Spatiotemporal Periodicity During Atrial Fibrillation in the Isolated Sheep Heart

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Background—The activation patterns that underlie the irregular electrical activity during atrial fibrillation (AF) have traditionally been described as disorganized or random. Recent studies, based predominantly on statistical methods, have provided evidence that AF is spatially organized. The objective of this study was to demonstrate the presence of spatial and temporal periodicity during AF.

Methods and Results—We used a combination of high-resolution video imaging, ECG recordings, and spectral analysis to identify sequential wave fronts with temporal periodicity and similar spatial patterns of propagation during 20 episodes of AF in 6 Langendorff-perfused sheep hearts. Spectral analysis of AF demonstrated multiple narrow-band peaks with a single dominant peak in all cases (mean, 9.4±2.6 Hz; cycle length, 112±26 ms). Evidence of spatiotemporal periodicity was found in 12 of 20 optical recordings of the right atrium (RA) and in all (n=19) recordings of the left atrium (LA). The cycle length of spatiotemporal periodic waves correlated with the dominant frequency of their respective optical pseudo-ECGs (LA: R²=0.99, slope=0.94 [95% CI, 0.88 to 0.99]; RA: R²=0.97, slope=0.92 [95% CI, 0.80 to 1.03]). The dominant frequency of the LA pseudo-ECG alone correlated with the global bipolar atrial EG (R²=0.76, slope=0.75 [95% CI, 0.52 to 0.99]). In specific examples, sources of periodic activity were seen as rotors in the epicardial sheet or as periodic breakthroughs that most likely represented transmural pectinate muscle reentry. However, in the majority of cases, periodic waves were seen to enter the mapping area from the edge of the field of view.

Conclusions—Reentry in anatomically or functionally determined circuits forms the basis of spatiotemporal periodic activity during AF. The cycle length of sources in the LA determines the dominant peak in the frequency spectra in this experimental model of AF. (Circulation. 1998;98:1236-1248.)

Key Words: atrium ■ arrhythmia ■ mapping ■ imaging ■ Fourier analysis

The activation patterns that underlie the irregular electrical activity during atrial fibrillation (AF) have traditionally been described as disorganized or random.1-7 Recent studies, based predominantly on statistical methods, have provided evidence that AF is not entirely random.8,9 Evidence for preferential routes of wave propagation during AF has been suggested by the demonstration of transient linking.9 Furthermore, the anatomy and electrophysiological characteristics of the atria are likely to constrain the patterns of wave propagation, resulting in some degree of underlying order. In fact, spatial organization has been demonstrated over short distances by statistical analysis of signals recorded by bipolar electrograms (EGs) during AF.5 However, little is known of the spatial characteristics of waves that might contribute to the underlying order.

Although some data suggest underlying spatial organization,10-12 little work has been done on the temporal characterization of AF. Analyses of recordings of the arrhythmia in the frequency domain have demonstrated multiple narrow-band peaks, often with a single dominant peak.10-12 The presence of a dominant peak suggests that a substantial portion of the atria is activated at that frequency, potentially in a spatially ordered manner. However, the spatial patterns of wave propagation that correspond to the dominant peak of the spectral analysis remain unknown. We used high-resolution video imaging in combination with ECG techniques and frequency analyses to simultaneously study electrical wave propagation on the surface of both atria in an acute model of AF in isolated Langendorff-perfused sheep hearts. We had 2 principal aims: (1) to identify patterns of wave propagation that demonstrate spatial and temporal periodicity and (2) to use spectral analysis to determine the contribution of the periodic activity to the frequency content of global measures of atrial electrical activity, especially in relation to the dominant frequency.

Methods

Langendorff-Perfused Sheep Heart Preparation

Young sheep (18 to 25 kg) were anesthetized with sodium pentobarbital (35 mg/kg). The heart was rapidly removed, placed in cold
cardioplegic solution (mmol/L: glucose 280, KCl 13.44, NaHCO₃ 12.6, mannitol 34, 4°C) for transportation, then connected to a Langendorff apparatus. This method has been described elsewhere in detail. Briefly, the coronary arteries were continuously perfused via a cannula in the aortic root with warm Tyrode’s solution under a constant flow of 115 to 140 mL/min and bubbled with 95% O₂/5% CO₂. We ensured that the heart was in sinus rhythm and contracting forcefully and rhythmically at the initiation of the experiment. In most cases, ventricular fibrillation (VF) occurred on rewarming shortly after the heart was connected to the Langendorff apparatus. If this occurred, the VF was allowed to continue throughout the experiment. Methoxyverapamil (D600, 2³10⁻⁶ mol/L) was added to the Tyrode’s solution as an electromechanical uncoupler and maintained throughout the experiment. A bolus injection of 5 to 10 mL of the potentiometric dye di-4-ANEPPS (10 mg/mL) enabled us to simultaneously image the fluorescence resulting from changes in transmembrane potentials from the epicardium.

High-Resolution Optical Mapping

The video imaging approach used for these studies is a modification of that described elsewhere in detail. We recorded simultaneously from 20,000 sites in the right atrial (RA) free wall and 10,000 sites in the left atrial (LA) appendage using 2 identical cameras. A diagram of the experimental protocol is presented in Figure 1. Briefly, quasi-monochromatic light (535 nm) was directed onto the epicardial surface of the atria. The emitted fluorescence was transmitted through a 645-nm filter and projected onto CCD video cameras (Cohu 6500) and acquired at a rate of 120 frames per second (sampling at 8.33-ms intervals). Both video cameras were triggered simultaneously by a delivered pulse. The areas of the mapped regions were as follows: 3³5 cm of the RA free wall and 3.5³3.5 cm of the LA appendage. This represents ~40% of the total surface area of the sheep atrium, including the septum.

Isochrone Maps

Isochrone maps were generated from the sequence of the video image of electrical activity on the heart surface by analysis of the value of each pixel over time in a manner previously described. Briefly, by a threshold technique, points for each pixel over time were labeled as part of the wave front if they were part of the fastest part of the upstroke of the action potential (maximum first derivative). In this way, 8.33-ms isochrone bands were formed.

Pseudo-ECGs

Pseudo-ECGs were constructed from optical recordings by integrating the transmembrane fluorescence signal over the left and right halves of the mapped region and taking the difference. This was done both for the entire mapped regions of both atria and for smaller areas when spatiotemporal periodicity was observed. Although the pseudo-ECG is different from the traditional ECG, it captures the important global aspects of a true ECG. The surface ECG leads record the extracellular potential arising from transmembrane potentials closest to the electrode. By integrating the transmembrane fluorescence signal from ~10,000 to 20,000 sites on the atrial epicardium and calculating the difference between left and right halves, the pseudo-ECG gives a comparable measure of electrical activity.

Bipolar EG

A continuous atrial bipolar EG was recorded as the difference between 2 epicardial leads, 1 located on the RA and the other on the LA. This method was chosen over a volume-conducted ECG to obtain an atrial signal minimally contaminated by ventricular signal (VF or sinus rhythm). The electrodes were connected to a Gould amplification system and filtered at 0.1 to 300 Hz. To ensure that the recorded bipolar EG was a true reflection of global activity within the atria, in 2 experiments (6 episodes), an intracavitary atrial EG was also recorded. This EG recorded the difference of 2 leads surrounded by cotton wicks and allowed to float freely in the Tyrode’s solution–filled cavities of the LA and RA away from their walls. Theoretically, the intracavitary EG should sample all areas of the atria with similar weighting. Correlation between the dominant peaks of frequency of the intracavitary EG and bipolar EG was strong (R²=0.94, slope=0.91; 95% CI, 0.74 to 1.08). Therefore, the bipolar EG was considered to contain most frequency components of the electrical activity of the atria; it was considered unlikely that large regions of high-frequency components were not represented in the bipolar EG.

Definition of AF

AF was induced by burst rapid atrial pacing from the epicardial surface of either the RA or LA after the addition of acetylcholine.
Spatiotemporal Periodicity During AF

(ACh; 0.1 to 0.5×10⁻⁶ mol/L) to the perfusate. We defined AF as follows: (1) the bipolar EG was required to demonstrate a rapid sustained irregular rhythm with variability of morphology and/or timing of EGs on a beat-to-beat basis and (2) the optical recordings of 1 or both atrial movies were required to demonstrate wave propagation and lines of block, which changed on a beat-to-beat basis. AF was considered sustained if it lasted 2 to 5 minutes. In most experiments, once initiated, AF lasted >20 minutes.

Analyses
In 6 experiments, 20 optical recordings were made; in 5 experiments, recordings were made from both atria simultaneously; and in 1 experiment, only the RA was recorded. For each experiment, episodes of AF obtained several minutes apart were selected for analysis on the basis of signal quality.

Periodicity Analysis
To identify sequential wave fronts that might demonstrate spatial and temporal periodicity, optical recordings were analyzed by recordings of both the timing and spatial direction of propagation of all new waves entering into the mapping area. Sequential wave fronts demonstrated spatiotemporal periodicity if a minimum of 4 sequential waves entered as new wave fronts or emerged as breakthroughs (1) from the same location (edge or breakthrough) and direction or (2) with a timing that varied by no more than ±1 frame (8.33 ms) from a mean period. As it turned out, no region was activated by 4 sequential periodic wave fronts alone without the return of the same spatiotemporal pattern of activation at a later time during the recording.

Signal Analysis
Spectral analysis was performed with fast Fourier transforms (FFTs) on measures of global atrial electrical activity (bipolar EGs and pseudo-ECGs of both atria) and pseudo-ECGs of discrete regions activated by periodic waves, as well as single-pixel recordings. The content in the 0.4- to 60-Hz band was analyzed, and peak frequencies from these sources were compared. The peak frequencies were also compared with the frequency of the periodic activity as determined visually from the optical recordings (see above under Periodicity Analysis). In each FFT, the total power varied according to the intensity of fluorescence. As such, the magnitudes of various peaks were not compared from one FFT to another. Rather, the relative amplitudes of peaks in each FFT were compared to determine the dominant peak. The power axes for each FFT were therefore not labeled. The bipolar EG was acquired at 1713 Hz for 10 seconds and filtered (bandpass, 0.1 to 300 Hz). This provided a spectral resolution of 0.1 Hz over the range of 0.4 to 60 Hz. Optical recordings were acquired at 120 Hz (8.33 ms) for 360 to 480 frames (≈3.0 to 4.0 seconds). This provided a spectral resolution of 0.33 to 0.25 Hz.

Statistical Analysis
Correlation of frequencies was performed by simple linear regression analysis (Statview 4.53, Abacus Concepts). Slopes are presented with 95% CIs. Correlation coefficients (R²) are also presented, with associated P values.

Results
Twenty episodes of AF were analyzed from 6 experiments; 1 episode was recorded from the RA only. In all cases, patterns of wave propagation were complex, with evidence of incomplete and complete reentry, epicardial breakthrough patterns, and wave collisions as in our previous work.¹⁴

Spectral Analysis of Global Measures of Activity
To evaluate the frequency content of episodes of AF in our model, we first performed spectral analysis of global measures of atrial activity, ie, the bipolar EG. A representative example is shown in Figure 2. The bipolar EG (Figure 2A) demonstrates irregular electrical activity characteristic of AF. Its corresponding FFT (2B) demonstrates multiple discrete narrow-band peaks. This is similar to the findings of previous reports of spectral analyses of AF.¹⁰⁻¹² A dominant narrow peak is seen at 8.3 Hz. A peak corresponding to a harmonic frequency is also seen at 16.7 Hz. Other smaller-amplitude narrow-band peaks were also found, including a narrow peak at 13.9 Hz that corresponded to ongoing VF. In all episodes of AF, spectral analysis of the bipolar EG demonstrated multiple narrow-band peaks with a single dominant peak. The dominant frequency of the bipolar EG ranged between 6.4 and 16.7 Hz, with a mean of 9.4±2.6 Hz (cycle length, 112±26 ms). The presence of a dominant peak suggests that a substantial portion of the atria is activated at that frequency, potentially in a spatially ordered manner.

Spectral Analysis of Pseudo-ECGs
To investigate the origin of the various peaks, especially the dominant peak seen in the narrow-band power spectrum of the bipolar EG, spectral analysis was performed on the pseudo-ECG constructed from the optical recording of each atrium. In Figure 2, the pseudo-ECG of the LA (Figure 2C) appears more regular compared with the bipolar EG (2A) and the pseudo-EG of the RA (2E). Spectral analysis of the LA pseudo-ECG demonstrates a discrete narrow-band dominant peak at 8.2 Hz. This corresponds to the dominant peak in the spectrum of the bipolar EG (8.3 Hz). The pseudo-ECG of the RA (2E) is characterized by more irregular but less rapid activation. This is corroborated by a narrow-band spectrum that demonstrates prominent peaks at 6.3 and 4.0 Hz, which are also seen on FFTs of the bipolar EG (2B). A small peak is also seen in the RA spectrum at 8.0 Hz.

Periodic Activity in Optical Recordings
Having identified the major peaks in the frequency content of the optical recordings from both LA and RA, we proceeded to locate the specific sources of activity at these frequencies. Figure 2G shows 4 sequential color isochrone maps of activation recorded from the LA over a period of 490 ms (see horizontal bar in 2A). It is clear from these maps that, during that interval, the LA was repetitively activated via the same spatially oriented wave front (lower right). The wave front was periodic throughout the entire 3 seconds of recording (2C). The cycle length of periodic waves was 120 ms (8.3 Hz), which corresponded to the dominant frequency peak seen in the bipolar EG. In fact, the frequency of activation in this region of the LA corresponded to the dominant peak in the power spectra of both the pseudo-ECG of the LA (2D) and the bipolar EG (2B). Nevertheless, analysis of the 4 consecutive LA isochrone maps (2G) revealed some variability in the pattern of wave front propagation from 1 map to the next, which translated into the somewhat variable morphology of complexes seen in the LA pseudo-ECG (2C) and probably resulted in additional minor peaks in the corresponding power spectrum (2D). Figure 2H shows three 8.3-ms color isochrone maps of the RA, which correspond in time to those in 2G. Despite spatiotemporal periodicity seen in the LA, no such periodicity was seen in the RA. As previously reported,¹⁴ isochrone maps of the RA showed incomplete reentry, breakthrough patterns, and ever-changing
lines of conduction block over the entire mapping region. This complex pattern of activation resulted in marked variability in the RA pseudo-ECG and contributed significantly to the irregular activity seen in the bipolar EG (2A).

The data in Figure 2 are representative of 19 episodes of spatiotemporal periodicity originating in the LA. Indeed, analysis of all episodes of AF demonstrated spatiotemporal periodic activity more often in the LA; spatiotemporal periodicity was seen in all 19 recordings of the LA and 12 of 20 (60%) of the recordings of the RA. Moreover, in recordings in which spatiotemporal periodicity was seen in both atria during the same episode of AF, the frequency of the LA sources of periodic activity was greater than or equal to the RA in all cases (see below and Figure 5).

To assess quantitatively the contribution of the frequency of the local periodic activation to that of the global measures of electrical activity in all analyzed episodes of AF, the cycle length of the periodic activations (in ms) was correlated with

Figure 2. EGs with corresponding spectral analyses and isochrone maps from a single episode of AF. A and B, Bipolar EG and its FFT. C through F, LA pseudo-EG and its FFT and RA pseudo-EG and its FFT, respectively. G, Four sequential LA isochrone maps during AF. H, Three sequential RA isochrone maps corresponding in timing to LA. Timing of isochrone maps is indicated by horizontal bars over EGs.
the inverse frequency (1/f in ms) of the dominant peaks of the RA and LA pseudo-EGs and the recorded bipolar EG. Figure 3A shows the correlation of the frequency of the periodic activations (n=12) found in the RA and the inverse of the dominant frequency from the RA pseudo-ECG. These were strongly correlated ($R^2=0.97$, $P<0.0001$). The slope of the regression line was 0.92 (95% CI, 0.80 to 1.03). A similarly strong correlation is shown in Figure 3B for the 19 LA recordings of AF ($R^2=0.99$, $P<0.0001$, slope=0.94; 95% CI, 0.88 to 0.99). The strong correlation between the cycle length of periodic activation and the dominant frequency of the pseudo-ECG of the respective atrium suggests that a significant portion of the mapped area was activated in a 1:1 pattern by these periodic activations.

To determine the contribution of the individual atria to the dominant peak of the bipolar EG, we correlated the frequency of the dominant peaks of the pseudo-ECGs with the dominant peak from the spectral analysis of the bipolar EG. These data for the LA are shown in Figure 3C ($R^2=0.76$, $P<0.0001$, slope=0.75; 95% CI, 0.52 to 0.99). As can be seen in Figure 3C, there are 3 clear outliers in the data. Two points lie above the unity line; the dominant frequency of the LA pseudo-ECG was higher than that of the bipolar EG. In these cases, the dominant frequency of the LA pseudo-ECG was represented in the FFT of the bipolar EG, not as the dominant peak but as a smaller-amplitude peak with a higher frequency. It is conceivable that an LA source might generate repetitive impulses that propagate with varying degrees of block (see below). If the region of 1:1 conduction from the source is sufficiently small compared with other regions, the frequency of the source will not be the dominant peak in the bipolar EG. The third outlier lies below the unity line; the dominant frequency of the bipolar EG is higher than that of the LA pseudo-ECG. In this case, the bipolar EG may be sampling activity beyond the mapping region, which is more rapid than that recorded from the LA appendage. As shown in Figure 3D, when these outliers were excluded, the correlation was higher: $R^2=0.98$, $P<0.0001$, slope=1.03; 95% CI, 0.94 to 1.12. No such correlation was seen for the RA ($R^2=0.17$, $P=0.11$). This occurred because the bipolar EG in 12 of 16 episodes had a peak that was higher in frequency than that of the corresponding RA, whereas the remaining 4 were similar (data not shown).

**Periodic Activity in Transmembrane Signal of Optical Recordings**

Detailed analysis of local activation in the individual movies revealed important information about wave propagation and frequency relations between neighboring sites within a given atrium and between the 2 atria. In Figure 4, we present recorded transmembrane fluorescent signals from selected regions of both LA and RA for the same episode of AF. Each recording represents the integrated signal from a region 16×8 pixels in size, located as indicated by the numbers in the respective LA and RA maps. In Figure 4A, site 1 corresponds to the region of spatiotemporal periodicity at the lower right corner of the LA appendage (see also Figure 2A). The transmembrane signal was periodic and relatively uniform in
amplitude throughout the 3.0 seconds of this AF movie (≈25 activations). The power spectrum of this signal had a single narrow peak at 8.2 Hz, which matches the dominant peaks of both the LA pseudo-ECG (8.2 Hz) and the bipolar EG (8.3 Hz). Site 2 was located at some distance away from the source of periodic activity in the LA (4A). Both the isochrone maps (Figure 2) and the transmembrane signal of site 2 (Figure 4) demonstrated an interval in time at which the frequency of activation was half of that at site 1 (ie, 2:1). The transmembrane signal recorded from regions at a greater distance from the source of periodic activity (marked 3 and 4) demonstrated increasing variability in period and amplitude of activity compared with that seen at site 1. The variability in the transmembrane signal activation seen in panels 3 and 4 resulted from beat-to-beat fluctuations in propagation patterns in these regions as seen from the isochrone maps in Figure 2. In Figure 4B, transmembrane signal is shown for 4 regions (marked 1 to 4) of the RA during the same AF episode. Marked variability in the signal is seen at all sites, which correlates with the complex patterns of activation seen in the color isochrone maps in Figure 2. Note that despite the complex activation patterns seen in the RA, spectral analysis of the RA pseudo-ECG showed dominant peaks at 4.0 and 6.3 Hz, compared with 8.2 Hz in the LA.

In Figure 4, when one relates the number of activations in the RA (Figure 4B) to the number of activations in the LA site 1 (4A), a pattern of 4:3 (8:6; LA1:RAn) is seen at sites RA1 to RA3 over the 2 seconds of recorded signal. Similarly, a relation of 2:1 (8:4) is seen for site RA4; hence, the overall pattern of activation in each of these regions is a simple ratio of the frequency of the source of periodic activity in the LA. These simple ratios also correspond to the ratios of peaks in
In summary, in all analyzed episodes, at least 1 segment of AF demonstrated spatiotemporal periodicity. Furthermore, the frequency of the spatiotemporal periodic activity in the LA was represented as the dominant peak in the frequency content of measures of global atrial electrical activity (ie, bipolar EG and LA pseudo-ECG). Complex patterns of wave propagation away from the source of periodic activity resulted in complex electrical activity, as seen in the optical recordings of the LA. Activation patterns seen from the optical recordings of the RA were highly complex, with incomplete reentry, breakthroughs, and wave collisions. However, despite the spatial complexity, spectral analysis suggested that the overall temporal pattern of activation of the RA resulted from simple ratios of the frequency of activation from the source. Our interpretation of the above data is that the AF activity in this model was maintained in the majority of cases by a source of periodic activity located beyond the mapped regions, most likely in the LA. Because the dominant frequency of the source was probably too high to be able to activate the RA in a 1:1 manner, it is likely that the additional frequency components, and thus activation ratios contained in the RA pseudo-ECG, were the result of a spectral transformation imposed by intermittent propagation from the LA through interatrial pathways (eg, Bachmann’s bundle and pectinate muscle network) into the RA free wall.

Sources of Periodic Activity

To gain insight into the underlying mechanism of spatiotemporal organization in AF, we attempted to identify sources of periodic activity in the 20 initial episodes surveyed. In most cases, periodic activity emerged from 1 of the edges into the mapping field. In several episodes, however, the specific source of the periodicity could be identified. Sources of spatiotemporally periodic activity could be seen from the epicardium as stationary rotors on the anterior wall of the LA appendage, as seen in Figure 6. Figure 6A shows on the left an 8.33-ms isochrone map for a single rotation. The corresponding pseudo-EG is shown in 6B. Spectral analysis of these signals (not shown) revealed a direct correspondence between the frequency of rotation of this source (9.6 Hz) and the dominant frequency in the global bipolar EG (6E). Yet, despite the highly periodic activity in the LA, the RA was activated in an apparently aperiodic fashion, as seen in the 2 sequential RA isochrone maps in 6C and by the highly irregular activity in the RA pseudo-ECG (6D). The global bipolar EG taken during the same period (6E) is consistent with AF, and the FFT (not shown) revealed a dominant peak at 9.6 Hz. Although in this specific example the entire mapping region of the LA was activated periodically, more commonly, regions demonstrating dominant periodic activity were smaller than that shown here.

Sources of periodic activity could also be seen as breakthroughs of activation, which were probably a result of endocardial activation via a pectinate muscle, with transmural propagation to the epicardium and reentry into the pectinate muscle. An example is shown in Figure 7, in which 2 breakthroughs of activity demonstrate continuous spatiotemporal periodicity. 7A shows 3 sequential isochrone maps; the breakthroughs are indicated by asterisks. In each map, the
location of the breakthroughs is maintained for many cycles. Furthermore, the color of the breakthroughs is constant, demonstrating the temporal periodicity. This is confirmed in 7B by the single-pixel transmembrane signal at site 1, corresponding to 1 of the breakthroughs. Its FFT is shown on the right; a dominant single peak is seen at 20.3 Hz. In contrast, as shown in 7C, the transmembrane signal of region 2 (same size) located at some distance below that breakthrough shows more complex variability in signal amplitude. Such variability is due to cycle-to-cycle changes in wave propagation away from the breakthrough and is manifested in the FFT as an additional narrow peak at 10.1 Hz. The dominant peak at 20.3 Hz is maintained. For comparison, the LA pseudo-ECG and its FFT are presented in Figure 7E. Note that the dominant peak in both was 20.4 Hz, which demonstrated that propagation to this region from the breakthrough site was not 1:1 but occurred at a ratio of ≈4:3. Thus, global activation of the LA in this AF episode was the result of an LA source that manifested as highly periodic epicardial breakthroughs.

**Duration of Spatiotemporal Patterns**

In some cases, spatiotemporal periodic activity could be seen on a continuous basis; ie, throughout the 3.0- to 4.0-second recording of AF (see Figures 2 and 6). These regions could be seen in the RA or LA. In general, the size of these regions varied from cycle to cycle, depending on propagation patterns from their source and in relation to their interaction with preexisting waves.

In most analyzed episodes, periodic activation was transient, consisting of 4 to 14 consecutive activations. In all cases, periodic activation from a specific region was inter-
ruptured by wave fronts propagating from a different region in a different pattern. The former pattern of spatiotemporal periodicity returned in all cases. That is, these recordings were characterized by segments in which the same periodic wave appeared for 4 to 14 sequential activations, separated by segments in which no periodic activity was seen or periodic activation occurred from another location and direction. An example is shown in the 4 isochrone maps in Figure 8. Initially, a wave front of activation near the left edge activated a portion of the LA appendage periodically for 8 sequential cycles at 71-ms intervals (only the last 2 are shown in 8A and 8B). After this, a wave front from the opposite direction propagated from the edge of the mapping area (indicated by an asterisk in 8C). This wave front propagated into the refractory tail of previous waves, resulting in the formation of a rotor. Because the formation of the rotor was out of phase with the source of periodic activity at the left edge, waves emanating from the rotor activated the entire mapping region, thus interfering with the original source of periodic activity. This rotor continued for 3 rotations (the second complete rotation is shown in 8D) at a period similar to that of the source of periodic activity (within \( \sim 8 \) ms, 1

Figure 7. Periodic breakthroughs of activity. A, Three sequential LA isochrone maps demonstrate 2 phase-locked breakthroughs of activation (*). B, Transmembrane signal from breakthrough on right (site 1, 16×8 pixels) with its FFT. Spatiotemporal activity is seen at this site. C and D, Transmembrane signal at sites 2 and 3 (away from site 1). E, LA pseudo-EG.
frame) and acted as the dominant source of periodic activity in the field of view for $\approx 240$ ms. Subsequently, the rotor was terminated by collision with another wave front propagating from the right edge of the field of view (not shown). At a later time, the periodic activity from the left edge returned. Hence, this example demonstrates the simultaneous occurrence of 2 independent but transient sources of periodic activity, occurring through potentially different mechanisms during 1 second of AF. Figure 8E shows the pseudo-ECG during this episode. Despite the presence of 2 sources of spatiotemporal periodicity, complex dynamics are produced by the transient interaction of the 2 sources of periodic activity, which results in complex electrical activity as seen in the pseudo-ECG.

Frequently, transient sources were seen to border on the edge of the mapping region (see Figure 8). In these instances, it was impossible to know the fate of the underlying source of periodic activity, ie, whether the underlying source itself was transient in nature (similar to rotor in Figure 8) or whether propagation pathways to the mapping region from the source had transiently been altered by the interaction with other propagating waves.

**Number of Apparent Sources**

It was also noted that multiple discrete sources of spatiotemporal periodicity with the same approximate cycle length could coexist. The number of sources of spatiotemporal periodicity found in the same atrium ranged from 1 to 3. These sources were found to be simultaneous and phase-locked (see breakthroughs, Figure 7) in specific cases, whereas in others, sources were intermittent, sequential, and not phase-locked (see Figure 8). In either case, these phenomena might result from the activation of these epicardial sites from a single source via multiple select routes of ongoing 1:1 propagation in the case of continuous phase-locked periodicity or in complex patterns of propagation, resulting in the sequential use of different routes of propagation in the case of intermittent, sequential non-phase-locked sources.

Irrespective of the mechanism, duration, and number of the sources of periodic activity in the LA, their cycle length correlated well with the dominant frequency of spectral analysis of the bipolar EG. As seen in Figure 9, $R^2=0.94$ ($P<0.0001$) and slope=0.88 (95% CI, 0.73 to 1.02). These data strongly suggest that the sources of periodic activity in the LA are the dominant source of activity in this model of AF.

**Discussion**

The most important result of this study is the demonstration of specific sequences of spatially similar temporally periodic
activity (ie, spatiotemporal periodicity) that were identified during complex patterns of activation seen in AF. Furthermore, the frequency of the spatiotemporal periodic activity found in the LA recordings was well correlated with the frequency of the dominant peak, as measured by spectral analysis performed on global atrial electrical activity in the bipolar EG. These data strongly suggest that the source or sources of these spatiotemporally periodic activations are the dominant source of activity maintaining AF in this model.

Previous Work on Organization
In the original description of the multiple-wavelet hypothesis of AF as put forward by Moe et al and later substantiated by Allessie et al, the wavelets were thought to move randomly throughout the atria. However, more recent studies that have applied rigid statistical methods to long episodes of endocardial recordings have provided evidence that AF is not random.8,9 Botteron and Smith showed that closely spaced bipolar recordings of human AF could be cross-correlated over distances of 1.5 to 6 cm (mean, 2.6 cm), and hence, spatial organization was demonstrated. Gerstenfeld et al demonstrated transient “linking” during 1-minute episodes of human AF by showing that the direction of activation of 6 successive EGs varied by <30° (ie, linked). The number of successive linked EGs ranged from 6 to 14. Schuessler et al used multiple epicardial EGs in isolated canine RA to study the activation patterns emerging after single extrastimuli at increasing concentrations of ACh. Their maps revealed that in the presence of large concentrations of ACh, rapid repetitive responses were characterized by multiple reentrant circuits. However, such multiple wavelets failed to perpetuate, and with time, sustained fibrillation was the result of a relatively stable single reentrant circuit. Moreover, 2 recent surgical studies using a small number of multielectrode recordings suggested that regular repetitive activations could be seen in the LA during AF in patients with isolated mitral valve disease undergoing valve replacement. Taken together, these studies provide strong evidence that AF is not an entirely random phenomenon. Given the anatomic and electrophysiologic substrate of the atria, it is not unexpected that repetitive activations seen during AF are constrained to preferred routes of propagation. However, our study is the first to clearly demonstrate that wavelets with similar spatial patterns of propagation can activate regions of the atrium with temporal periodicity during AF.

Mechanism Underlying the Periodicity
Commonly, the periodic activity that was identified bordered on the edge of the mapping region. For this reason, it was difficult to know the underlying mechanism of their periodicity. In several episodes, however, the mechanism of the underlying periodicity could be identified from the optical recordings. Both rotors in the plane of the epicardial sheet and breakthrough patterns were identified.

Reentry in the Plane of the Epicardium
Rotors in the plane of the epicardium could be transient, in general lasting 3 to 5 rotations before termination (see Figure 8), or continuous throughout the entire recording (see Figure 6). This latter phenomenon has been reported by Schuessler et al in a canine model of AF in which a stable, anatomically based flutter circuit rotated around 1 pulmonary vein while the recorded activity in the RA changed moment to moment. The recorded ECG was consistent with AF.

Transmural Reentry
Several episodes of AF demonstrated transient or continuous breakthroughs of periodic activity (see Figure 7). These sources probably represented reentry in specific pectinate bundles. This is supported by the work of Gray et al, who demonstrated breakthroughs of activation in optical recordings of the RA free wall during AF. Furthermore, computer simulations performed in our laboratory have also provided evidence that the natural asymmetries found in the pectinate muscle network can provide the substrate for unidirectional conduction block and pectinate bundle reentry (data not shown).

Other Possible Mechanisms
Most commonly, the regions that demonstrated periodic activity bordered on the edge of the mapping region and propagated into the field of view. These regions most likely represented preferential routes of propagation (1:1, or in some cases 6:5, 5:4, etc) from a source of periodic activity beyond the mapping region. It is difficult to infer the nature of the underlying source. Clearly, because rotors in the plane of the epicardial sheet and breakthroughs of periodic activity have been identified within the mapped areas, these phenomena are also likely to underlie periodic activity beyond the mapping field. Another possible explanation for the underlying periodicity comes from recent observations in humans in which a single, repetitive focal source of activity propagated from an individual pulmonary vein to the remainder of the atrium as fibrillatory waves. However, because of the continuous presence of ACh in our experiments, it seems unlikely that if such focal activity was present, it was the result of spontaneous or triggered activity. Therefore, we favor functional or anatomically based reentry as the underlying mechanism of these sources. In cases in which periodic activity was seen to propagate from the edge of the mapping area, the source could be located at sites not mapped by our technique, such as the pulmonary vein region or interatrial septum. Regardless of the underlying rhythmic process, spontaneous activity is likely to manifest as a rotunda (ie, focal pacemaker). The rotunda’s rate will be determined by the location of the ACh source; it must be slow enough to allow the propagation of subsequent wavelets. Therefore, the periodicity may be determined by a balance between the rate of the rotunda, the time required for wavelet propagation, and the distance between the rotunda and the mapping region.
of the underlying mechanism of the source, periodic waves might propagate to our mapping field in a spatially similar fashion, depending on the interaction with other intervening wave fronts.

Transient Nature of the Source
Periodic activity that was seen to border the edge of the mapping area was often transient but recurrent, i.e., these recordings were characterized by segments in which the same periodic wave appeared for 4 to 14 sequential activations separated by segments in which no periodic activity was seen. The transient nature of these regions is interesting. It is possible that the underlying source of these activations was itself transient, or it is just as likely that the route of propagation from a constant stable source changed because of complex interactions with other wave fronts. Because we have been able to identify both transient and continuous rotors in the epicardial sheet as well as transient and continuous breakthroughs of activity during AF, both explanations for the transient nature of some periodic activity seem plausible.

LA Versus RA
Several studies in different models have demonstrated that activity in the LA is more rapid than in the RA during AF. Indeed, this is the case in our study. Moreover, periodic activity was seen more often in the LA than the RA, and the frequency of activation of LA periodic activity was greater than or equal to that found in the RA in all cases. Our data strongly support the contention that a source of periodic activity was located in the LA during AF and that activation in the RA resulted from complex patterns of propagation through interatrial pathways. In 5 cases in which the dominant frequency of the RA pseudo-EG was similar to the dominant frequency of the LA pseudo-EG, it is possible that the origin of periodic activity could have been located in either atrium. As such, sources of periodic activity can undergo spectral transformation through the fractionation of periodic waves into multiple wavelets. Although complex, it appears that this process is restrained by relatively simple deterministic principles; simple patterns of wave propagation change over space and time, resulting in a complex global picture that characterizes AF. The detailed mechanisms by which this occurs require further study.

Implications for the Mechanism of AF
Clearly, the demonstration of spatiotemporal periodicity during AF has implications for the underlying mechanism. We have seen transient and continuous sources of periodic activity spatially represented as rotors in the epicardial plane and breakthroughs representative of transmural reentry. As such, the level of organization during AF may lie on a spectrum depending on the number of sources and the degree of fractionation of the periodic activity into independent wavelets, as well as their subsequent fate. A single rapid source of activity (see Figure 6) that undergoes substantial fractionation will manifest as AF. This was recognized as early as 1925 by Sir Thomas Lewis, who used the term “impure” flutter. However, if the newly created wavelets, once formed, initiate rotors of activity, new sources of periodic activity will be created secondary to the ongoing primary source. The life span of the secondary rotors may be short, in which case the maintenance of the AF will continue to depend on the primary source, the wavelets serving only to add to the overall complexity of activation. However, if the life span of the newly formed rotors is sufficiently long, the primary source of periodic activity need not continue for the maintenance of the arrhythmia. In the latter scenario, the rate of creation and termination and the life span of the individual wavelets may be critical for the degree of complexity and maintenance of AF.

Limitations
The high spatial resolution and large field of view of the video camera provide an invaluable tool for the study of cardiac arrhythmias in the isolated heart. However, our study has some limitations that must be considered and may be summarized as follows.

1. Temporal resolution: Optical recordings were acquired at 120 Hz for ∼3 to 4 seconds with a resolution of 0.25 to 0.33 Hz. As such, we were unable to distinguish absolutely periodic sources of activity from those that might be quasi-periodic, i.e., 1 source frequency modulated by another frequency. Long recordings of AF at high sampling frequencies have demonstrated quasi-periodicity. To confirm or disprove that various spatiotemporal sources in this study were quasi-periodic rather than periodic was beyond the capabilities of the system. Furthermore, it is also possible that higher-frequency sources of periodic activity beyond our mapping region propagated with some degree of block (i.e., 8:7 or 6:5). The resultant beat-to-beat changes in cycle length might be beyond the temporal resolution of our method.

2. Bipolar EG: Because spectral analysis of the atrial electrical signal was an important tool in this study, an atrial signal minimally contaminated by ventricular signal was required. On rewarming, VF often occurred. Therefore, to minimize contamination by VF signal, we chose a bipolar EG over a volume-conducted ECG. The frequency content of the bipolar EG correlated well with an intracavitary ECG, a recording method that theoretically should sample from all regions of the atria (see Methods and Results). Moreover, a separate peak corresponding to VF could be identified in all experiments surveyed.

3. Experimental model: The experimental studies were carried out in an acute animal model of AF under the artificial conditions of isolation and crystalloid perfusion. Clearly, although previous studies in some patients with AF have shown that the ECGs of these patients have a narrow-band frequency spectrum, suggesting some degree of organization, the relevance of these data to human AF remains to be studied.

4. Voltage-sensitive dye: The limitations of this technique resulting from the usage of a voltage-sensitive dye and mechanical uncoupler have been discussed repeatedly and in detail elsewhere.

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References


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