general Pol II transcription factors[7,20,25,26]. All protein fractions were dialysed against buffer B (25 mM Tris-HCl, pH 7.9, 50 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EDTA and 20% glycerol (v/v)). 25-μl reactions contained either 25 ng 17M/5pAL7 and pG1 (ref. 5) or 100 ng pTEF(Δ-138) or pTEF(Δ-138_{TATA})[15], with aliquots of TFTC, TFIIDβ and recombinant TBP[5]. Where indicated, 200 ng purified anti-TBP monoclonal antibody 1C2 was also included in the reactions before the other factors were added. GAL-VP16-activated transcription was performed as described[5]. After the preincubation steps (30 min), transcription was initiated by addition of nucleoside triphosphates to 0.5 mM and $MgCl_2$ to 5 mM. Transcriptions were incubated at 25 °C for 45 min. Correctly initiated transcripts from the different promoters were analysed by quantitative S1 nuclease analysis[15,27].

DNase I footprinting. DNase I footprinting was performed as described[18,19]. The labelled AdMLP-containing probes were amplified by polymerase chain reaction on either the 17M5/pAL7 (ref. 28) (Fig. 3a) or the pM677 (ref. 29) (Fig. 3b) templates. For the footprinting experiments, ten times more TBP, TFIIDβ, and TFTC was used than in the transcription reactions.

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corrections

Structure of the αβ tubulin dimer by electron crystallography

Eva Nogales, Sharon G. Wolf & Kenneth H. Downing

Nature **391,** 199–203 (1998)

In this Letter, the numbers for the secondary structure elements involved in Taxol binding are incorrect (page 202, second-to-last paragraph of main text). The sentences giving the correct numbers are, "In our model, the C-3′ is near the top of helix H1 (that is, between β:15–25), and the C2 group near H6 and the H6–H7 loop (that is, between β:212–222). The main interaction of the taxane ring is at L275, at the beginning of the B7–H9 loop." □

Spatial and temporal organization during cardiac fibrillation

Richard A. Gray, Arkady M. Pertsov & José Jalife

Nature **392,** 75–78 (1998)

The *x*-axis of Fig. 1d was mislabelled: the frequency values should instead read 0, 10, 20, 30, 40 Hz. □

bootstrap replicates. ML analyses of these two trees showed that they are not significantly different.

Effects of long-branch taxa. To identify taxa with long apparent branch lengths, we performed a four-taxon NJ analysis (using gamma-corrected Kimura distances) using *Tripedalia* (a diploblast), *Antedon* (a deuterostome) and *Glycera* (a protostome) with each nematode taxon in turn. We recorded the inferred distance from the protostome–nematode node to the nematode taxon. MP distances were derived from the phylogeny presented in Fig. 1. These long-branch-length taxa often have extreme base-composition biases, but not all taxa with extreme base compositions have long branch lengths (for example, *Brugia* has an AT content of 79% but one of the shorter inferred branch lengths). NJ and MP analyses were re-performed with successive trimming of the long-branch taxa (distance >0.19 from root in four-taxon NJ analysis) from the dataset. As would be expected[29], exclusion of these taxa had effects on the bootstrap support for some clades. In particular, re-analysis excluding one or all of *Panagrellus*, *Panagrolaimus*, and *Strongyloides* yielded stronger bootstrap support for the cephalobid–steinernematid clade IV (<50% to 68%), and analyses excluding the long-branch rhabditid taxa *Bunonema*, *Teratorhabditis* and *Pellioditis* gave increased support for the Diplogasterida–Rhabditina clade V (51% to 93%). Figure 2a shows a consensus of these analyses: branchpoints that were supported by >60% in bootstrap long-branch taxa resampling of NJ or MP trees from the trimmed datasets were accepted. When there was no support for a resolved branching order, we collapsed nodes to form polytomies. Major clades supported by all analytical methods are shown and are numbered I–V. The association of Secernentea (clades II, IV and V) with the Plectidae has not been unequivocally resolved and is shown as a polytomy. We could not place the long-branch-length taxa in our trees with any certainty. We assessed statistical support for the placement of the vertebrate-parasitic taxa into four clades by calculating ML values for six-taxon subsets from the data. Each of the placements was strongly supported.

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Spatial and temporal organization during cardiac fibrillation

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Cardiac fibrillation (spontaneous, asynchronous contractions of cardiac muscle fibres) is the leading cause of death in the industrialized world[1], yet it is not clear how it occurs. It has been debated whether or not fibrillation is a random phenomenon. There is some determinism during fibrillation[2,3], perhaps resulting from rotating waves of electrical activity[4–6]. Here we present a new algorithm that markedly reduces the amount of data required to depict the complex spatiotemporal patterns of fibrillation. We use a potentiometric dye[7] and video imaging[8,9] to record the dynamics of transmembrane potentials at many sites during

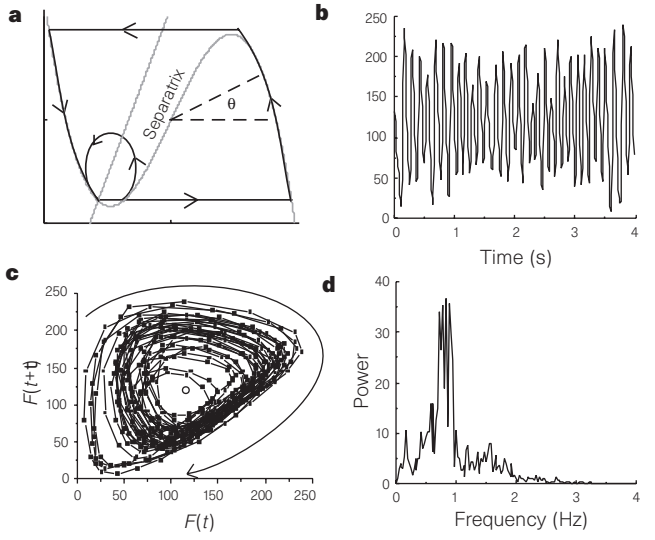

Figure 1 Temporal organization. **a**, Phase portrait of an excitable element incorporating two state variables[30]. A stable fixed point occurs at the intersection of the nullclines (dotted lines)[30]. **b**, Fluorescence signal (F) from a site on the surface of a rabbit heart during fibrillation. **c**, Phase portrait reveals trajectories circling around a centre (F_{mean}, F_{mean}), shown as a circle. **d**, The fluorescence signal exhibited a periodic component centred near 8 Hz, as observed in the corresponding power spectra. The frequency band 8 ± 3 Hz was different from equivalent white noise; $P < 0.00001$ for each heart (all sites combined).

fibrillation. Transmembrane signals at each site exhibit a strong periodic component centred near 8 Hz. This periodicity is seen as an attractor in two-dimensional-phase space and each site can be represented by its phase around the attractor. Spatial phase maps at each instant reveal the 'sources' of fibrillation in the form of topological defects, or phase singularities[10], at a few sites. Using our method of identifying phase singularities, we can elucidate the mechanisms for the formation and termination of these singularities, and represent an episode of fibrillation by locating singularities. Our results indicate an unprecedented amount of temporal and spatial organization during cardiac fibrillation.

It is still uncertain whether rotors underlie cardiac fibrillation. Self-organized rotors giving rise to spiral waves have been observed in various excitable media[11–13] including cardiac muscle[8]. Although stationary spiral waves occur in isolated thin pieces of cardiac tissue, in the whole heart, as in many excitable media, they tend to move throughout the heart. If these spiral waves move rapidly (at >30% of the wave speed), they give rise to fibrillatory activity[4]. The mechanisms of cardiac fibrillation vary[4,15], however, and fibrillation is usually the result of multiple three-dimensional electrical waves, sometimes described as meandering wavelets, propagating throughout the heart[16,17]. Cardiac fibrillation has been described in terms of

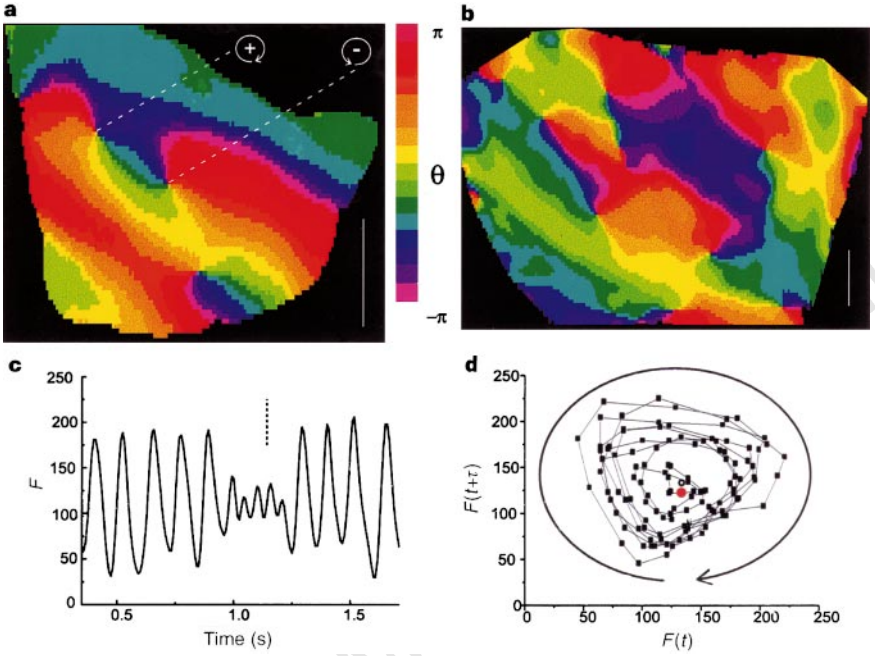

Figure 2 Snapshots of phase from the heart surface of the rabbit and sheep during sustained fibrillation. **a**, Rabbit; **b**, sheep. We classify rotor chirality as '+' for clockwise and '–' for anticlockwise[25]. At these instants, three phase singularities (two clockwise and one anticlockwise) were observed on the rabbit heart and nine (five clockwise and four anticlockwise) on the larger sheep heart. Signals (F) demonstrate (**c**) low amplitude and (**d**) remain near the centre of their phase portraits when a spatial phase singularity site is nearby. Dashed line and red circle indicate the time of the corresponding snapshot. Vertical white line represents 1 cm.

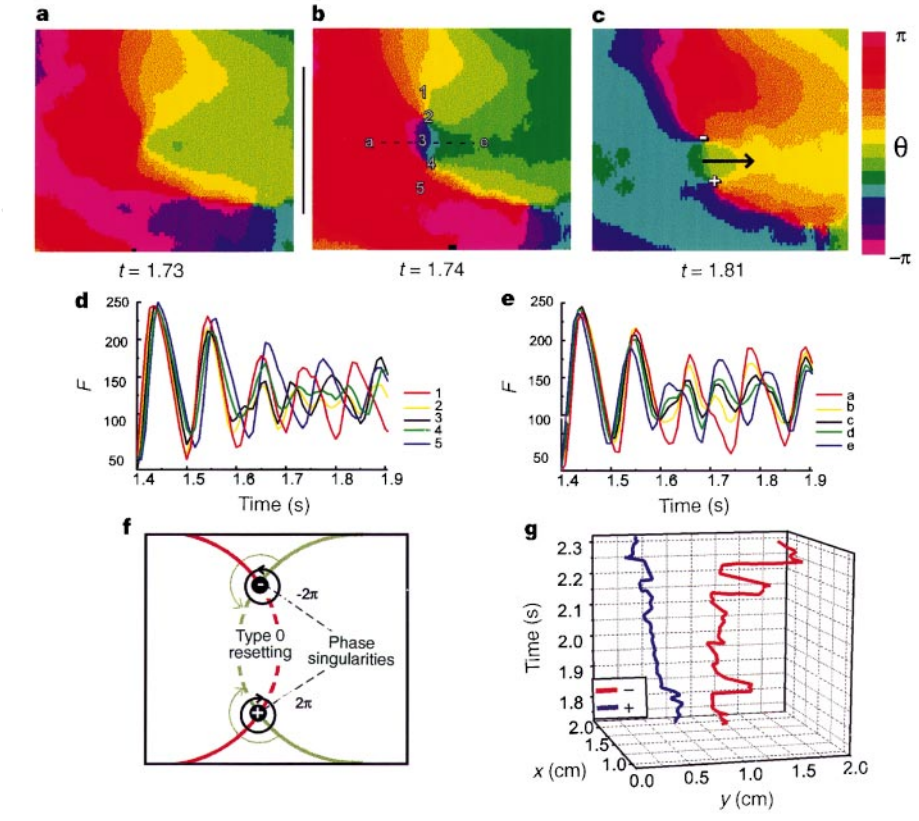

Figure 3 Initiation of a pair of spatial phase singularities. Snapshots of phase before (**a**), during (**b**), and after (**c**) the formation of a pair of spatial phase singularities during sustained fibrillation in the sheep heart. **d**, **e**, Transmembrane signals (F) measured at sites a–e and 1–5 labelled in **b**. **f**, A pair of singularities form when the local phase gradient becomes large (in other words, the excitation wave, $\theta \approx 0$ (green), approaches regions not fully recovered, $\theta \approx \pm\pi$ (red)). The excitation wave cannot proceed into the recovered region, and hence breaks, forming two phase singularities. The two excitation waves rotate around these newly formed singularities. Sustained rotation in the form of a pair of rotors occurs only if this excitation wave causes type 0, or even, phase resetting at the site of the initial wave break. Type 0 resetting (suprathreshold) advances the phase of this region into a new cycle, generating a new excitation wave (in the opposite direction to the previous wave; see arrow in **c**), resulting in the formation of a pair of self-sustaining rotors. Type 1 resetting (subthreshold) does not create this new excitation wave, and the phase singularity pair lasts less than one rotation. Notice the 'extra' cycle in the central region of block, sites 3 & c in **d** and **e**, indicative of type 0 resetting. **g**, Trajectories of '+' and '–' rotors following their initiation plotted in x, y, t space. Vertical line between **a** and **b** represents 1 cm.

rotors[4–6,14,15], on the basis of the long-held view that the heart is an example of a generic excitable medium[10,18–20]. There are many theories about fibrillation in excitable media, but only recently have experimental techniques become available to study the complex spatial patterns observed during sustained fibrillation[4,6,21–23]. Results from these experiments indicate that rotating waves are observed during fibrillation; however, they appear infrequently, and their initiation, termination and interaction have not been characterized.

An excitable element (for example, a cell, a patch of membrane, or a localized region in a spatially distributed system) can often be represented in phase space, which the element spends most of its time at a fixed point. A suprathreshold stimulus pushes the state of the excitable element past the separatrix and it continues along a closed-loop trajectory; however, if the stimulus is subthreshold, the state of the element does not cross the separatrix (Fig. 1). In periodic dynamics, it is simple and useful to represent the state of an element by its phase (θ) around the loop. The responses of single elements to external stimuli have been extensively studied by analysing the induced changes in θ (that is, phase resetting). Two fundamentally different responses to stimuli occur, namely, type 0 or even resetting, where a suprathreshold response gives rise to a new cycle, and type 1 or odd resetting, which effects a subthreshold response to stimuli[10,24]. In spatially extended excitable systems, the stimulation of individual elements are provided by neighbouring elements (usually through diffusion).

A rotor is composed of a wave of excitation propagating around a topological defect, which is known as a phase singularity. A spatial phase singularity is a site in an excitable medium at which the phase of the site is arbitrary; the neighbouring elements exhibit a continuous progression of phase that is equal to $\pm 2\pi$ around this site. As shown in Fig. 1, transmembrane signals (F) recorded from the surface of rabbit and sheep hearts during fibrillation exhibited attractors in reconstructed two-dimensional phase space, when $F(t)$ was plotted against $F(t+\tau)$ where t is time and τ is the embedding delay (see Methods). Periodicity of each site was near 8 Hz. Although trajectories for subsequent cycles did not coincide in phase space, the trajectories circulated around a central region, allowing us to construct a new variable, the phase along the attractor, θ. The phase variable (θ) calculated at each site rotated clockwise ($d\theta < 0$) 89 ± 4% of the time, indicating that the trajectories were encircling the centre. The distance of points from the centre was smaller for $d\theta \geq 0$ compared with $d\theta < 0$ ($P < 0.0001$), as would be expected if the phase were ambiguous at the centre[10]. This new variable, θ, has certain advantages over the fluorescence signal (F) that simplify the analysis of fibrillation. First, use of θ eliminates the need to pick activation times, which is difficult during fibrillation, especially in the important regions of slow propagation and block. Second, we can test directly whether spatial phase singularities exist and are necessary to maintain fibrillation.

With this new method to represent fibrillation by phase, $\theta(x, y, t)$, we could study directly the detailed dynamics of spatial phase singularities and rotors, including their initiation and termination. The spatial phase patterns during sustained fibrillation (Fig. 2) concurred with theoretical predictions (for example, isophase lines connect phase singularities of opposite chirality or end on a no-flux boundary)[10,25]. Spatial phase singularities are easily identified as sites at which all phase values ($-\pi$ to π) converge. The continuous spatial phase changes reflect waves propagating on the heart surface as a result of processes of excitation, recovery, and diffusion, and indicate that each site of the heart surface can be represented by its phase around a two-dimensional attractor. We elucidated the mechanism of rotor formation and termination by analysing successive frames of $\theta(x, y, t)$, (representative examples are shown in Figs 3 and 4). Movement of existing phase singularities created Doppler-shifted[4,8,26] short cycle lengths, and thus created large local phase gradients in front of moving singularities. The formation of phase singularities was necessary, although not sufficient, for sustained rotation (that is, for rotor formation). In addition to the formation of phase singularities, a new excitation wave must be generated[27] (that is, type 0 or even resetting must occur)[10,24] to form a rotor. Lifespan histograms for both rabbits and sheep indicate that the majority (80% for rabbit and 84% for sheep) of phase singularities lasted <100 ms, which is less than one rotation[4,14]. Therefore,

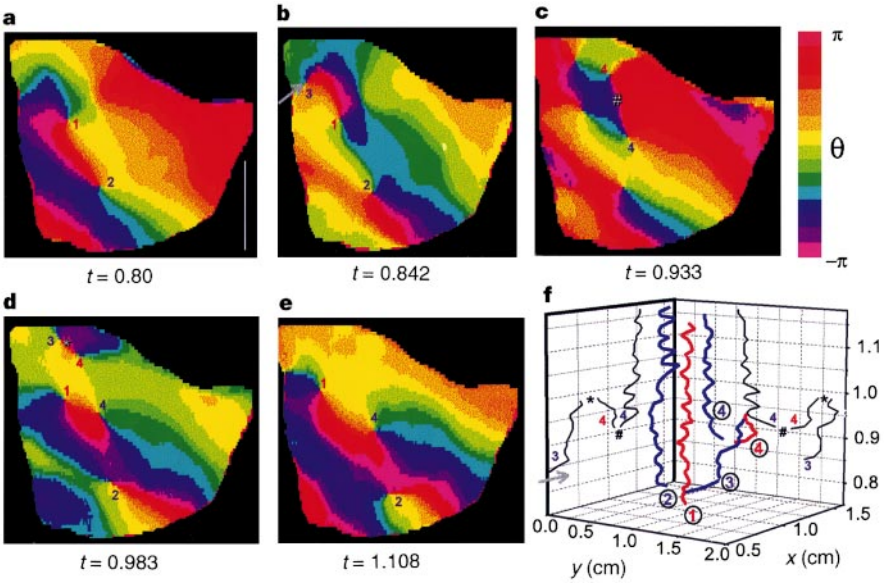

Figure 4 Dynamics of phase singularities. **a–e**, Snapshots of phase illustrating rotor dynamics on the surface of the rabbit heart during sustained fibrillation. **f**, Trajectories of the clockwise and anticlockwise rotors shown in **a–e** plotted in x, y, t space. Each rotor is numbered (1–4) and coloured (clockwise, blue; anticlockwise, red). The x and y projections in time are shown in black in **f** (except for rotors 1 and 2 for clarity). **a**, Rotors 1 and 2 formed separately before the time interval shown here (0.75–1.17 s). **b**, At t = 0.842 s a clockwise rotor (3) enters the field of view from the left and moves rapidly toward the right (grey arrow in **b** and **f**). This movement creates a convergence of phase values ahead of rotor 3, and (**c**) a pair of phase singularities (4) form (# in **c** and **f**) when the excitation wave ($\theta \approx 0$) reaches the high-phase-gradient region. Both of these newly formed singularities move; the clockwise one collides with anticlockwise-rotating rotor 3, resulting in mutual annihilation (asterisk in **d**), whereas the anticlockwise one survives in the form of a rotor. Vertical white line represents 1 cm.

only ~20% of phase singularities formed rotors.

Rotor termination occurred when rotors of opposite chirality merged or when a single rotor collided with a boundary (both of these occurrences are topologically equivalent for a non-flux boundary)[10]. Specifically, rotor pairs were mutually annihilated if the phase gradient between the rotors, perpendicular to the line connecting the phase singularities, was sufficiently large to stop propagation. On the basis of the number of rotors observed in our recording array, each rotor occupied on average $12 \pm 4\,cm^2$. According to rough measurements of heart surface area, we estimate that the total number of rotors during fibrillation would be approximately 1–2 for rabbits, 5 for sheep, and 15 for humans (assuming the rotor density is the same in humans).

These results indicate that analysing the complex spatiotemporal patterns seen during fibrillation on the surface of the heart can be greatly simplified by identifying and analysing phase singularities. This analysis reveals topological restrictions to the dynamics of fibrillation[10]: first, phase lines do not intersect; second, phase singularities are joined via isophase lines to two other singularities with opposite chirality (or a boundary); and third, phase singularities form and terminate as oppositely rotating pairs (Fig. 4). Under certain conditions, phase singularities give rise to rotors, which sustain fibrillation. The direct observation of phase singularities has led, for the first time, to the quantification of fibrillation in terms of rotor number and lifespan, and to the elucidation of the mechanisms underlying the formation and termination of rotors. □

Methods

The experimental protocols, video imaging-recording system, and signal processing have been described previously[14,28]. We acquired video images (typically 200×100 pixels) from the ventricular surface at a rate of 120 frames s^{-1} ($\Delta t = 0.00833$ s). We applied spatial and temporal filtering to improve the signal-to-noise ratio[28]. Two-dimensional phase portraits were obtained by plotting $F(t)$ versus $F(t + \tau)$ (ref. 29), where $t + n\Delta t$ and n is the frame number. The value of τ was chosen to be roughly one-quarter of the cycle length during fibrillation ($\tau = 25$ ms); this value roughly corresponds to the first zero crossing of the autocorrelation of F, indicating linear independence. A new variable phase, $\theta(t)$, was computed as $\mathrm{atan}(F(t + \tau) - F_{mean}, F(t) - F_{mean})$. Isolated hearts from rabbits of ~3 kg ($n = 3$) and from sheep of ~20 kg ($n = 3$) body weight were maintained at 36–38 °C. Ventricular fibrillation was initiated by rapid pacing, and 4-s recordings were obtained at least 5 min after initiation (perfusion was maintained during fibrillation). In Figs 2–4, white and black bars near images reflect a distance of 1 cm. Values are presented as mean ± s.d.m. Comparisons were made with paired student t-tests.

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Spatiotemporal evolution of ventricular fibrillation

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Sudden cardiac death is the leading cause of death in the industrialized world, with the majority of such tragedies being due to ventricular fibrillation[1]. Ventricular fibrillation is a frenzied and irregular disturbance of the heart rhythm that quickly renders the heart incapable of sustaining life. Rotors, electrophysiological structures that emit rotating spiral waves, occur in several systems that all share with the heart the functional properties of excitability and refractoriness. These re-entrant waves, seen in numerical solutions of simplified models of cardiac tissue[2], may occur during ventricular tachycardias[3,4]. It has been difficult to detect such forms of re-entry in fibrillating mammalian ventricles[5–8]. Here we show that, in isolated perfused dog hearts, high spatial and temporal resolution mapping of optical transmembrane potentials can easily detect transiently erupting rotors during the early phase of ventricular fibrillation. This activity is characterized by a relatively high spatiotemporal cross-correlation. During this early fibrillatory interval, frequent wavefront collisions and wavebreak generation[9] are also dominant features. Interestingly, this spatiotemporal pattern undergoes an evolution to a less highly spatially correlated mechanism that lacks the epicardial manifestations of rotors despite continued myocardial perfusion.

Ventricular fibrillation is a complicated, often lethal, but poorly understood, high-frequency mode of electrical activity. It can be