Frequency Analysis of Ventricular Fibrillation in Swine Ventricles

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Abstract—It has been suggested from frequency analysis that cardiac fibrillation is driven by stable intramural reentry, with wavebreak occurring due to failure of 1:1 propagation. We tested this hypothesis with a combined experimental and theoretical approach. Optical mapping was performed on epicardial, endocardial, and transmural cut surfaces of fibrillating swine ventricles. Wavelets were characterized, the frequency content of optical signals analyzed, and space-time plots (STPs) constructed to detect Wenckebach-like conduction. The findings were compared with simulations in 2D and 3D cardiac tissue using the Luo-Rudy action potential model. The incidence of reentry in the cut transmural surface (11.8% in right ventricle, 14.3% in left ventricle) was similar to that on the endocardial surface (13.1%, \( P=\text{NS} \)) but greater than on the epicardial surface (7.7%, \( P<0.01 \)). Frequency spectra of optically recorded membrane voltage were organized into spatial domains with the same dominant frequency, but these domains were nonstationary. In STPs, pseudo-2:1 conduction block was caused by double potentials arising when reentry occurred on the recording site rather than true Wenckebach conduction. The latter was observed in 11 of 166 STPs but did not occur at borders of high-to-low frequency domains. In simulations, similar findings were obtained when action potential duration (APD) restitution slope was steep. Stationary dominant frequency domains with Wenckebach conduction patterns were observed only in the presence of shallow APD restitution slope and marked nonuniform tissue heterogeneity. In conclusion, stable intramural reentry as the engine of fibrillation was not observed. Our findings support the idea that dynamic wavebreak plays a fundamental role in the generation and maintenance of ventricular fibrillation. (Circ Res. 2002;90:213-222.)

Key Words: fibrillation ■ Fourier transform ■ restitution ■ reentry

The mechanisms underlying ventricular fibrillation (VF) remain incompletely understood. The original hypothesis of Moe et al described multiple, disorganized wavelets. Dispersion of refractoriness was shown to lead to reentry and increase the vulnerability to VF, leading to the concept that tissue heterogeneity was the cause of VF. Later, functional reentry was demonstrated in the form of spiral waves during VF. Furthermore, it was shown in simulations that even in homogeneous tissue, spiral waves could break up, mimicking real VF in which reentry is typically transient and relatively rare. These theoretical predictions suggested that dynamically induced heterogeneity, in addition to preexisting heterogeneity, may play an important role in causing the wavebreaks that initiate and maintain VF. The most important determinant of the purely dynamically induced component of heterogeneity has been identified in theoretical and experimental studies as electrical restitution, ie, the variation of action potential duration (APD) and conduction velocity (CV) with the diastolic interval.

Recently, Chen et al and Zaitsev et al used fast Fourier transform (FFT) analysis of membrane voltage signals during VF and presented evidence that a stable, high-frequency, intramural rotor, rather than wavebreak, is the engine of VF. In this focal source mother rotor paradigm, wavebreak is primarily a result of fibrillatory conduction, ie, Wenckebach-like conduction in regions that cannot follow the mother rotor with 1:1 conduction.

Conclusive proof of the mother rotor hypothesis is still lacking. A recent mapping study of transmural cut surfaces during VF failed to show stable intramural reentry, and frequency analysis failed to detect single stable dominant frequencies (DFs) in the FFT spectra of optical signals, instead finding multiple peaks and complex frequency spectra.

We addressed these issues using a combined experimental and theoretical approach. Optical mapping of the endocardial, epicardial, and cut transmural ventricular surfaces was performed during VF. We characterized activation wavelets, analyzed the frequency content of optical signals to determine the spatial and temporal stability of the regional DFs, and constructed space-time plots (STPs) to detect Wenckebach-
like conduction. Experimental studies were complemented by simulations in 2D and 3D cardiac tissue using the Luo-Rudy I action potential model.\textsuperscript{15}

**Materials and Methods**

**Right Ventricle (RV) Preparation**

The experimental model has been previously described.\textsuperscript{16} The hearts of 15 farm pigs of either sex (purchased from S&S Farms, Ranchita, Calif) were removed via thoracotomy, the RV wall was excised and placed in a tissue bath, and the right coronary artery was perfused. In 12 tissues, the endocardium faced upward, an oblique cut was then performed at the distal edge, exposing the transmural surface\textsuperscript{13} and the right coronary artery was perfused. In 9 preparations that always contained at least part of the posteromedial obtuse marginal artery was excised, leaving an inverted L-shaped endocardium. For comparison of transmural versus epicardial or endocardial reentry, 3 RVs were mapped in the epicardium and 3 in the endocardium. The research protocol was approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center and followed guidelines of the American Heart Association.

**Left Ventricle (LV) Preparation**

Our LV wedge preparation has been previously described.\textsuperscript{13,17} In 9 tissues, a rim of tissue surrounding the left circumflex and the second obtuse marginal artery was excised, leaving an inverted L-shaped preparation that always contained at least part of the posteromedial PM. The tissue was placed in the bath with the transmural cut surface facing upward. In 3 tissues, the left circumflex artery was ligated proximally and a wedge of tissue surrounding the left anterior descending artery was cut, exposing the transmural surface of the interventricular septum.

**Optical Mapping**

Tissues were stained for 20 minutes with 1 to 2 \( \text{mol/L} \) di-4-isothiocyanate (500±30 nm) or laser (532 nm) light. The fluorescence was collected with a CCD camera, at either 270 or 435 frames per second, for either 4.3 or 2.3 seconds (1200 and 1000 frames, respectively). To test the short-term time dependency of frequency domains, several 5000-frame (11.5 seconds) recordings were performed in 3 tissues.

**Transmembrane Potential Recording**

One-minute recordings of single-cell transmembrane potentials (TMPs) were performed in 6 RV endocardial tissues, using a standard glass microelectrode, digitized at an acquisition rate of 5000 Hz.

**Data Analysis**

Optical signals were processed to reduce noise as described previously.\textsuperscript{13} Wavelets were identified using our previously described depolarization and repolarization detection algorithm.\textsuperscript{13} Points where depolarization and repolarization met were defined as wavebreak points. Reentry was defined as wavefront rotation around a wavebreak point completing a 360° cycle (although a stationary center of rotation was not required). The fraction of reentrant wavelets was determined as the ratio of the number of wavelets participating in a reentrant circuit over the total number of wavelets in the mapping field. Isochronal maps were generated based on the location of wavefronts in each frame, coded to different colors over time.

**Computer Simulations**

We simulated cardiac arrhythmias using the following partial differential equation\textsuperscript{18}:

\[
\partial V / \partial t = - I_{ion}/C_m + \nabla \cdot \vec{D} = \nabla V
\]

where \( V \) is the transmembrane potential and \( C_m \) the membrane capacitance. \( I_{ion} \) is the total ionic current density of the membrane, which was generated from phase I of the Luo and Rudy (LR1) action potential model.\textsuperscript{15}

\[
\vec{D} = \sigma \mathbf{S} \cdot C_m
\]

is the diffusion tensor, where \( \sigma \) is the conductivity tensor and \( \mathbf{S} \), the surface-to-volume ratio of the cell.

Details of incorporating fiber rotation into Equation 1 are described in our previous study.\textsuperscript{18} In the 2D simulation, we assumed that diffusion in Equation 1 was isotropic. However, we incorporated electrophysiological heterogeneities into the tissue as previously described.\textsuperscript{16} Methods for detection of reentry, FFT analysis, and pseudo-ECG generation were the same as in the experiments.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

**Results**

**Wavelet Characteristics During VF**

The Table summarizes the number of wavelets per mapped area, the mean wavelet lifetime, the incidence of complete reentry, and the mean number of reentry cycles per reentry episode. The combined (RV and LV) incidence of full-loop reentry was 11.9% (of all activation pathways), with a mean number of reentry cycles of 6.5±5.7 per reentry episode. The shortest stable rotor from 83 epochs of VF in 12 LV and 15
RV preparations lasted 69 ms (1 cycle) and the longest lasted 3423 ms (54 cycles). The incidence of transmural reentry in both LV and RV was similar to that of the endocardium but higher than that of the epicardium.

**Characteristics of DF Domains**

DFs, defined as the largest peak in the FFT spectra of the optical voltage signal at each pixel, were detected in all VF episodes and were spatially localized in well-circumscribed domains. However, these DF domains were not stationary over time, and, except at gross anatomical structures (see below), shifted their location continuously. This instability was present not only in different VF acquisitions but also within segments of a prolonged VF recording. Figure 1 shows a typical example of a 9.2-second (4000 frames) epoch of VF recorded from RV epicardium. Figure 1A shows 5 to 10 spatially discrete domains with DFs ranging from 11.9 to 14.0 Hz, which did not remain stationary over time. In fact, the spatial patterns of DF domains, when analyzed in consecutive segments, not only differed from each other, but also differed from the pattern obtained when the corresponding segments were analyzed as a whole (Figure 1A, compare panels a and b versus panel c, and panels d and e versus panel f). Subtraction maps, cross-correlation, and statistical analysis of the frequency distributions confirmed the differences between consecutive DF maps (see the online data supplement available at http://www.circresaha.org). Figure 2A shows another example.

Only at locations corresponding to gross anatomical structures such as PM insertions and endocardial trabeculae in the RV were boundaries between DF domains relatively stable. However, the DF values on either side of the boundary often changed, and the direction of DF gradients across such boundaries was variable. Figures 2A and 3A show examples: despite generally shifting DF domain boundaries, there was one location (black dotted line in Figure 2A, red arrows in Figure 3A) that consistently showed a domain boundary, regardless of the frequencies it separated. Moreover, the high-DF region could be either above or below the boundary. Of all VF acquisitions, 73% exhibited one boundary in a stable location (defined as present in at least 4 of 5 frequency maps). The mechanism of boundary formation at this location was due to the increased incidence of reentry at these locations, leading to double potentials (a characteristic of the core of reentry) generating shifts in DFs (see next section).
Local DF Instability

Local DF instability underlay frequency domain instability. Figures 1A and 2B illustrate this finding. Figure 1A shows representative FFT spectra from three pixels (labeled 1, 2, and 3) obtained from different segments of the acquisition. In Figure 1A, at the pixel labeled 1, the FFT spectrum had a DF of 11.9 Hz during the first 4.6 seconds of VF (Figure 1Ac) that shifted to 13.0 Hz during the next 4.6 seconds (Figure 1Af). This pixel had shown other DFs during other segments of the VF episode (13.2 Hz in Figures 1Aa and Figure 1Ad, 12.3 Hz in Figure 1Ab, and 12.7 Hz in Figure 1Ad). However, when all 9.2 seconds were analyzed together, the resulting DF was 13.3 Hz (Figure 1C).

Figure 2B, left, shows, in a different tissue, FFT spectra of a single pixel calculated at 2.3-second intervals throughout 11.5 seconds of VF. Five different DFs are present. DF was unstable consistently in all tissues. Figure 2B, right, shows the DF time course of single pixels for 5 different tissues. To show that the changing DF was not due to filtering of the optical signal, we also analyzed the FFT of prolonged single-cell TMP recordings, which revealed a similar second-to-second variability in DF (Figure 2C).

The DF as a Single Peak: Impact of FFT Resolution

Although a largest peak (DF) could always be identified, the FFT spectra were multipeaked and exhibited significant broadband power. This was especially true when long intervals were analyzed, as illustrated by the comparison of FFT spectra of 2.3-second intervals (0.4-Hz resolution, Figure 1A, panels a, b, d, and e) with those of 4.6-second intervals (0.2-Hz resolution, Figure 1A, panels c and f), 9.2-second intervals (0.1-Hz resolution, Figure 1C), and finally, 1-minute recordings from TMPs (0.019-Hz resolution, Figure 2C).

Apparent Conduction Block Patterns Caused by Scroll Wave Cores

Figures 3 and 4 illustrate the most common form of apparent conduction block observed in the STP. Between 150 and 400 ms, the STP (Figures 3C and 4B) shows a discontinuity in activation sequence with branching bands in the lower third. The DF corresponding to these regions (pixels 3 through 5 in Figure 4) was 11.04 Hz, with the adjacent sites on either side having lower values of 9.77 to 10.19 Hz. One possible interpretation, consistent with the mother rotor hypothesis, is that reentry (or
breakthrough activation arising from a rotor underneath the mapped surface) with a frequency of 11.04 Hz was driving the adjacent domains with 11:10 conduction block occurring on either side. However, optical activation maps did not reveal such a pattern. As shown in Figure 3, no stable rotor was visualized on the transmural surface. Instead, two colliding wavefronts (Figure 3B, at arrows in a and b) resulted in the formation of a reentrant circuit (Figure 3B, c through i). The core of this reentrant circuit was located in the 11.04-Hz DF domain (sites 3 through 5 in Figure 4A). There was no evidence of conduction block in the surrounding domains with lower DF. Figure 4 illustrates that the shift to higher DF at the core compared with surrounding sites could be attributed to double potentials in the core of the scroll wave (as seen in the voltage traces at sites 3 through 5 in Figure 4C). In addition, there was a small (nondominant) peak in the FFT spectra at 22 Hz, corresponding to the double-potential frequency, which coincided with the DF harmonic. As described previously, this pattern of branching bands in the STP (Figures 3C and 4B) is characteristic of the core of a rotating scroll wave. This reentry was unstable both spatially (note the subtle core displacement to the right in the isochronal maps, Figure 3B, c through i) and temporally, as it was successively interrupted (Figure 3B, g and i) and resumed (Figure 3B, h and j). In Figure 3B, k through l, another wavelet invaded this area and the reentrant circuit was terminated and the branches in the STP fused together. Note that the DF in the region where this core formed was only 11.04 Hz, not double the DF of the adjacent regions (9.77 to 10.19 Hz). This reflects the fact that the segment during which double potentials were present in the voltage traces (150 to 400 ms) was only part of the total segment (0 to 2300 ms) from which the FFT spectrum was obtained and thus did not have sufficient power to become the DF. However, the high-frequency segment was sufficient to alter the relative powers of the multiple peaks in the FFT spectrum near 10 Hz so that the DF was shifted to a higher value. We confirmed this explanation by simulating the sequence with sine waves of mixed frequencies and examining the FFT spectra (data not shown).

Domain Boundaries and Apparent Wenckebach Conduction
We examined STPs for evidence of conduction block. No consistent direction of propagation gradient was present (Figures
Although rare, we occasionally identified Wenckebach-like conduction patterns in the STP (11 of 166 STPs). Figure 5 shows an example. Wenckebach-like conduction occurred once at ≈400 ms and then again at 550 ms on the STP (Figure 5B). Figure 5A shows the corresponding isochronal activation maps, and Figure 5D the optical voltage traces from sites 1 through 3 as indicated. In Figure 5Aa, a wavelet spread downward from the left upper portion of the tissue in a planar fashion, with wide isochrones across the STP line, reflecting rapid conduction. In the following activation (Figure 5Ab), this wavelet formed a reentrant circuit, whose core was near the vertical STP line. This reentry persisted for two additional rotations (Figure 5A, c and d), and then was interrupted by a wavelet spreading downward from the top (Figure 5A, e and f). The subsequent activation, also from the top (Figure 5Ag), blocked between site 1 and sites 2 and 3 (Figure 5Bg), causing a 7:6 Wenckebach-like cycle. Later, a second Wenckebach-like block occurred by a similar mechanism, but this time was due to an upward planar wave (Figure 5Ak).

Therefore, a 7:6 Wenckebach-like conduction pattern was followed by a 4:3 Wenckebach-like cycle. Of note, the apparent block occurred in both directions (from top-to-bottom in the first case and from bottom-to-top in the second), which is inconsistent with the concept of one stable domain driving the neighboring regions. However, in both episodes, the apparent block occurred during propagation from a region of low DF (10.61 Hz at sites 1 and 3) to a region of high DF (11.46 Hz at site 2), as shown in the accompanying FFT spectra (Figure 5C). This does not support the idea that high-DF domains correspond to regions that are better able to sustain 1:1 conduction, as postulated by the mother rotor hypothesis.

**Computer Simulations**

We performed computer simulations to examine the relative importance of tissue heterogeneities and APD restitution steepness. In simulated 3D tissue with a physiological degree of fiber rotation but otherwise homogeneous conditions, steep APD restitution slope in the cardiac action potential model caused spontaneous scroll wave breakup, with multiple wavelets coursing through the tissue similar to VF. The Table summarizes the average number of wavelets per mapped area, the mean lifetime of wavelets, the incidence of completed reentry, and the mean number of reentry cycles per reentry episode. The incidences of
reentry on the epicardial/endocardial surfaces were similar and matched the incidence on the endocardial surface in the biological tissue experiments. In contrast, the incidence of transmural reentry was lower than on the surfaces in simulated tissue, which contrasts with the similar incidence in biological tissue. This discrepancy is probably due to the heterogeneities such as PM attachments and trabeculae in the biological experiments, which our previous mapping studies showed acted as transient anchoring points for reentry. These findings also indicate that the lack of morphologically identifiable scroll waves on the tissue surface does not reliably exclude scroll wave dynamics as the underlying mechanism.

We performed frequency analysis in 2D simulated tissue (due to the computational intractability for 3D tissue). In heterogeneous tissue with physiological APD restitution (slope \( H_1/10^2 \)) (Figure 6), we obtained similar findings as in the tissue experiments, with spatially discrete DF domains that shifted rapidly over time and space (Figure 6A). STPs across the boundaries of these domains commonly showed the branching 2:1 patterns characteristic of spiral wave cores, and patterns resembling Wenckebach-like conduction were also observed (Figure 6B). Homogeneous tissue gave similar results, except that the DF domains were smaller in size and varied over a smaller range (data not shown).

To reproduce DF domains whose boundaries and DF values remained stationary required heterogeneous simulated tissue with flattened APD restitution slope (Figure 7). Under these conditions, DF domains were well-defined and remained stationary in time and space (Figure 7A). STPs across these boundaries often showed clear Wenckebach-like conduction patterns as impulses propagated from a region of high-to-low DF (Figure 7B). The most rapid spiral wave, the mother rotor, was fairly stationary and located in a region with the highest DF (at the lower left corner in the spatial DF maps in Figure 7A).

**Discussion**

The hallmark of cardiac fibrillation is ongoing wavebreak, traditionally speculated to be the engine that sustains fibrillation. The recent hypothesis\(^{11,12}\) that wavebreak may be an
epiphenomenon, related to Wenckebach-like conduction as impulses originating from a relatively stable mother rotor unable to sustain 1:1 conduction through heterogeneous tissue, is an intriguing one. In this case, the mother rotor, rather than ongoing wavebreak, is the engine of fibrillation. The clinical implications are significant, because efforts directed at preventing ongoing wavebreak might then be therapeutically useless. The major pieces of evidence supporting the mother rotor hypothesis are as follows: (1) the correlation between DF and the frequency of reentry and breakthrough periodic activations; (2) the presence of DF domains that remain stable in space and time over many seconds\(^2\); (3) the observation in STPs of Wenckebach-like conduction at the borders between different DF domains\(^1,2\); (4) the relative infrequency of reentry on the surface of the heart during fibrillation,\(^6\) favoring an intramural location of the mother rotor; and (5) simulations suggesting that scroll waves may prefer to align their filaments along an intramural axis.\(^2\)

However, we were unable to substantiate these findings. Our major observations are as follows: (1) the uniqueness of DF, defined as the highest peak in the FFT spectrum, depended on the FFT resolution. With longer acquisition times, multiple peaks were present and selection of a single DF became ambiguous (Figures 1C and 2C). These findings bring into question the significance and utility of DF as a descriptive parameter in VF\(^14\); (2) DFs were unstable, and although discrete DF domains were present, they were neither temporally nor spatially stable over a time course of seconds (Figures 1 and 2). An exception occurred at certain anatomically distinct regions, such as PM and trabecular insertions, in which DF domain boundaries remained constant in location. However, the DF values on either side of the boundary varied freely (Figures 2A and 3); (3) Additionally, the specific value of the DF and the domain boundaries also depended on the duration of the FFT analysis; for example, the DF domains for successive 2-second epochs of VF neither resembled each other nor the DF domains obtained from the combined epochs (Figure 1); (4) Optical mapping failed to identify stable intramural reentry occurring at a higher incidence than on the endocardial surfaces (Table), although it

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**Figure 6.** Spatiotemporal instability of frequency domain distribution during VF in simulated 2D heterogeneous cardiac tissue (10×10 cm) with steep APD restitution slope, reproducing the findings in Figure 1. Data from 8 seconds of simulated VF are shown. A, panels a through f, DF maps in successive 2.0- (a, b, d, and e) and 4-second (c and f) intervals for a 5×5-cm area. No consistent pattern stationary in time or space is present. Red vertical line is the line sampled for STPs. Adjacent to each panel are FFT spectra at sites labeled 1, 2, and 3. B, STPs during 0 to 4 seconds of simulated VF. Discontinuities resembling conduction block result from drifting spiral wave cores and wave collisions, but no true Wenckebach patterns are present. C, Action potentials at sites 1 through 3 and pseudo-ECG corresponding to the STPs above. FFT spectra are shown at the far right.

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was greater than on the epicardial surface. An important limitation, however, is that we could map only a limited portion of intramural myocardium, so stable reentry could have existed elsewhere and been missed; and (5) Apparent conduction block patterns on STPs most typically showed a pseudo-2:1 pattern. However, activation mapping and identification of double potentials on optical traces in these cases showed that this pattern was due to the core of a spiral/scroll wave migrating through this region rather than conduction block (Figures 3 and 4). Wenckebach-like conduction was observed rarely (Figure 5), but when it was, it did not consistently occur at high-to-low DF domain borders. In summary, our findings suggest that in the fibrillating arterially perfused swine ventricle, DFs, frequency domains, and boundaries are dynamically generated by wavelet behavior rather than by anatomically determined conduction block.

The discrepancies between our findings and those of Chen et al and Zaitsev et al may be due to experimental conditions and/or species differences. Our computer simulations provide some insights into possible explanations. The first point is that when a wavebreak-driven fibrillation-like state is produced in tissue that is homogeneous except for fiber rotation, completed reentrant circuits were observed with a similarly low incidence (4% to 12%, Table). When wavebreak occurred in this setting, the broken end (tip or filament) tried to form a morphological spiral/scroll wave but usually could not complete a full loop of reentry due to interactions with other wavelets. Thus, failure to observe reentrant circuits does not exclude spiral/scroll wave dynamics as the underlying mechanism. Also, the lower incidence of reentry on the transmural surfaces, compared with the epicardial or endocardial surfaces in simulated 3D tissue, could not substantiate a preference for filaments to align parallel to the epicardial and endocardial surfaces, as described by Berenfeld et al. In biological tissue, anchoring to anatomical features such as PM insertions and trabeculae is a known factor accounting for the majority of reentrant circuits on the transmural surface.

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Spatiotemporal stability of frequency domain distribution during VF in simulated 2D heterogeneous cardiac tissue (10x10 cm) with shallow APD restitution slope, reproducing previously reported findings. Data from 8 seconds of simulated VF are shown. A, panels a through f, DF maps in successive 2.0- (a, b, d, and e) and 4-second (c and f) intervals for a 5x5-cm area. DF domains are stable over both time and space. Red vertical line is the line sampled for STPs. Adjacent to each panel are FFT spectra at sites labeled 1, 2, and 3, illustrating the DF stability of each region. B, STPs during 0 to 4 seconds of simulated VF, showing Wenckebach conduction block patterns. C, Action potentials at sites 1 through 3 and pseudo-ECGs corresponding to the STPs above. FFT spectra are shown at the far right.
To reproduce our experimental findings of unstable DF domains required the combination of steep APD restitution and tissue heterogeneity. Under these conditions, DF domains had similar characteristics to those observed experimentally, including pseudo-2:1 Wenckebach conduction patterns produced by the cores of spiral/scroll waves (Figure 6). Higher-order Wenckebach-like conduction was also observed, but as in the swine ventricle, did not occur exclusively at high-to-low DF domain borders.

Finally, to reproduce the findings reported by Chen et al.11 and Zaitsev et al.12 required flattening APD restitution slope to <1, as well as introducing nonuniform heterogeneities. The latter type of heterogeneity is different from the uniform heterogeneity (base-to-apex14 or endocardial-to-epicardial17) that characterizes normal ventricular myocardium. In these conditions, DF domains became spatiotemporally stationary, and Wenckebach-conduction block occurred at borders between high-to-low DF domains (Figure 7). Most of the experiments of Zaitsev et al.12 were performed in the presence of the excitation-contraction uncoupler diacetyl monoxime (DAM), which is known to flatten APD restitution slope.9 However, recognizing this drawback, they performed additional experiments in the absence of DAM and obtained similar results.12 In the arterially perfused swine ventricle, APD restitution slope during VF or pacing is typically >1, but whether this is true under their experimental conditions11,12 is unknown. One factor that can make APD restitution slope shallower is acute ischemia, which also promotes nonuniform heterogeneity in regional electrophysiological properties.22–24 Both alterations would favor the mother rotor mechanism.

In conclusion, both the restitution-based dynamic wavebreak mechanism and the mother rotor mechanism, coupled with preexisting tissue heterogeneity causing dispersion of refractoriness,25 may be relevant clinically in the maintenance of VF. Our observations suggest that during the initial phases of VF, dynamic wavebreak is likely to be very important in maintaining VF. However, as VF proceeds, the heart becomes ischemic, flattening APD restitution22 and promoting nonuniform regional electrophysiological heterogeneity, conditions under which we speculate that the mother rotor mechanism may become increasingly important.

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Methods

RV preparation

The experimental model has been previously described. Briefly, the hearts of 15 farm pigs of either sex (25 to 32 kg) were used for the study. After the hearts were extracted, the right coronary artery was perfused with oxygenated Tyrode’s solution. The composition of the Tyrode’s solution was as follows (mM): NaCl, 125; KCl, 4.5; MgCl₂, 0.5; CaCl₂, 0.54; Na₂HPO₄, 1.2; NaHCO₃, 24; and glucose, 5.5, along with albumin 50mg/L. The RV wall (weighting 28.3 to 34.6 g) was then excised and placed in a tissue bath. The endocardium faced up in 12 tissues. VF, which occurred during tissue manipulation, persisted in vitro in the isolated RV. An oblique cut was then performed at the distal edge, exposing the transmural surface. The cut was performed so that it would include the papillary muscle (PM). Optical mapping (see below) was performed in the cut transmural surface as well as the adjacent endocardial surface. For comparison of transmural vs. epicardial or endocardial reentry 3 RVs were mapped in the epicardium and 3 in the endocardium.

LV preparation

While experiments were conducted with RV, the LV was preserved by infusion of ice-cold cardioplegic solution (St Thomas’s Hospital). The composition of this solution was as follows (mM): NaCl, 110; KCl, 16; MgCl₂, 16; CaCl₂, 1.2; NaHCO₃, 10; with a pH of 7.8. A 3-minute infusion was performed every 20 minutes through a cannula into
the left main coronary artery. Our LV wedge preparation has been previously
described. In 9 tissues a rim of tissue surrounding the left circumflex and the second
obtuse marginal artery was excised, leaving an inverted L-shaped preparation that always
contained at least part of the posteromedial PM. The angle formed by the obtuse marginal
and circumflex arteries was opened and the tissue was placed in the tissue bath with the
transmural cut surface up, allowing mapping studies. In 3 tissues, the left circumflex
artery was ligated proximally and a wedge of tissue surrounding the left anterior
descending artery was cut, exposing the transmural surface of the interventricular septum.
Once the tissue was cut, warm, oxygenated Tyrode’s solution was substituted for the
cardioplegic solution. VF persisted in these tissues for 63 to 79 minutes. These 12 LV
wedges (weighting 37.6 to 42.8 g) were used for the experiments.

Optical Mapping

The optical mapping system was similar to the one described previously. The
tissues were stained for 20 minutes with 1 to 2 μmol/L di-4-ANEPPS (Molecular Probes,
Inc) in the Tyrode’s solution. In 15 tissues, the tissue was illuminated with
quasimonochromatic light (500±30 nm) from a 250 W tungsten-halogen lamp (Model
66196, Oriel Corp, Stratford, CT). The induced fluorescence was collected through a 600
nm long-pass glass filter (R60, Nikon, Tokyo, Japan) and a 25 mm/f-stop 0.85 video lens
(Fujinon CF25L, Fuji Photo Optical Co., Omiya City, Japan) with a 12-bit CCD camera
(Dalsa Inc, Ontario, Canada) operating at = 279 frames per second, acquiring 96 x 96
sites simultaneously. Each acquisition lasted for 4.3 s (1200 frames). In 9 tissues, solid
state, frequency doubled laser light was used (532 nm, Verdi, Coherent Inc., Santa Clara,
CA), the CCD camera operated at 435 frames per second (2.3 ms sampling interval) acquiring 128 x 128 sites, and each acquisition lasted for 2.3 s (1000 frames). To test the short-term time dependency of frequency domains, several 5,000-frame (11.5 s) recordings were performed in 3 tissues. The digital images were transferred to a personal computer with a frame grabber (IC-PCI-DIG16, Imaging Technology, Bedford, MA). No electromechanical uncouplers were used in the study.

**Transmembrane potential recording**

In 6 RV endocardial tissues, single-cell transmembrane potentials (TMP) were recorded using a standard glass microelectrode filled with 3 M KCl, coupled with an Ag-AgCl wire leading to a high input impedance and variable-capacity neutralization (Am-2 and ME-3221, Biodyne Electronics Laboratory, Los Angeles, CA). Data were acquired with an acquisition rate of 5000 Hz by AXON TL-1-40 A/D acquisition hardware and Axoscope 7.0 software (Axon instruments, Inc, Foster City, CA), and digitized with 12 bits of accuracy. In all six tissues, data were acquired prior to the staining with voltage-sensitive dye.

**Data Analysis**

The optical signals were temporally filtered and spatially averaged to reduce noise.\(^\text{2,5}\) For temporal filtering, we applied a 5-point time median filter to each pixel. We took the original first 5 data points (i.e. frames 1, 2, 3, 4, 5), found the median value of those points and used that as the new value for point (frame) 3. Then we took the next
original 5 points (i.e. frames 2, 3, 4, 5, 6), found the median value of those and use that as
the new value for point 4, and so forth. We then inverted the signals, reset the baseline at
zero (by subtracting the average of the 5 lowest fluorescent values recorded by that
pixel), and range-normalized each pixel from 0 to 255 (scaled using the average of the 5
lowest and 5 highest points, respectively). For spatial averaging, we averaged the
fluorescent values at each pixel with its 8 nearest neighbors. The temporal and spatial
filters were then applied a second time. Fluorescence values were displayed on a gray-
scale, with the maximal signal amplitude coded white (representing the fully depolarized
state) and the minimum signal amplitude coded black (representing the fully repolarized
state).

The activation wavelets were detected as follows: the computer first detected
adjacent pixels in each frame with pre-defined threshold value (corresponding to 50% of
the maximum value), and generated lines between them; if the values increased over the
subsequent frame, the edge was identified as the activation wavefront and colored red. If
the intensity decreased, the edge was identified as the repolarization waveback and
colored green. The area between a red and a green line constituted a wavelet. We defined
a wavebreak point in a propagating wavelet as a point where the activation wavefront
(red line) and the repolarization waveback (green line) intersected. Reentry was defined
by a wavefront rotating around a wavebreak point to complete a 360° cycle. The fraction
of reentrant wavelets was determined by the ratio of the number of wavelets participating
in a reentrant circuit over the total number of wavelets in the mapping field. Wavelets
that were smaller than 8 pixels, discontinuous (mosaic-like pattern), or not present in
subsequent acquisition frames were considered artifacts and were excluded from the analysis.

Isochronal maps were generated based on the location of wavefronts in each frame, coded to different colors over time.

FFT, STP and pseudo-ECGs were derived from optical recordings as described elsewhere. STP sampling lines were generated vertically and horizontally dividing the maps in two halves (see Figure 1), and these lines always crossed 2-5 frequency domains. Wenckebach-like conduction was defined to occur when the number of activations across one domain exceeded the number across the neighboring domain with a consistent ratio or with apparent decremental conduction.

The resolution of the FFT depended on the length of the acquisition analyzed: for optical mapping data it was 0.4 Hz, 0.2 Hz and 0.1 Hz when segments of 2.3 s, 4.6 s and 9.2 s were analyzed, respectively. For TMP data, the resolution was 1.2 Hz and 0.07 Hz for segments of 1 and 10 seconds, respectively.

Data are presented as mean ± standard deviation. \( \chi^2 \) test was used to compare percentages of reentrant wavelets in epicardium, endocardium, and transmurally. Kruskal-Wallis ANOVA was used to compare wavelet descriptors. A \( p < 0.01 \) was considered significant.

DF maps were obtained from 2.3 s segments of a given acquisition. Quantitative comparison of DF maps was made in 3 ways. First, subtraction maps were obtained where the values of one DF map (128 x 128 pixels) were subtracted from the values of a consecutive DF map. The differences were plotted in a new \( \Delta \text{DF} \) map. Second, we compared the distribution of frequencies between consecutive maps to determine whether
the two patterns could have arisen from a rearrangement of the same frequency
distribution. Histograms of different frequencies were generated for each map, a 5x2
contingency table was generated, and frequency distributions were compared using $\chi^2$
test. Third, cross-correlation was performed by plotting each pixel’s DF in one map
against the same pixel’s DF in the consecutive map. Correlation coefficient was
calculated.

**Computer Simulation**

We simulated cardiac arrhythmias using the following partial differential
equation:\(^8,^9\)

$$\frac{\partial V}{\partial t} = -I_{ion} / C_m + \nabla \cdot \vec{D} \nabla V \quad (1)$$

where $V$ is the transmembrane potential, $C_m$ the membrane capacitance. $I_{ion}$ is the total
ionic current density of the membrane. $\vec{D} = \bar{\sigma} / S_v C_m$ is the diffusion tensor, where $\bar{\sigma}$ is
the conductivity tensor, $S_v$ the surface-to-volume ratio of the cell. We used the phase I of
the Luo and Rudy (LR1) action potential model\(^10\) to generate the ionic current in Eq.(1).
The total ionic current in the LR1 model is: $I_{ion} = I_{Na} + I_{si} + I_k + I_{K1} + I_{kp} + I_h$.

$I_{Na} = \bar{G}_{Na} m^3 h j(V - E_{Na})$ where $I_{Na}$ is the fast inward Na$^+$ current; $I_{si} = \bar{G}_{si} df(V - E_{si})$ is
the slow inward current, assumed to be the L-type Ca$^{2+}$ current; $I_k = \bar{G}_k x h j(V - E_K)$ is
the slow outward time-dependent K$^+$ current; $I_{K1} = \bar{G}_{K1} K1 \omega(V - E_{K1})$ is the time-
independent K$^+$ current; $I_{kp} = 0.0183 K_p (V - E_{Kp})$ is the plateau K$^+$ current; and
$I_h = 0.0392 (V + 59.87)$ is the total background current. $m$, $h$, $j$, $d$, $f$, and $x$ are gating
variables satisfying the following type of differential equation:
\[
\begin{align*}
\frac{dy}{dt} &= (y_\infty - y)/\tau_y \\
\text{where } y &\text{ represents the gating variables. } y_\infty \text{ and } \tau_y \text{ are functions of } V. \text{ The ionic concentrations are } [\text{Na}]=18 \text{ mM, } [\text{Na}]_c=140 \text{ mM, } [\text{K}]=145 \text{ mM, and } [\text{K}]_o=5.4 \text{ mM, while the intracellular } \text{Ca}^{2+} \text{ concentration obeys:}
\end{align*}
\]
\[
\frac{d[Ca]}{dt} = -10^{-4}I_x + 0.07(10^{-4} - [Ca]).
\]
Details of the LR1 action potential model were presented in Luo and Rudy’s paper.\textsuperscript{10} We fixed $G_{s,i} = 16$ mS/cm\textsuperscript{2} and modified other channel conductance to achieve desired properties as described below.

In 3D simulation, the only heterogeneity is the fiber rotation. We assume the fibers are parallel and uniform in the x-y plane but rotate along the z-direction. Therefore, $\tilde{D}$ has the following matrix structure:\textsuperscript{8}

\[
\begin{pmatrix}
D_{xx} & D_{xy} & 0 \\
D_{yx} & D_{yy} & 0 \\
0 & 0 & D_{zz}
\end{pmatrix}
\]

where

\[
\begin{align*}
D_{xx} &= D_1 \cos^2 \theta(z) + D_\perp \sin^2 \theta(z) \\
D_{yy} &= D_1 \sin^2 \theta(z) + D_\perp \cos^2 \theta(z) \\
D_{xy} &= D_{yx} = (D_1 - D_\perp) \cos \theta(z) \sin \theta(z) \\
D_{zz} &= D_\perp
\end{align*}
\]

$D_1$ is the diffusion constant along the fiber direction, and $D_\perp$ is the transverse diffusion constant. We chose $D_1 = 0.001 \text{cm}^2/\text{ms}$ and $D_\perp = 0.0002 \text{ cm}^2/\text{ms}$. $\theta(z)$ is the fiber rotation angle along the z-direction. We used a uniform fiber rotation angle $\theta(z)=\alpha z$ with $\alpha=6.8^\circ/\text{mm}$. Tissue size was set as 5 cm x 5 cm x 2.2 cm. Changes of maximum
conductance in LR1 model for the 3D simulations are: $G_K = 0.423$ mS/cm$^2$ and $G_n = 0.045$ mS/cm$^2$, at which a scroll wave breaks up in the 3D tissue with presence of fiber rotation.

In 2D simulation, we assume there is no diffusion anisotropy so that we chose the following diffusion matrix:

$$\tilde{D} = \begin{pmatrix} D_{xx} & 0 \\ 0 & D_{yy} \end{pmatrix} = D \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$$

and we set D=0.001 cm$^2$/ms. Tissue size is 10 cm x 10 cm. To achieve desired restitution properties and electrophysiological heterogeneities, modifications to the LR1 model are as follows: 1) steep APD restitution causing spiral wave breakup in homogeneous tissue: $G_n = 0.049$ mS/cm$^2$ and $G_K = 0.45$ mS/cm$^2$; 2) Steep APD restitution plus electrophysiological heterogeneities causing spiral wave breakup: $G_n = 0.049$ mS/cm$^2$ but $G_K$ ranges from 0.38 mS/cm$^2$ to 0.5 mS/cm$^2$ to generate heterogeneities; 3) Electrophysiological heterogeneities with shallow APD restitution causing spiral wave breakup: $G_n = 0.049$ mS/cm$^2$ and $G_K = 0.45$ mS/cm$^2$, but $G_{K1}$ ranges from 0.24 mS/cm$^2$ to 0.6 mS/cm$^2$ to generate heterogeneities. The reason that we choose $G_{K1}$ instead of $G_K$ to generate heterogeneity is to keep the APD restitution in the shallow regime throughout the whole tissue.\(^{11}\) The generation of heterogeneity by randomly distributing $G_K$ or $G_{K1}$ in a certain size area is the same as in our previous study.\(^{11}\)

We used our advanced numerical method\(^{8,9}\) to integrate Eqs.(1)-(3). Simulation for 3D was carried out in the San Diego Supercomputer Center, and simulation for 2D was
carried out in a workstation. Methods for detection of reentry, pseudoECG generation, and FFT analysis are the same as in the experiments.
RESULTS

Statistical comparison of DF maps

Thirty-one DF maps from successive segments of individual acquisitions (five 2.3 s and two 4.6 s in the 11.5 s acquisitions, or two 2.15 s in the 4.3 s acquisitions) were analyzed and compared. Subtraction maps showed a $\Delta F > 0$ in 81 ± 8% of the pixels. Comparison of histograms of DF distribution revealed significantly different distributions by $\chi^2$ in all 31 pairs, indicating that successive DF patterns could not have arisen from a rearrangement of the existing DF’s. Cross-correlation also demonstrated poor correlation between consecutive DF values of each pixel, with an $r$ value averaging 0.19 ± 0.12 for 31 comparisons. Figure 1 of this supplement shows an example using the same acquisition in Figure 1 of the printed manuscript.
REFERENCES


FIGURE LEGEND

Figure 1. DF map comparison. $Aa$ and $Ab$ show two consecutive DF maps of 4.6 s segments within one 11.5 s acquisition. $Ac$ shows a subtraction map, coded in shades of red if DF increased or green if it decreased (with a 0.2 Hz resolution). Black pixels are those which did not change in DF. $Ad$ and $Ae$ show histograms of the distribution of DFs for each DF map, with the corresponding $\chi^2$. $Af$ shows poor cross correlation of DFs for each pixel in the consecutive maps, with the regression line distant from the identity line (gray dotted line).