Distribution of Excitation Frequencies on the Epicardial and Endocardial Surfaces of Fibrillating Ventricular Wall of the Sheep Heart

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Abstract—Tissue heterogeneities may play an important role in the mechanism of ventricular tachycardia (VT) and fibrillation (VF) and can lead to a complex spatial distribution of excitation frequencies. Here we used optical mapping and Fourier analysis to determine the distribution of excitation frequencies in 20 000 sites of fibrillating ventricular tissue. Our objective was to use such a distribution as a tool to quantify the degree of organization during VF. Fourteen episodes of VT/VF were induced via rapid pacing in 9 isolated, coronary perfused, and superfused sheep ventricular slabs (3×3 cm²). A dual-camera video-imaging system was used for simultaneous optical recordings from the entire epicardial and endocardial surfaces. The local frequencies of excitation were determined at each pixel and displayed as dominant frequency (DF) maps. A typical DF map consisted of several (8.2±3.6) discrete areas (domains) with a uniform DF within each domain. The DFs in adjacent domains were often in 1:2, 3:4, or 4:5 ratios, which was shown to be a result of an intermittent Wenckebach-like conduction block at the domain boundaries. The domain patterns were relatively stable and could persist from several seconds to several minutes. The complexity in the organization of the domains, the number of domains, and the dispersion of frequencies increased with the rate of the arrhythmia. Domain patterns on the epicardial and endocardial surfaces were not correlated. Sustained epicardial or endocardial reentry was observed in only 3 episodes. Observed frequency patterns during VT/VF suggest that the underlying mechanism may be a sustained intramural reentrant source interacting with tissue heterogeneities. (Circ Res. 2000;86:408-417.)

Key Words: ventricular fibrillation ▪ optical mapping ▪ frequency analysis

The spatial distribution of excitation frequencies during ventricular fibrillation (VF) is important for understanding the mechanism of this complex arrhythmia.1–3 It has been suggested that such a distribution reflects the dispersion of refractory periods, a feature that, according to the theory of Moe et al,4 plays a key role in the initiation and maintenance of fibrillation. Until recently, technical constraints have significantly limited our ability to measure the frequency distribution in any significant detail. In this study, we overcome these limitations by using the following combination of experimental techniques.

Instead of the traditional multiple-electrode mapping, we have made use of optical methods. With a high-resolution charge-coupled device video camera,5,6 we have recorded spatiotemporal variations in the fluorescence of a voltage-sensitive probe. In contrast to a conventional electrogram that reflects the complex distribution of extracellular currents, an optical signal is proportional to the cardiac action potential.7 This fact makes optical recordings particularly advantageous during VF when extracellular signals become complicated and hence difficult to interpret.

Another methodological difference that distinguishes this study from previous work on VF is our use of isolated coronary–perfused preparations of free ventricular wall8–10 capable of maintaining complex fibrillatory activity.11,12 An advantage of this experimental preparation is the accessibility of both endocardial and epicardial surfaces to optical recordings. By simultaneously using 2 video cameras, we were able to map the entire surface of the preparation with uniformly high spatial resolution and thus avoid ambiguity in the interpretation of experimental data. Clearly, such ambiguity is unavoidable in experiments using whole-heart preparations when significant areas of the heart remain unmapped.

Finally, to determine the local frequencies of excitation, we used a recently developed technique based on dominant frequency (DF) analysis13 instead of the traditional approach based on measuring local activation times. The frequency of excitation was derived from Fourier spectra calculated for each pixel of the epicardial and endocardial images. Using the DF method eliminated the need for manual selection of activation times, which is often required during analysis of VF based on more traditional approaches. The availability of fully automated algorithms was crucial for this study, given the fact that our typical frequency maps contain information from thousands of pixels.
The major finding of this study is that the spatial distribution of local frequencies of excitation during VF appears to be unexpectedly simple and organized. A typical frequency map (DF map) consists of few relatively large domains with uniform DFs within each domain. The ratios of DFs in adjacent domains are often close to 1:2, 3:4, or 4:5 as a result of intermittent Wenckebach-like propagation block at boundaries between domains. The domains persist for a relatively long time as compared with the excitation cycle. Our findings suggest the possibility that fibrillation in our experimental model is driven by a stable intramural high-frequency source of excitation.

Materials and Methods

Isolated Coronary-Perfused Sheep Ventricular Preparation

All experimental protocols conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

Young sheep (n=9) were heparinized (500 IU, IV) and subsequently anesthetized with sodium pentobarbital (35 mg/kg IV). The heart was rapidly removed and Langendorff-perfused with cold (4°C) cardioplegic solution.14 The right (n=6) or left (n=3) free ventricular wall was quickly excised, and one of the large coronary arteries was cannulated. Nonperfused tissue was removed, and the preparation was stretched on a plastic frame as shown in Figure 1B.

Preparations were perfused with a standard oxygenated Tyrode solution at 80 mm Hg and superfused with the same solution at a rate of 40 mL/min. We added diacetyl-monoxime (DAM) to the Tyrode solution (15 mmol/L) to stop contractions. The voltage-sensitive dye di-4-ANEPPS (15 μg/mL) was added as described elsewhere. Areas with inadequate perfusion, if present, did not stain normally with fluorescent dye and could be immediately identified as dark regions in the background fluorescence images. The preparations with inadequate perfusion were excluded from the study.

The preparation was paced at a basic cycle length (BCL) of 500 ms at 2 times diastolic threshold. The shortest cycle length maintaining 1:1 ventricular capture (BClim) was determined for each preparation. To induce tachycardia, decremental pacing at the Stimulus intensity 1.0 to 4.0 mA was applied; the cycle length was progressively shortened from BCL in 5- or 10-ms steps. A bipolar electrogram was monitored on a digital storage oscilloscope (Hita-chi) and recorded on videotape (Neuro-Corder DR-484).

Optical Setup and Signal Processing

The optical setup consisted of 2 identical video imaging systems16 for simultaneous imaging of the endocardial and epicardial sides of the preparation (Figure 1A). The magnification and field of view of both video cameras were adjusted in a such a way that they observed the same area of the preparation from opposite sides. The video images (typically 200×100 pixels) were acquired at 120 frames per second, and the background fluorescence was subtracted from each frame. After spatial filtering,16 the effective spatial resolution of the method was 0.4–0.8 mm, depending on magnification.

Fast Fourier transform (FFT) was applied to the 2.13-second raw images (typically 200×100 pixels) were acquired at 120 frames per second, and the background fluorescence was subtracted from each frame. After spatial filtering,16 the effective spatial resolution of the method was 0.4–0.8 mm, depending on magnification.

The complexity of the DF maps was characterized by the coefficient of variation of DF over the mapped area (σDF/DFmax ×100%). DF maps were compared using coefficient of cross-correlation. The power of the DF (PDF) of the individual spectra was derived for each pixel of the epicardial and endocardial images, which provided a spectral resolution of 0.47 Hz. The position of the largest spectral peak (DF) was determined for each pixel, and maps of spatial DF distribution were constructed for each recording of arrhythmia (Figure 1C).

Results

Overview

Using the decremental pacing protocol (see Materials and Methods), we were able to induce sustained (>3 minutes) arrhythmias in all 9 preparations. A total of 14 tachyarrhythmias (25 recordings) of various degrees of complexity, ranging from monomorphic ventricular tachycardia (VT) to complex polymorphic tachycardias reminiscent of VF were analyzed. The characteristics of the sample are given in the Table.

Breakthrough pattern and the absence of a complete reentry on either surface during arrhythmia were typical findings in our preparations. Sustained reentrant circuits (see asterisks on the Table) were observed in only 3 of 25 recordings (1 VT and 2 VF). Figure 2 shows representative

Figure 1. Experimental setup. A, Dual-video camera system for simultaneous endocardial and epicardial optical mapping. In each system, the collimated light from the 250-W tungsten-halogen lamp was passed through a heat and a bandpass (520±30 nm) filter, reflected 90° from a dichroic mirror (560 nm), and projected onto the surface of the vertically hanging preparation. Emitted light was collected via a 50-mm objective lens, transmitted through the emission filter (645 nm), and projected onto a respective charge-coupled device video camera. B, Schematics showing the mounting of the perfused slab of sheep ventricle in a tissue bath. C, Diagram explaining the DF mapping. Using FFT, the DF and PDF were derived for each pixel of the epicardial and endocardial images.

Time-space plots (TSPs) and pseudo-ECGs were derived from optical recordings as described elsewhere.6,17

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examples of isochronal maps during monomorphic VT and polymorphic VT (PVT)/VF. Panel A shows the endocardial and epicardial propagation in control conditions during epicardial pacing at BCL = 500 ms. Epicardial longitudinal and transverse conduction velocities were 0.84 and 0.40 m/s, respectively, which is within the normal range for ventricular myocardium. Panel B shows activation during VT (cycle length = 192 ms) in the same preparation. A multiple breakthrough pattern was revealed on both surfaces, and no reentrant activity was seen. Panel C shows isochronal maps after conversion of VT shown in panel B into VF by burst pacing at cycle length of 130 ms. Multiple breakthroughs, incomplete reentrant circuits, and multiple lines of conduction block can be seen. Note the marked difference between the epicardial and the endocardial patterns of activation.

The cycle length of monomorphic VT in our experiments was 196±18 ms. This was significantly longer than the average cycle length during VF (135±21 ms, \(P<0.0001\)) measured as the inverse value of the average DF over the preparation (DFmean). It is of interest that the average cycle length during VF was very close to BCLmin (see the Table). BCLmin, DFmean, and pseudo-ECG patterns for individual recordings are given in the Table.

The spatial distribution of the DF was studied in all episodes of arrhythmia. Epicardial and endocardial DF maps constructed for an episode of monomorphic VT are shown in panel A of Figure 3 (the same episode as in Figure 2B). As one might expect, the DF was spatially uniform and identical on both surfaces of the preparation, which reflects homogeneous distribution of the cycle length. The DF was 5.2 Hz throughout the preparation, which corresponds to a cycle length of 192 ms. The spectra of individual signals (Panel A, bottom) featured a pronounced dominant peak at 5.2 Hz. The spectrum of the pseudo-ECG (Panel A, top) was very similar to the local spectra.

Surprisingly, the DF maps of VF also appeared very simple. They consisted of few relatively large areas (“domains”) of uniform DF. Panel B of Figure 3 shows such maps for the episode of VF shown in Figure 2C. A major part of the epicardial surface was occupied by a continuous domain with a DF of 6.1 Hz. The rest of the surface had a DF of 8.0 Hz,

### Descriptive Statistics of Experiments

<table>
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<th>Experiment No.</th>
<th>Preparation</th>
<th>BCLmin, ms</th>
<th>Arhythmia No.</th>
<th>Optical Recording No.</th>
<th>DFmean, Hz</th>
<th>1/DFmean, ms</th>
<th>Pseudo-ECG Pattern</th>
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RV indicates right ventricle; LV, left ventricle; BCLmin, shortest cycle length at which 1:1 excitation was maintained; DFmean, mean DF for a given recording.

*Occurrence of sustained reentry.

Descriptive Statistics of Experiments
with a large domain in the upper-right corner and small domains in the center and in the lower left corner of the preparation. On the endocardium, the DFs were the same as on the epicardium (6.1 and 8.0 Hz). However, the location and shape of the domains were different from those on the epicardium.

The power spectrum of the pseudo-ECG (Panel B, top) also had 2 major peaks at 6.1 and 8.0 Hz, which corresponded to the 2 prevalent local DFs. Unlike monomorphic VT, during which all local power spectra contained only the DF and its harmonics, during VF the local spectra were more complex. For example, the power spectrum in epicardial point a1 (see panel B, bottom) had 2 peaks, one at 6.1 Hz (dominant) and the other at 8.0 Hz. Note that the second peak corresponds to the DF on the opposite point (a2) on the endocardial surface. Such bimodal spectra were usually observed near the boundaries between different DF domains (see below).

The average number of domains >3 mm² in a given VF episode was 8.0±3.6 on the epicardium and 8.27±3.9 on the endocardium (NS). Given that the area of the preparation was ≈3×3=9 cm², the average area of a single DF domain was ≈1.1 cm². The number of different frequencies in a given VF episode was usually smaller (4.84±1.95) than the number of domains, which means that there were several domains with the same DF (on average, 1.6 domains per frequency). In addition to major domains, there were also a number of small (<3 mm²) domains randomly scattered throughout the preparation. Their location correlated to sites of low amplitude, such as areas adjacent to lines of conduction block and low illumination at the boundaries of the wedge. The origin of such domains was most likely the result of noise. However, their relative contribution was always <10% of either the epicardial or the endocardial surface. We did not find a significant difference in domain organization of arrhythmias in the left and right ventricles.

An important question is how stable the DF domain pattern is and how it changes with time. To address this question, in 4 experiments we compared DF maps of short (2.13-second) consecutive segments of the same long (8.56-second) recording of VF. The analysis shows that the spatial distribution of DF domains remained largely unchanged over time intervals of 8 to 10 seconds. In all of these experiments, the coefficient of cross-correlation between the consecutive DF maps was >0.9. Figure 4A shows the DF maps of 2 segments (I and II) separated by a 2.13-second interval from the same episode of VF shown in Figures 2 and 3, as well as a map including the entire episode, as indicated by the top thick lines. It is seen that the shape of the DF domains is highly preserved. Moreover, the DF distribution during the whole recording (III) is also remarkably similar to those of the short segments (I and II).

Temporal patterns of the DF changes at individual pixel locations are shown in Figure 4B. The plots were obtained by shifting the 256-frame analysis time window (2.13 seconds) in 16-frame steps (133 ms). In the majority of points, the DF either was stable (traces b, e, f, and h) or flickered between the 2 main frequency levels, 6.1 and 8.0 Hz (traces a and c). The flickering was observed in those points in which the spectra had 2 distinct peaks with comparable amplitudes (see, for example, point a1 in Figure 3B). Fluctuations in the amplitude of these peaks could lead to a change in the spatial extension of a given domain. In general, more variability was observed closer to the boundaries between DF domains and/or in the areas of conduction block.

Nature of the DF Domains

Analysis of impulse propagation near the boundary between adjacent DF domains often revealed Wenckebach-like conduction block patterns. A representative example is shown in Figure 5, which shows a fragment of endocardial DF map from Figure 3B (map rotated 90°). To visualize the propagation pattern, we constructed a TSP for a column of pixels (vertical white line) across the boundary between 8.0- and 6.1-Hz domains (Figure 5A). The TSP (Figure 5B) reveals 3:4 conduction block with a Wenckebach-like activation pattern. We infer that the excitation travels from the 8.0- to 6.1-Hz domain. Every fourth impulse originating in the 8.0-Hz domain is blocked. The position of the block (dashed line) coincides with the boundary of the 6.1-Hz domain. The individual recordings (a through e in Figure 5D) show a
reduction in action potential amplitude from point e to point c on every fourth cycle of points e and d. The block occurred between points d and c. The spectra from points a through e (Figure 5C) show a bimodal distribution of power between peaks at 8.0 and 6.1 Hz. The amplitude of the 8.0 Hz peak gradually decreased from point e to point a, whereas the amplitude of 6.1 Hz peak gradually increased. The change of the dominance, demarcating the boundary between the DF domains, occurred between point d and c. The activation pattern (TSP) and spectral pattern showed a good agreement with respect to the localization of the conduction block. In this, as well as in other experiments, we observed that domains with lower DF often had longer action potential duration (APD). Figure 5E shows individual action potentials optically recorded from different domains during regular pacing and during VF. One can see that in both cases APD recorded in the domain with higher DF (point f) was shorter than the one at point a located within a low frequency domain (see Figure 5E). Although absolute difference in APD between these 2 points was larger during regular pacing, the relative difference (normalized to the APD) was larger during VF (27% versus 18%). Panel F shows the profile of APD across the domain boundary during control pacing with a

Figure 3. Frequency analysis of monomorphic VT (A) and PVT/VF (B) recorded in the same preparation. Top, Integral optical signal (pseudo ECG) and its power spectrum. Middle, DF maps. Corresponding isochrone maps are shown in Figure 2B and 2C, respectively. Bottom, Individual epicardial (a1, b1, and c1) and endocardial (a2, b2, and c2) signals are shown together with their respective power spectra. During VT, DF is uniform on both epicardial and endocardial surfaces of the preparation (5.2 Hz). During PVT/VF, there are several discrete DF domains at DF = 8.0 Hz and 6.1 Hz.

Figure 4. Short-term stability of DF distribution. A, Comparison of DF maps of 2 separate 2.13-second segments (I and II) and DF map of the whole 8.8-second recording (III) of the same PVT/VF as shown in Figures 2 and 3. Similarity of these maps indicates that the frequency domains are stationary. B, Plots of DF vs time in selected points, of which the position is marked on DF map III of panel A. In the majority of pixels, DF either is stable or flickers between 2 main levels (8.0 and 6.1 Hz).
superimposed profile of 1/DF during VF. Note the abrupt change in 1/DF despite a gradual variation in APD.

The most common DF ratios observed at boundaries between domains were 3:4, 1:2, and 4:5. The latter were found in 6, 5, and 3 of 17 VF recordings, respectively. The ratios 2:3, 5:6, and 6:7 were observed less often (in 2, 1, and 1 out of 17 VF recordings, respectively). The resolution of our method (0.47 Hz) was not sufficiently high to resolve ratios of higher order. TSP analysis revealed Wenkebach patterns in 76% of cases with simple DF ratios. Although Wenkebach block patterns were easily seen at the boundaries of larger domains, they were less obvious between smaller domains. The degree and localization of block could fluctuate in time (see Figure 4B), causing shifts of domain boundaries.

**Complexity of Arrhythmia as a Function of Its Rate**

The complexity of the domain organization correlated with frequency. To quantify this correlation, we calculated the coefficient of spatial variation of the DF (see Materials and Methods) and plotted it versus DF mean for each recording of monomorphic VT and VF as shown in Figure 6A. A significant increase in coefficient of variation was seen as DF mean increased ($r=0.79; P<0.001$). The clusters of VT points (open symbols) and VF points (closed symbols) practically do not overlap; most of the VT points are to the left, whereas all VF points are to the right of the fastest frequency in control (calculated as 1/BCL min (see Table)). Note that the complexity of VF increases gradually with the average frequency of arrhythmia, and no features can be identified that allow the selection of subranges of PVT versus VF.

As demonstrated in Figure 6B, the same conclusion can be drawn from the analysis of the PDF (see Materials and Methods) that shows an inverse correlation between the PDF mean and DF mean ($r=-0.87; P<0.001$). Large PDF indicates a high degree of periodicity in which most of the energy is contained in the dominant peak. A less ordered process, with a broader spectrum, will have lower PDF. In the same arrhythmia, the PDF usually decreased when the rate of the arrhythmia increased. This was due to the appearance of additional peaks and general broadening of the local spectra.
limited number of domains were relatively stable over 8- to 10-second intervals. Figure 8 shows the DF maps of 3 consecutive segments (I–III) from a 10.24-second recording of VF in the absence of DAM. There are 4 to 5 large DF domains on the epicardium and 3 to 4 domains on the endocardium. It is seen that the shape of the DF domains is relatively stable, especially during segments I and II. The domain with the highest frequency (9.8 Hz) on the epicardium (red “horseshoe”) is the most stable; other domains are more variable. The coefficient of cross-correlation between maps I and II was 0.86; between maps II and III it was 0.77. A TSP in Figure 8 shows Wenkebach patterns at the boundaries between 9.8- and 8.0-Hz domains in the upper part of the epicardium. Note intermittent 5:4 and 6:5 Wenkebach patterns giving rise to average frequency ratio 9.8 Hz/8.0 Hz = 11/9. It should be noted, however, that experiments without DAM have certain limitations. Despite significant reduction of mechanical activity during VF, which made optical measurements possible, the contractions were not completely eliminated. In certain areas, we still observed a noticeable effect of mechanical activity on the shape of recorded action potentials, which could have affected the domain patterns.

Discussion

The major finding of this study is that the spatial distribution of local frequencies of excitation during VF appears to be much simpler and more organized than one might expect from the theory of Moe et al. A typical frequency map (DF map) consists of a small number (∼5) of relatively large (∼1 cm²) domains with uniform DFs within each domain. The ratios of DFs in adjacent domains are often close to 1:2, 3:4, or 4:5. The domains persist for a relatively long time as compared with the excitation cycle. There is usually no significant correlation between domain patterns on the epicardial and endocardial surfaces.

What do these data tell us about the mechanism of VF? Before attempting to answer this important question, we discuss a mechanism by which DF patterns similar to those described above can emerge. For the sake of simplicity, we assume a 1-dimensional strip of myocardium with a continuous distribution of refractory periods (see Figure 9), and we determine the DF patterns that emerge in such a strip during high-frequency pacing. The source with a constant period (T₁) is located on the left end (x=0) of the strip. It is easy to see that if T₁ is sufficiently small (smaller than the maximum refractory period T₁< Rmax), a frequency pattern with discrete domain organization will emerge. Indeed, at the right of point x₁, the refractory period is greater than T₁, and 1:1 propagation is impossible. This causes the development of an intermittent Wenkebach-like block pattern with a stepwise reduction in frequency of excitation at points distal to x₁ (see panel A).

Hence, 2 domains emerge; one is located proximally to x₁, and the other is located distally to point x₂, with frequencies f₁=1/T₁ and f₂=1/T₂, respectively. The ratio of excitation frequencies (4:3 in this example) is selected as the ratio T₂/T₁ of the 2 smallest integers that satisfy the inequality T₂/T₁ ≥ Rmax/T₁ (the cycle length T₁ must be greater than Rmax).

The mechanism described above explains why the domain organization becomes more complex when the frequency of
excitation increases. Panel B shows a new domain pattern formed in the same refractory profile after a decrease in $T_1$. Similar to what we observe experimentally, the number of domains increases and they become smaller. Now, instead of 2, 3 domains emerge. The left domain (0$<$x$<$x$_1$) has a frequency $f_1$ = $1/T_1$, the central domain ($x_1$ $<$ x $<$ x$_2$) has a frequency $f_2$ = $1/T_2$, and the right domain ($x_2$ $<$ x$<$) has a frequency $f_3$ = $1/T_3$. The second domain is formed near the first small peak of the refractory profile. However, the increase in period that occurs as a result of the block is insufficient to overcome the second larger peak. As a result, a second block (3:2) develops near point $x_2$, giving rise to a third domain with the slowest frequency.

The domain organization of VF may strongly depend not only on frequency but also on the location of the hypothetical source. For example, if the source were located in the “dip” of refractory profile between points $x_1$ and $x_2$, the fastest domain would be located between 2 slower domains and the actual local frequencies in each domain would be quite different from the examples presented in Figure 9. Therefore, even with the same spatial distribution of refractoriness, the domain organization may be dissimilar in different episodes of VF.

The examples discussed above show that a simple model with a single periodic source of excitation described above can reproduce qualitatively the broad spectrum of the observed phenomena, including the discrete domain organization, the stability of domain boundaries, and the correlation of complexity with frequency. It is tempting to hypothesize that fibrillation in our experimental model has a similar mechanism and is driven by a stable high-frequency source of excitation. Such a mechanism has been considered in relation to atrial fibrillation as an alternative to the Moe et al.'s multiple-wavelet hypothesis and is termed “fibrillatory propagation.” Similar to previous findings in atrial fibrillation, in our study the area with the fastest frequency during VF often showed more orderly activation than sites with slower frequencies (see Figure 7). In a 3-dimensional myocardial wall, fibrillatory propagation can result in very complex activation patterns away from the source. Multiple wave breaks caused by intermittent block at the boundaries of refractory domains can generate activity patterns indistinguishable from VF. The fibrillatory propagation hypothesis is consistent with recent phase-mapping data. The short-lived phase singularities, reported on the surface of the fibrillating ventricles, could represent the formation of wave breaks at boundaries of refractory domains.

Although the fibrillatory propagation hypothesis seems plausible, its validation will require the identification of the excitation source and its mechanism. An extremely high rate of excitation (significantly higher than the fastest possible rate in control) favors a reentrant mechanism. To the best of our knowledge, none of the known mechanisms of normal or abnormal automaticity can account for such a high rate of excitations. On the contrary, the spiral wave activity is capable of undergoing frequencies that are usually higher than those achievable during spontaneous pacemaker activity or rapid pacing. Recent computer simulations indicate that the core of the spiral wave may have a strong repolarizing influence on the surrounding tissues. Under these conditions, the APD of the cells near the core is shorter than the APD of the cells away from the core. In theory, this may account both for the extremely high rate near the excitation source and rhythm transformations away from that source, where the tissue cannot keep up with the rate of the spiral source.

In the majority of cases, we did not see sustained reentrant activity on either the endocardial or the epicardial surface. This suggests the possibility that, if the fibrillatory propagation hypothesis is correct, the driving reentrant source should be intramural. Recent theoretical analysis shows that 3-dimensional reentrant activity in the myocardial wall tends to organize in space in such a way that its rotation occurs around the long axis of the myocardial fibers, which would predict that reentrant activity is likely to be hidden inside the ventricular wall. Indeed, it has been shown experimentally that intramural reentrant circuits do not manifest on the surface. This may explain why sustained reentrant activity is rarely seen in intact ventricular preparations as opposed to epicardial slices and the thin epicardial rim surviving myocardial infarction. The fact that there are...
large differences between endocardial and epicardial activation and DF patterns (see Figure 3) is additional evidence for 3-dimensional VF in our model.

Limitations
The major limitation of optical mapping is the interference of mechanical contractions with the optical signal. This interference can be eliminated by using electromechanical uncouplers such as DAM, verapamil, or cytochalasin D.\textsuperscript{28,30,31} However, uncouplers not only eliminate contractions but also alter the electrical properties of cardiac myocytes.\textsuperscript{32–34} Quite recently, it was shown that, in some species, DAM may significantly affect the characteristics of VF; in coronary-perfused slabs of the dog right ventricle, DAM can convert VF into VT.\textsuperscript{12} However, in our preparations, as well as in the isolated rabbit heart,\textsuperscript{35} DAM does not eliminate complex fibrillatory activity. Our data show that, although the domain organizations and DF\textsubscript{mean} in the presence and absence of DAM may differ, the main findings of this study are not affected by DAM. Specifically, the number and stationarity of domains and the frequency relationships between adjacent domains are similar in both cases. It should be noted, however, that using uncouplers cannot be completely avoided. Although mechanical artifact is significantly reduced during VF, it still has noticeable effects on the optical recordings in some areas and may influence the domain patterns. A more thorough analysis of the influence of electromechanical uncouplers and calcium blockers on the spatiotemporal organization of VF is required.

Our explanation of the domain organization is based on spatial heterogeneity of refractoriness or APD. Indeed, in our experiments we observed general correlation between APD and the fibrillation cycle length (1/DF), which is consistent with previous reports.\textsuperscript{1–3} However, fiber organization may also influence the shape of DF domains and the position of interdomain boundaries, given that, at fast frequencies associated with VF, occurrence of block may depend on fiber orientation.\textsuperscript{36,37}

Finally, we would like to emphasize that even though our data suggest fibrillatory propagation as a mechanism of VF, they do not exclude other mechanisms. Theoretically, the multiple-wavelet hypothesis\textsuperscript{4} and spiral wave-breakup mechanism\textsuperscript{38} may also result in similar DF patterns. A more quantitative comparison between modeling and experimental data are required before definite conclusions can be drawn. It appears from our results, however, that DF analysis will become a useful tool for such quantitative comparisons.

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References


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