Cardiac Microstructure
Implications for Electrical Propagation and Defibrillation in the Heart


Abstract—Our understanding of the electrophysiological properties of the heart is incomplete. We have investigated two issues that are fundamental to advancing that understanding. First, there has been widespread debate over the mechanisms by which an externally applied shock can influence a sufficient volume of heart tissue to terminate cardiac fibrillation. Second, it has been uncertain whether cardiac tissue should be viewed as an electrically orthotropic structure, or whether its electrical properties are, in fact, isotropic in the plane orthogonal to myofiber direction. In the present study, a computer model that incorporates a detailed three-dimensional representation of cardiac muscular architecture is used to investigate these issues. We describe a bidomain model of electrical propagation solved in a discontinuous domain that accurately represents the microstructure of a transmural block of rat left ventricle. From analysis of the model results, we conclude that (1) the laminar organization of myocytes determines unique electrical properties in three microstructurally defined directions at any point in the ventricular wall of the heart, and (2) interlaminar clefts between layers of cardiomyocytes provide a substrate for bulk activation of the ventricles during defibrillation. (Circ Res. 2002;91:331-338.)

Key Words: bidomain model ■ defibrillation ■ finite elements ■ anisotropy ■ discontinuous conduction

There is now compelling evidence that ventricular cardiomyocytes are not arranged in a uniformly connected continuum as has often been assumed in the past. Microscopic studies of ventricular myocardium have revealed that myocytes are assembled in distinct layers1–2 with extensive clefts, or cleavage planes, between these layers. Such laminar architecture has been observed in hearts of different species3–7 and is characterized as an ordered system of interconnected muscle layers or sheets2,5,6 that have a predominantly orthogonal anisotropy with electrical propagation across muscle layers slower than propagation transverse to the myofiber axis within layers.6 This hypothesis has not previously been tested.

Structural discontinuity has been widely invoked to explain the myocardial response to electrical shock.13–17 It is acknowledged that defibrillating shocks applied to the surface of the heart would not depolarize a sufficient volume of tissue to achieve cardioversion if myocardium behaved as a continuum.16 An explanation is that discontinuities such as gap junctions, collagenous septa, and blood vessels give rise to localized regions of depolarization during defibrillation, thereby activating a critical mass of tissue.13,15–17 These local depolarizations are termed secondary sources of excitation, where the primary source is at the electrode-tissue interface. Secondary sources arise at positions where the current flux between the stimulating electrodes is forced to cross the cellular membrane in order to traverse discontinuity of the intracellular resistive network (see Figure 7 of Reference 17). Fast et al13 have suggested that the cleavage planes between muscle layers may provide an important substrate for the formation of such secondary sources. This hypothesis was supported by studies using cultured cardiac myocytes, but it has yet to be addressed in three-dimensional ventricular myocardium.

In the present study, we approach these questions using a novel combination of extended-volume confocal reconstruction of tissue microstructure7 and finite element computer
modeling of electrical propagation. This combination provides a powerful means of relating structure to function and overcomes the lack of an experimental technique for examining three-dimensional electrical events in the heart at appropriate spatial resolution. We describe a model of electrical propagation solved in a structurally discontinuous domain, which accurately represents the measured three-dimensional microstructure of a transmural segment of left ventricular myocardium. The model describes discrete discontinuities at the interlaminar cleft level while averaging the effects of gap junctional discontinuities at the cellular level. The governing equations are the bidomain equations that have been well validated in a number of experimental studies. We use the model to address the hypotheses that (1) early propagation from a focal activation can only be accurately described by a discontinuous model of the myocardium, (2) the laminar organization of myocytes determines unique propagation velocities in three microstructurally defined directions at any point in myocardium, and (3) interlaminar clefts, or cleavage planes, provide a means by which an externally applied shock can influence a sufficient volume of heart tissue to terminate cardiac fibrillation.

Materials and Methods

Structural Representation
Extended confocal microscopy was used to reconstruct the three-dimensional architecture of myocytes in a transmural segment of rat left ventricular myocardium. The image volume was 0.8 x 0.8 x 3.7 mm and consisted of 6.07 x 10^6 cubic voxels with 1.56-µm sides. The reconstructed volume (Figure 1; see movie 1 in the online data supplement, available at http://www.circresaha.org) confirmed that ventricular myocytes are arranged in branching layers separated by extensive interlaminar clefts (or cleavage planes). Myofiber orientation varied smoothly through the ventricular wall from around 75° below the circumference at the epicardial surface to 75° above the circumference at the endocardial surface. Three orthogonal unit vectors (a_t, a_n, and a_l) that reflect this microstructure can be identified at any point: a_t lies parallel to myofibers, a_n is transverse to the myofibers but in the plane of the muscle layer, and a_l is normal to the layer. For further analysis, the tissue reconstruction was reduced to a stack of 18 transmural images, each with square pixels of side dimension 4.69 µm. One of these images is shown in Figure 1. These images were used to reconstruct the three-dimensional geometry of the cleavage planes that coursed through the tissue volume. A network of bilinear finite elements describing the cleavage plane geometries was generated from the image stack by manual assignment of element nodes to regions devoid of myocyte-to-myocyte connections. Each finite element spanned a distance of 47 µm in the z direction (Figure 1), which was the distance between successive images in the stack. In total, 1540 elements and 2803 nodes were required for accurate geometrical description of the cleavage planes (Figure 1).

Discontinuous Bidomain Model Formulation
A bidomain approach was used to model electrical propagation in the reconstructed tissue volume so that the effects of the interlaminar clefts (cleavage planes) on the spread of activation could be investigated. With this approach, the coupling between ventricular myocytes and extracellular space is represented on a continuum scale by defining two domains that coexist at each point throughout the volume. Conductivity tensors G_l and G_e for these intracellular and extracellular domains, respectively, are defined by

\[
G_{ij} = \begin{bmatrix}
0 & 0 & a_t^i \\
0 & 0 & a_n^i \\
0 & 0 & a_l^i
\end{bmatrix}
\]

where \(g_{i,t}, g_{i,n},\) and \(g_{i,l}\) are effective intracellular and extracellular conductivities along the local microstructural axes \(a_t, a_n,\) and \(a_l\).

Within this context, interlaminar clefts must be viewed as discontinuities in the intracellular domain, across which there is zero intracellular flux. We sought a solution to the active bidomain equations that allowed accurate spatial representation of the interlaminar clefts, in order to apply the condition of zero intracellular flux across these boundaries. A Galerkin finite element method was used on a mesh of trilinear elements representing the reconstructed tissue volume. Disruption of cellular connections across cleavage planes was achieved by the removal of elements of the trilinear mesh from the intracellular domain and application of no-flux (Neumann) boundary conditions along the resulting internal intracellular domain boundaries. Examples of the procedure are illustrated in Figure 2. Representation of cleavage planes required removal of \(\approx 11\%\) of the solution mesh volume from the intracellular domain. The entire mesh consisted of 483 840 nodes. The finite element method generates systems of equations in \(V_e\) (transmembrane potential) and \(\Phi_e\) (extracellular potential), which were solved with a semi-implicit time integration scheme utilizing the conjugate gradient method. A finite element solution of the bidomain equations
was used in preference to a finite difference approximation, as the Neumann condition could be applied at all internal intracellular boundaries with a minimum number of degrees of freedom.

The model incorporated the following linear variation of myofiber angle (x) through the ventricular wall based on measurements from the reconstructed tissue volume: \( \alpha = 0.83x - 1.28 \text{ radians} \) (see Figure 1 for direction of x).

Intracellular and extracellular conductivities were assumed to be transversely isotropic with respect to fiber direction \( g_{n;i} = g_{e;i} \). Any possible transverse anisotropy of the intracellular domain was explicitly modeled by the inclusion of the cleavage plane discontinuities.

Two sets of simulations were carried out to investigate both the response of the tissue to propagation after point stimulation (part I) and the response to defibrillation-strength shock (part II).

Part I: Propagated Response

The reconstructed tissue volume was stimulated at twice diastolic threshold by applying transmembrane current for 2 ms at its center. The propagated response was followed through time, until the volume was fully depolarized (~18 ms). A simple cubic ionic current model was used in the simulation to reduce computational expense. Neumann boundary conditions were applied along all external boundaries of the reconstructed tissue in both intracellular and extracellular domains, and \( \Phi_i \) was measured relative to position \((x=3.7, y=0, z=0.8)\) (see Figure 1). Solutions required ~85 hours of CPU time with 8 processors of an SGI Origin 2000 (250 MHz R10000 CPU units).

A major premise underlying this part of the study is that electrical propagation through myocardium perpendicular to muscle layers is slower than propagation within layers transverse to the myofiber axis because of the discontinuities introduced by interlaminar clefts. To obtain spatially averaged estimates of propagation in the discontinuous bidomain model, results were matched with a continuous model in which orthotropy was incorporated through unequal transverse intracellular conductivities \( g_{n;i} \neq g_{e;i} \). Comparison of these results also resolved the effects of structural discontinuity on local propagation. In the continuous model, a conventional bidomain formulation was used and the following continuous description of the transmural variation in cleavage plane angle (\( \beta \); defined in Costa et al84) was used: \( \beta = -0.0892x^2 + 0.5595x - 0.814x + 0.8669 \text{ radians} \).

The microstructural axes \( \alpha_n \) and \( \alpha_e \) were then oriented according to the cleavage plane angle description. The intracellular conductivity normal to the cleavage plane, \( g_{n;i} \), was set at a value \( (g_{n;i})_{\text{def}} \) that minimized the root mean square (rms) difference between the continuous and discontinuous model solutions for transmembrane potential while the same value for \( g_e \) was used for both models.

Part II: Shock-Induced Response

A defibrillation-strength shock was applied across the reconstructed tissue volume. Constant current (10 ms in duration; uniform density of 0.14 mA/mm\(^2\)) applied to the extracellular domain at the epicardial (cathodal) and endocardial (anodal) surfaces induced an extracellular potential gradient of ~1 V/mm across the ventricle wall. The shock response was modeled using the Drouhard-Roberge-modified Beeler-Reuter ionic current model (BRDR)\(^{25,24}\) with recent revisions.\(^{23}\) During the shock, the transmural boundaries of the reconstructed tissue volume acted to redistribute current between the extracellular and intracellular spaces because of the oblique angle of approach of the myocyte laminae to these boundaries. To minimize these artifactual boundary effects, the domain was expanded laterally (in y and z directions; expanded volume = \(4 \times 4 \times 3.7 \text{ mm} \)), allowing continuity of current at the transmural boundaries of the reconstructed volume. For computational efficiency, a continuous description of the laminar structure was included in this additional region bounding the central tissue volume in which the discontinuities were explicitly represented.

As in part I, the shock-induced response of this discontinuous model was compared with that of a fully continuous model in which the same cubic description of cleavage plane angle was applied throughout the entire tissue volume.

Model Parameters

The model parameters used in this study are given in the Table and discussed in the online data supplement, available at http://www.circresaha.org.
Results

Part I

The activation sequence after point stimulation at the center of the tissue volume is shown for the discontinuous and continuous models in Figures 3A and 3B, respectively. Potential maps are given on seven planes through the heart wall at 2.7-ms intervals after stimulation (see movie 2 in the online data supplement for the complete activation sequence). Discontinuous propagation around the cleavage planes is evident from the irregularity in isopotential lines in Figure 3A. Important differences between the two models are apparent. First, activation in the discontinuous model is markedly asymmetric about the site of stimulation in its early stages (see the 5.4-ms panel of Figure 3A) compared with the symmetrical activation of the continuous model. Second, the insulating boundaries formed by cleavage planes allow juxtaposition of fully depolarized and nondepolarized tissue regions in the discontinuous model (eg, at 10.8 ms).

Despite local differences between the two models, the continuous model reproduces key features of the global pattern of excitation in the discontinuous model. For example, earliest activation skews toward the edges $(y=0, z=0)$ and $(y=0.8, z=0)$ as the wavefront propagates through the tissue volume in both models. The conductivity parameter $g_{n_{\text{MIN}}}$ used in the continuous model of Figure 3B was that which minimized the $rms$ difference between the continuous and discontinuous solutions over the 18 ms required for full activation of the tissue block. The $rms$ difference between models is 5.2 mV, and the best-fit parameter $g_{n_{\text{MIN}}}$ was 0.0107 S/m, giving the ratio $g_{c}/g_{n_{\text{MIN}}}=2.44$. With this optimization procedure, it is assumed that $g_{n}$ is uniform throughout the block in the continuous model. However, there were fewer cleavage planes in subepicardial and subendocardial regions than in the midwall, suggesting that $g_{n_{\text{MIN}}}$ may overestimate conductivity normal to muscle layers in the midwall region. Excluding the final 6 ms of propagation (where the wavefront enters subepicardial and subendocardial regions) from the optimization procedure, reduced $g_{n_{\text{MIN}}}$ to 0.0080 S/m ($g_{c}/g_{n_{\text{MIN}}}=3.25$, minimized $rms$ difference $=4.95$ mV). The nonunity value of $g_{c}/g_{n_{\text{MIN}}}$ indicates that the tissue block behaves in an electrically orthotropic fashion: propagation across layers of myocytes is substantially slowed because of the convoluted path the wavefront must take around cleavage planes. In fact, one effect of this orthotropy is the tendency of earliest activation to skew toward the upper and lower left edges of the domain as the wavefront progresses (Figures 3A and 3B).
It is well recognized that discontinuity in wavefront propagation may lead to complex polyphasic (fractionated) extracellular potential recordings. To investigate the possibility of fractionation in extracellular recordings from ventricular tissue, we plotted extracellular potential waveforms at 28 sites throughout the rat tissue volume. The signals from the discontinuous and continuous models are shown in Figure 4 (top and bottom panels, respectively). All signals are referenced to the point \((x=3.7, y=0, z=0.8)\) and hence approximate unipolar recordings as might be performed in real tissue. The distance of each recording location from the central site of stimulation is given by the signal color (red indicating close; blue indicating far; see Figure 4 legend), while the \(y\) and \(z\) coordinates of the recording locations are shown above the traces in parentheses. Several observations can be made from these traces. First, nearly all signals from the discontinuous model show some degree of fractionation. The extent of fractionation appears highest in the early stages of propagation. Second, it is evident that the downstroke duration (time over which signal derivative is negative) of signals recorded close to the stimulus site is considerably longer in the discontinuous model than in the continuous model. Downstroke duration was measured by the time required for the downstroke to change from 90% to 10% of its maximum amplitude. The mean duration across red and yellow signals (Figure 4) was 7.25 and 2.81 ms for the discontinuous and continuous models, respectively. A comparable difference in transmembrane potential upstroke duration (measured by the time required for the upstroke to change from 10% to 90% of its maximum amplitude) was not seen at the same recording locations (mean times were 3.23 and 2.77 ms for discontinuous and continuous models).

Traditionally, the time of maximum negative slope \((-V'_{\text{max}})\) of a unipolar extracellular recording is used to pick the time of activation at the site of measurement. In the fractionated recordings of Figure 4, there are often two distinct regions of similar negative slope. To test the ability of \(-V'_{\text{max}}\) to discern local activation time, we compared the time at \(-V'_{\text{max}}\) (open circles, Figure 4) with the time of maximum positive slope of the transmembrane potential recording from the same site (closed circles, Figure 4). The two markers of activation are shown on each trace where time differences were greater than the interval used in the slope calculation (0.1 ms). Local time of activation was correctly determined by \(-V'_{\text{max}}\) in all but two traces of the continuous model. There were more cases of inaccurate assignment in the discontinuous model; however, in only two cases was the error >0.6 ms.

Part II

The activation sequence during the shock delivered across the ventricular wall is shown for the discontinuous model in Figure 5A and for the continuous model in online Figure 1, available in the online data supplement at http://www.circresaha.org. Transmembrane potentials are mapped on a central plane \((z=0.4 \text{ mm})\) at each time step, and the color spectrum is scaled to distinguish depolarized from hyperpolarized regions. Numerous islands of depolarization are formed on the endocardial side of the cleavage plane discontinuities. These localized excitatory regions, or secondary sources, arise from redistribution of current between intracellular and extracellular compartments, allowing current flow around the insulated cleavage plane boundaries of the intracellular domain. They are responsible for activation of the bulk myocardium within the 10-ms duration of the
applied shock. In the continuous model (online Figure 1), transmembrane potential varies smoothly, and most of the tissue remains hyperpolarized during the shock. Figure 5B shows the three-dimensional architecture of secondary sources 2.5 ms into the shock period. An extensive set of surfaces on which transmembrane potential is -60 mV is distributed throughout the tissue volume (see movie 3 in the online data supplement for the full activation sequence). The largest region of secondary source depolarization arises at the junction between midwall and subendocardial regions (Figure 5A, 4 ms), where there can be maximal redistribution of current between intracellular to extracellular spaces because of the lack of further discontinuity toward the endocardium.

Figure 6 presents transmembrane potentials along a line drawn through the center of the reconstructed tissue volume (from epicardium to endocardium), for both discontinuous and continuous models, at 6 ms into the shock. This figure demonstrates the full range of transmembrane potentials present in the tissue volume. The continuous model shows an activation wavefront moving away from the depolarized epicardium and located at \( t \approx 1 \) mm from it.

It is clear from Figures 5 and 6 that cleavage plane discontinuities facilitate the synchronous activation of a large volume of myocardium during shock application, through the formation of localized (secondary) regions of depolarization.

**Discussion**

Cardiac excitation and contraction have previously been modeled in our laboratory on global representations of cardiac anatomy using the finite element numerical solution technique. In the present study, we extend this approach to investigate how excitation processes could be influenced by structure at a much finer scale. We extracted an accurate representation of ventricular microstructure for use in the model from a high-resolution dataset previously assembled by sequential sectioning and extended confocal imaging of a transmural block of rat left ventricular myocardium. These data clearly exhibit the laminar arrangement of myocytes in ventricular myocardium reported elsewhere. Application of the bidomain equations to the finite element representation of this microstructure yielded interesting insights into the effects that discontinuities associated with muscle layers have on excitation.

**Implications for Propagation**

The principal finding of the study of electrical propagation in ventricular myocardium is that discontinuities associated with the laminar arrangement of cardiac myocytes could markedly
influence the spread of electrical activity from a focal source of excitation. The spread of activation is predicted to be nonuniform and potentially asymmetric about the focus of stimulation. Moreover, propagation is not transversely isotropic but moves most slowly through the myocardium perpendicular to muscle layers. It is noteworthy that slow and nonuniform propagation are factors implicated in the genesis and maintenance of reentrant cardiac arrhythmia.

Nonuniform local propagation can be modeled only by explicit inclusion of structural discontinuities, and this should be taken into account in future computer models of reentrant arrhythmia. However, this study also shows that the global spread of electrical activation from a point excitation source is represented with reasonable accuracy by continuous orthotropic models in which separate electrical conductivity values are associated with three microstructurally defined material directions.

We have previously estimated the relative velocities of midwall electrical propagation in each of the three material directions based on images of myocardial structure and two-dimensional measurements of the pathlength a propagating wave might take to traverse cleavage planes. Pathlength measurements do not take into account the possibility of direct stimulation of myocardium across cleavage planes due to current flows in the extracellular domain. Spread of activation between two completely disconnected intracellular spaces by such electrotonic effects was observed in the model studies reported here. For physiological extracellular conductivity, however, the delay of propagation at interlaminar clefts slows propagation in the direction normal to myocyte laminae. A conductivity ratio \( c_t/c_n \) of 2 in the midwall was fitted in this study for propagation in the midwall. Our previous pathlength measurements suggested a ratio of propagation velocities \( c_t/c_n \) of 2 in the midwall.

To compare these results, we use the following relationship:

\[
c_t \approx \frac{g_{nc} + g_{ni}}{g_{ne} g_{ni}} \left( \frac{g_{te} g_{ti}}{g_{te} + g_{ti}} \right)
\]

and obtain a velocity ratio \( c_t/c_n \) of 1.68. These results appear consistent given that the pathlength measurements were made in two-dimensional tissue sections and probably overestimate the effective length of propagation pathways in three-dimensional myocardium.

In addition to anisotropy of conduction velocity, we expect discontinuous propagation in the ventricle to be reflected by other measures. Polyphasic extracellular potential waveforms have previously been observed in human ventricular tissue, and in infarct border zone or ischemic regions of myocardium. The results of this study indicate that extracellular recordings from sites close to a midwall focal stimulus, in healthy ventricular tissue, may also exhibit complex multiple inflections. These recordings may also show a much reduced rate of downstroke than previously expected. These two characteristics may lead to inaccurate assignment of the local time of activation based on the maximum negative derivative of the extracellular signal.

### Implications for Defibrillation

Electrical shock applied to the heart achieves defibrillation by simultaneously resetting myocardium into a uniform electrical state. It is necessary for the applied shock to influence the membrane potential of a substantial volume of tissue. We have presented striking new evidence that interlaminar clefts within the ventricles are critical to this process, allowing activation of the bulk of the myocardium during the applied shock.

Over the past decade, many mechanisms by which an applied shock to the heart may alter transmembrane potential have been elucidated. “Virtual electrode” is now common jargon to refer to any site of shock-induced transmembrane potential change distant from the site of current injection. It has been shown that virtual electrodes may be induced by unequal anisotropy of intracellular and extracellular spaces, myofiber curvature, fiber narrowing, spatial inhomogeneity of intracellular volume fraction, and discontinuity associated with gap junctions and intercellular clefts. Any geometrical condition that induces current redistribution across the cell membrane during stimulation will lead to formation of a virtual electrode. The present day challenge is to ascertain how all these mechanisms affect the ability of a shock to terminate fibrillation.

We assert that the most important form of structural discontinuity in terms of cardioversion is the interlaminar cleavage plane not the gap junction. Modeling studies of gap junction–induced virtual electrodes have indicated spatial fluctuations in transmembrane potential of a few millivolts; however, these sawtooth patterns have not been seen experimentally. There is further previous evidence for our assertion. The threshold required for diastolic cathodal point stimulation in the midwall has been measured as 0.8 V/cm. It is necessary to assign discontinuity at the level of bundles of myocardial fibers rather than gap junctions to match this value in a discontinuous syncytial model of myocardium. Our structural model incorporates transmural fiber rotation and unequal anisotropy, as well as structural discontinuity. Under conditions of uniform field stimulation, it is the cleavage plane discontinuities that are responsible for the activation of the bulk of the myocardium within the duration of the shock.

It is expected that a shock applied to the body surface will induce a relatively uniform current density field within the myocardium. The shock field from an implantable cardioverter-defibrillator (ICD) is likely to be less uniform, however, and the relative role of structural discontinuity in this form of far-field stimulation remains to be investigated. In the setting of nonuniform field stimulation, unequal anisotropy between intracellular and extracellular spaces can by itself generate virtual electrodes in the form of a dog-bone shape of polarization, with adjacent regions of opposite polarization. It is interesting to note that the experimental studies confirming these patterns have been carried out on the epicardial surface of the heart, where coupling between myocytes is increased with respect to the midwall, and large intercellular clefts are absent. It is yet to be seen whether a dog-bone pattern of polarization is in fact visible at all, deep within the midwall, during nonuniform field application. The development of a transmural probe able to record transmembrane potentials deep within myocardium may yield exper-
Phyllis Paykel Trust of New Zealand. In summary, this study contributes two observations that we have obtained an overestimate of on the slowing of propagation. As a result, it is likely that we obstacles, in turn enhancing the effect of the cleavage planes available paths of propagation around the cleavage plane (Figure 3). The presence of the transmural boundaries acts to reduce the available paths of propagation around the cleavage plane obstacles, in turn enhancing the effect of the cleavage planes on the slowing of propagation. As a result, it is likely that we have obtained an overestimate of $g_e/g_{li}$ for this tissue block. The boundary effect does not, however, change our overall conclusion that the tissue behaves in an electrically orthotropic fashion. Other limitations of the study relate to the piecewise cube approximation of the gap space between myocyte laminae that was used, and the assumption of isotropy of the extracellular space in the plane orthogonal to myofiber direction that was made. These limitations are discussed further in the online data supplement.

Conclusions
In summary, this study contributes two observations that we believe are of primary importance to cardiac electrophysiology. First, we have shown that the standard notion that myocardium is electrically isotropic in the plane orthogonal to myofiber direction is incorrect; the laminar organization of myocytes leads to orthogonal anisotropy of electrical properties within the ventricular wall. Second, we have demonstrated that the discontinuities associated with muscle layers could play a significant role in the termination of fibrillation by an externally applied shock.

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Model parameters

The values selected in this study for the conductivities $g_{Li}$, $g_{Le}$, $g_{Lc}$, and $g_{Tc}$, follow suggestions made in a recent review\(^1\) of experimental determinations of electrical constants in the heart. It should be noted that the relative values of $g_{Li}$ and $g_{Tc}$ used are derived from heart surface potential measurements.

Supplement Movie 1. Three-dimensional reconstruction of rat left ventricular free-wall myocardium, and the finite element description of cleavage plane discontinuities. The myocardial cleavage planes are most easily distinguished on thin tissue sections. The full reconstructed volume is less amenable to their visualization.

Supplement Movie 2. Propagation in a discontinuous (upper) and continuous (lower) model following point stimulation. Transmembrane potentials are plotted through time (0 – 22ms) on 7 equally spaced planes. The continuous model was fitted to the first 12ms of the discontinuous model only, in order to examine the spatially averaged behaviour of the discontinuous propagation through the ventricular midwall. Cleavage planes are sparse in the subepi- and subendo- cardiac regions of the discontinuous model. Hence, as the wavefront enters these regions the velocity of propagation increases due to the relative lack of cleavage plane obstructions to intracellular current flow. The continuous model conductivities are set to be uniform throughout the block, and hence this increase in conduction velocity is not matched by the continuous model; the model begins to ‘lag’ the discontinuous model in the final few milliseconds of the movie.
Supplement Figure 1. Progression of activation in the continuous model during the 10ms of applied shock. Transmembrane potentials are mapped on a single mid-volume plane (z=0.4mm) at 2ms time increments following onset of shock. The color spectrum is scaled to emphasize boundaries between depolarised and hyperpolarised regions, and does not cover the full range of potentials within the area shown.
Supplement Movie 3. Development of secondary sources of activation during shock application. The movie shows surfaces on which membrane potential is \(-60\text{mV}\), and their progression until 9ms following the shock onset.

Limitations of the study
The main limitations of the study arise from the dimensions of the reconstructed tissue block used in the simulations. It is evident from Fig. 1 that some cleavage planes extend across the full width of the rat tissue volume. Study of larger volumes of tissue will allow us to reconstruct the full extent of single cleavage planes. At present there is no evidence to suggest that cleavage planes in the normal ventricle play a role in producing arrhythmia due to conduction blocks. However, full reconstruction of cleavage planes, along with models of age and disease related processes such as myofiber uncoupling\(^2\) and depressed conduction may yield further insights into the possible role of the laminar organization of myocytes in providing a substrate for re-entrant activity.

Initially Part II of the study was carried out using just the rat tissue volume. The transmural boundaries acted to redistribute current between intra- and extra- cellular spaces to an extent that obscured the secondary sources of activation at the cleavage plane discontinuities. In order to reduce these boundary effects and better approximate the behavior of tissue within an intact ventricle, it was necessary to enclose the reconstructed tissue volume within a region were cleavage plane orientation was represented by a continuous description. Ideally, we may wish to examine the effects of cleavage planes on defibrillation by establishing a re-entering wavefront within the discontinuous model, and examining the conditions required for termination of re-entry. However, both the volume of reconstructed tissue, and the computational expense of such a model precluded this approach. Acquisition of the extended confocal image was highly labor intensive, and in order to image larger volumes of tissue in reduced time we are currently developing an automated approach to this imaging process.
As indicated in Fig. 2A, the finite element model made a piecewise cube approximation to discontinuities in the intracellular space. This representation gave rise to the artifact that the gaps between adjacent myocyte laminae had a volume which was determined by the resolution of the finite element mesh. For example, in the discontinuous model of Part I a total of 483,840 nodes were used, giving trilinear element dimensions of 0.014 x 0.017 x 0.017mm. This resulted in ~11% of the total intracellular domain being removed from the solution (assigned as “gap space”). It is suggested from studies of collagen volume fraction in normal rat ventricle that connective tissues may constitute approximately 3.4% of the total ventricular volume. Not all of this collagen volume would be expected to lie between myocyte laminae. Thus our model may over-estimate the fractional volume of the gaps between myocyte laminae. Further refinement of the mesh used, however, was prohibited by computational time.

A further assumption of our model is that extracellular conductivity is isotropic transverse to myofiber direction (i.e. $g_{t,e}=g_{n,e}$). The close relationship between myocyte and collagen networks suggests that the extracellular space is likely to exhibit orthogonal anisotropy along the same microstructural axes as its intracellular counterpart. The model gave no means of testing this prediction. Moreover, the significance of discontinuity of the extracellular space in secondary source formation during defibrillation was not investigated. It is likely that spatial changes in collagen fraction and interstitial volume between adjacent myocytes may also contribute to secondary sources.

The application of this work to the human heart requires analysis of cleavage plane geometries in healthy and diseased human hearts. To date no such study has been carried out; we base our understanding of the laminar organization of myocytes from measurements taken from rat, dog, and pig hearts (unpublished observations).

Direct validation of the model predictions reported in this paper is technically difficult. It would be necessary to measure electrical potential with high spatial resolution to relate myocardial structure to the spread of activation from a point stimulus. Although
membrane potential can be recorded with appropriate resolution from heart surfaces, the density of cleavage planes in subepicardial (and subendocardial) regions is low and myocytes are tightly coupled. Attempts to reconstruct the 3D spread of electrical activation through the ventricular wall using extracellular plunge electrodes can provide relatively coarse global information at best. Within this context, sophisticated computer models that incorporate realistic representations of cardiac microstructure may provide the most reliable information. Nonetheless, preliminary studies utilizing arrays of extracellular plunge electrodes in an experimental pig heart model have been carried out in our laboratory. Fractionated extracellular potential waveforms are typically observed adjacent to a point stimulus, though not in sinus rhythm. In addition, reconstructed activations wavefronts often appear to be asymmetric about a site of point stimulation.

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