic structures, its occurrence was still unpredictable and subject to dynamic wavelet behavior. Whenever the PS was not visiting these structures, fully developed potentials could be recorded. This effectively rules out inadequate or artifactual signal recording. Autocorrelation (Figure 5) showed a first peak corresponding with the cycle length of the occasional fully developed potentials but no other consistent tall peaks. In contrast, in locations where anatomy is homogeneous (Figure 5), nearly periodic activation patterns were detected, the phase portraits had hollow ring-like patterns, and autocorrelation showed peaks corresponding with the periodicity cycle length, its double, triple, and so forth, suggesting a higher degree of regularity.

**PS and Wavelet Behavior**

PSs were continuously formed and extinguished, with variable chirality and unstable spatial locations. Despite their many variations, their cumulative spatial distribution was stable, consistently clustered at anatomic structures. The wavelet manifestations included reentry and wave splitting but most commonly were simply a wavelet delimiter. Figure 6 shows an example where multiple PSs were present. Local anatomy (epicardial arteries in this example) generated PSs at consistent locations but led to widely variable activation patterns, depending on the PS chirality and inter-PS interactions, which were subject to dynamic behavior. Regardless of the varying activation patterns, the spatial distribution of PSs remained relatively stable.

**Dynamic Versus Anatomic PSs**

Previous theoretical and experimental studies have suggested that dynamic instabilities of cardiac tissue are a sufficient substrate for sustaining fibrillation. However, the location of PSs was not determined in those studies. In this study we found that PSs without a structural basis were rare (370 of 2202 in 36 episodes, 16.8% of all detected; 175 of 1008 in 12
Transmural PS Gradient

Previous studies have suggested a critical role of the endocardium in the generation of fibrillation. An endocardial-to-epicardial activation rate gradient and more complex frequency distribution have been proven in the endocardium relative to the epicardium. We found a higher incidence of PSs in the endocardium than in the epicardium (42.3 ± 9.2 PSs per second, compared with 23.5 ± 11.6 in the epicardium, P < 0.01), which may explain the previous findings. The transmural surface had an intermediate incidence of 28.1 ± 12.6 PSs per second (P < 0.01 compared with the endocardium). The spatial density of PSs was highest in the transmural surface (5.6 PSs/cm²), followed by the endocardium (4.7 PSs/cm², P = NS), and lowest in the epicardium (2.6 PSs/cm², P < 0.01 compared with both endocardium and transmural surface). These differences may be attributable to different degrees of histological complexity in the 3 preparations.

Discussion

There are several major findings of our study. First, detailed quantitative analysis and cumulative display of PSs showed close colocalization of PSs with underlying anatomic heterogeneities, suggesting that most have anatomic determinants. Second, PS meandering was determined by underlying anatomic heterogeneities. Third, spatial autocorrelation analysis demonstrated spatially arranged local periodicity and irregular dynamics. Fourth, spatial PS distribution was relatively stable in the presence of varying activation patterns.

Isolated episodes of reentry and wave splitting have been shown to occur in certain anatomic heterogeneities. Despite their relevance, reentry and wave splitting are relatively rare phenomena during fibrillation. PSs, however, are the necessary engines of fibrillation whether maintained by a mother rotor or dynamic wavebreak and only lead to these phenomena on a probabilistic basis. The colocalization of most PSs with these structures suggests an enhanced role of anatomic substrates from mere anecdotal inducers of reentry
and wave splitting to key players in the maintenance of fibrillation.

The origin of mapped multiple wavelets in VF is disputed. The multiple wavelet hypothesis proposed by Moe et al.\textsuperscript{21} relied on preexisting dispersion of refactoriness to promote wavebreaks. Wavebreaks can also arise from dynamic oscillations in the recovery of excitability.\textsuperscript{8,16–18} Electrical restitution (the variation of action potential duration and conduction velocity with the diastolic interval) has been shown to be a major determinant of dynamically induced wavebreaks.\textsuperscript{17,22–24} Pharmacological modulation of electrical restitution may convert fibrillation into tachycardia in isolated ventricular tissues\textsuperscript{23,24} by eliminating spiral wave breakup.\textsuperscript{24} The present study suggests that normal anatomic heterogeneities play a key role in either generating or attracting wavebreaks (PSs). Our findings are consistent with simulations showing that preexisting heterogeneities significantly reduce the level of dynamic instability required to create PSs.\textsuperscript{25,26} However, it is impossible to discern whether PSs are primarily formed at these locations or simply attracted to them. The fact that PSs persist for longer periods of time when in a particular anatomic substrate suggests at least a stabilizing effect and supports the relevance of this functional-anatomic interaction. Functional dynamic heterogeneities, which can determine spiral wave meandering in simulated cardiac tissues,\textsuperscript{8,16,17,22} are likely to be a determining factor in PS meandering and inter-PS interactions. Anatomic heterogeneity, on the other hand, may exert a stabilizing effect and lengthen the life span of these PSs.

The focal source hypothesis\textsuperscript{4,5,27} postulates that fibrillation is maintained by a stable, rapid reentrant circuit (the “mother rotor”) from which activation wavelets emanate but fail to conduct 1:1 to the surrounding tissues because of preexisting heterogeneities. Fibrillatory conduction originates wavebreak and leads to multiple wavelets, which are considered an epiphenomenon rather than the origin of fibrillation. A limitation of the focal source hypothesis has been the inability to identify a stable rotor in isolated pieces of tissue.\textsuperscript{10,18} Nevertheless, our data are compatible with this paradigm and may explain situations in which dominant frequency borders are stationary because of clustering of PSs at locations corresponding to anatomic features.\textsuperscript{27} These frequency domain boundaries have also been correlated with certain anatomic locations.\textsuperscript{10} Our study ties these 2 findings together.

The potential mechanisms for the colocalization of PSs with anatomic heterogeneity include the potential of relative inexcitability. Pinning of scroll waves to unexcitable elements is a well-documented phenomenon in excitable media.\textsuperscript{28} Epicardial and intramural vessels can anchor reentrant excitation.\textsuperscript{12,13} The papillary muscles and endocardial trabeculae may alter propagation by the additional current sink caused by the increased tissue thickness. Abrupt fiber orientation changes\textsuperscript{10} as seen transmurally and in the subepicardium can lead to anisotropic (resistive) discontinuities.\textsuperscript{29}

**Figure 6.** PS variability but consistent location. a through f, Consecutive (5 frames apart) phase maps from epicardial rabbit Langendorff preparation (top) with corresponding voltage maps (bottom). White arrows show direction of activations. a, Two adjacent PSs of opposite chirality are present, but no reentry is completed. b, The wavelet on the bottom (lower arrow) rotates clockwise (contrary to the chirality of the initial PS, probably attributable to fusion with another wavelet breaking through to the epicardium). This lower wavelet activates the lower portion of the tissue and completes 1 rotation (b through f) around a PS that appears in b. Meanwhile, the wavelet on top activates the right superior portion. On collision with the lower wavelet, 2 PSs are generated (l). Later in the same episode, a similar PS location is seen (compare g with a). This time a figure-eight reentry is completed (g through k). Other wavelets are seen arising from the lower portion of the tissue but do not interfere with this rotation. k, Another wavelet collides with the lower wavelet of the figure-eight circuit and eliminates the counterclockwise PS (not present in l). The upper wavelet completes one more clockwise rotation alone. m and n, Cumulative PS display during intervals depicted in a through f (in m) and g through l (in n), showing very similar patterns despite the changing activation patterns. o, Cumulative PS display of 1 second of fibrillation, outlining the epicardial vessels. p, Local electrograms of sites visited by the PS in interval a through f (site 1) and the PS in interval g through l (site 2), as well as a site away from both (site 3).
factors versus dynamical restitution-based factors on PS formation. Future studies will be needed to test this idea by examining the effects of flattening APD restitution on PS formation at anatomic structures.

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