Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution

Short Title: Engelman; Structural Heterogeneity and Dynamic Restitution

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ABSTRACT

Background: Marked changes in ventricular APD restitution and associated alternans rhythm have been demonstrated in structural heart disease (SHD). However, whether this is due to structural heterogeneity or regional variation in cellular properties remains uncertain. In this study, we address the hypothesis that the structural heterogeneity associated with SHD is sufficient to alter dynamic restitution and increase the probability of electrical instability.

Methods and Results: Activation was simulated in a 14x14mm² domain in the presence and absence (control) of a central region containing nonuniform discontinuities resembling patchy fibrosis. A modified LR1 cardiac activation model was used in a bidomain formulation with isotropic conductivities. Bipolar stimulation was imposed above the central region with coupling intervals decreasing progressively from 500ms and then maintained at 105ms. Structural discontinuities had little effect on electrical activation at low stimulus rates, but activation time and APD distributions became highly nonuniform within and adjacent to the discontinuous region at high rates. Discordant APD alternans occurred in both “fibrosis” and control, but at lower stimulus rates and with markedly greater extent in the former. Tortuous conduction through the discontinuous region resulted in large fluctuations of diastolic intervals giving rise to regional electrical instability, which modulates dynamic conduction velocity and APD restitution. This led to heterogeneous conduction block and reentry not observed in control.

Conclusions: We show that structural discontinuities can amplify discordant alternans and provide a rate-dependent substrate for reentry. This work provides new insights into the mechanisms by which fibrosis may contribute to arrhythmogenesis.

Keywords: Arrhythmia, Computers, Reentry, Remodeling, Ventricles,
Introduction

Structural heart disease (SHD) can occur as a result of either ischemic or nonischemic cardiomyopathy and is associated with myocyte necrosis, proliferation of interstitial fibrosis and disruption of cardiac cellular organization.\(^1\) These structural changes lead to increased heterogeneity of electrical properties with marked local perturbations in electrical anisotropy and in the extent of discontinuous conduction.\(^2\) Structural remodeling is thought to provide a substrate for reentrant electrical activation and ventricular fibrillation.\(^3,4\) Wavebreak and fibrillation are also linked with beat-to-beat alternation in heart rhythm and contractility\(^5-7\) and the incidence of T wave alternans is an established predictor of sudden cardiac death in SHD.\(^8,9\)

Clinical, experimental and computer modelling studies suggest that structural discontinuities across a range of scales interfere with the spread of cardiac activation and contribute to initiation of spiral waves and wavebreak.\(^2,10-12\) They indicate that both the extent and the organization of interstitial fibrosis increase the risk of electrical reentry. In particular, wavebreak and fibrillatory activity are most likely to occur in the presence of patchy, non-uniformly distributed fibrosis.\(^11-13\) Related studies also provide complementary insights into the dynamic processes that generate alternans rhythm. This is viewed as being driven by one of two mechanisms, both cellular in origin; either action potential duration (APD) restitution slope, which reflects ion channel kinetics,\(^7\) or altered intracellular calcium handling.\(^14\) On the other hand, discordant alternans, in which electrical rhythm in adjacent heart regions is out of phase, is caused by spatial variation of conduction velocity (CV)\(^7\) or nonuniform activation delays due to the presence of structural barriers. There is evidence that structural barriers alter CV restitution\(^15,16\) and give rise to increased instability during APD alternans.\(^17-20\) Kawara et al\(^2\) have shown that, in the human heart, patchy fibrosis is associated with substantial activation delays. Additionally, marked changes in APD restitution have been demonstrated in patients with SHD.\(^21\) However, the extent to which this is due to structural remodeling (fixed heterogeneity) as distinct from regional variation in cellular properties due to electrical remodeling (dynamic heterogeneity) remains uncertain. In general, perturbations in dynamic restitution relationships\(^21\) and the development of discordant alternans\(^19\) as well
as the heterogeneity of restitution relations constructed for patients with ischemic heart disease\textsuperscript{22} have been attributed to dynamic heterogeneity.

In this paper, we hypothesize that the structural heterogeneity associated with SHD may be sufficient to alter electrical restitution and increase the probability of reentrant activation. To test this, we have investigated the rate-dependent behaviour of a 2D computer model that incorporates anatomic discontinuities typical of those observed in patchy fibrosis, but in which cellular electrical properties are uniform.

**Methods**

A 14x14 mm\textsuperscript{2} domain was discretized into a regular mesh of 0.05x0.05 mm\textsuperscript{2} finite elements with discontinuities of scale and arrangement similar to those observed in patchy fibrosis\textsuperscript{2,4} in a 10x4 mm\textsuperscript{2} region at the centre (Figure 1). The bidomain equations were solved\textsuperscript{23} on this domain with an the LR1 action potential model\textsuperscript{24} modified to produce desired APD properties.\textsuperscript{6} The LR1 model parameter $G_{Si}$ was changed to 0.06 mS/cm\textsuperscript{2}. Steep and flat APD restitution were obtained respectively by increasing the time constant $\tau_j$ of the slow $I_{Na}$ inactivation variable five-fold or by decreasing the calcium current time constants $\tau_d$ and $\tau_f$ by factors of 0.3. Membrane properties were uniform and isotropic conductivities of 0.02 mS/mm in both domains were chosen match velocities observed in cardiac cell culture experiments. Intracellular current flux was assumed to be zero within discontinuities. Transmembrane potentials ($V_m$) and extracellular potentials were solved at 0.02 ms time steps. The model was solved without discontinuities to provide control data.

Bipolar extracellular stimulation was applied via an anode and cathode, each 0.4x0.4 $\mu$m\textsuperscript{2} in area, located 2 and 4 mm respectively from the upper boundary of the domain. Stimulus strength was 20 $\mu$A/cm\textsuperscript{2} (~2x diastolic threshold). The pacing protocol consisted of 17 stimuli, in which coupling interval (CI) was progressively reduced from 500 to 105 ms in increments from 100 to 5 ms, followed by a steady stimulus train at CI=105 ms (see Figure 1).
Activation time (AT) and repolarisation time (RT) were defined as the times at which $V_m= -35$ mV and -75 mV in depolarization and repolarization, respectively. Restitution relations were characterized by fitting a three parameter mono-exponential function to APD and diastolic interval (DI) data\textsuperscript{16} using a nonlinear least squares technique. Fitted curves were excluded from analysis when the normalized best-fit residual error was $> 0.1$ which occurred with local conduction block. Maximum slopes were determined at the minimum DI. Local conduction velocity (CV) was determined at each point from ATs in a small window centered on that point. The complexity of local conduction pathways was quantified by evaluating the normalized centre-line length of tissue surrounding discontinuities in a 1x1 mm window at each point across the central region of the tissue domain. This measure is defined as "tortuosity". The distribution of CVs for sites with different tortuosity was depicted using a boxplot, which shows the lower (Q1), median, and upper (Q3) quartiles along with the smallest and largest datum within a 1.5 interquartile range (Q3-Q1) of Q1 and Q3, respectively.

A detailed outline of methods is provided in the Online Supplement.

Results

The effects of structural discontinuity on wave propagation and action potential characteristics are summarized in Figure 2. At slow stimulus rates (CI = 500 ms), the activation wavefront is perturbed only marginally as it propagates through the discontinuities. While there is little qualitative difference in RT relative to control (Figure 2A), propagation delays due to the structure result in slightly longer APDs. At CI = 110 ms (Figure 2B), there is marked variation in AT within and beyond the region of discontinuity. Propagation is delayed by structural heterogeneities, while localized wavefront collision is also observed. However, activation propagates relatively quickly through the centre of the region which is structurally more uniform. Finally, complex asymmetric dispersion of APD is seen below the discontinuous zone, because the distribution of RT is more uniform than that of AT in this region.

When CI was reduced progressively, APD alternans occurred in the presence and absence of structural discontinuities once the threshold CI = 130 ms was reached. We examined
APD alternans during a train of stimuli at CI = 105 ms and the first four beats at this rate are presented in Figure 3. In the absence of discontinuities (Figure 3A), the spatial distribution of APD alternates with successive stimuli in a non-steady fashion. Figure 3B shows the effects of fixed heterogeneities for corresponding stimuli. Inactive tissue (a combination of tissue voids and regions of conduction block) is indicated by white space. APD distributions are asymmetrical proximal to regions of greatest heterogeneity (see inset ellipses) and they change substantially with successive beats. Also noteworthy is the beat-to-beat variability in the extent of local conduction block in the region immediately above the inset ellipses.

APD gradients are steepest, and conduction block most likely to occur, adjacent to the nodal lines that define the boundary between regions of spatially discordant APD alternans. Figure 3C shows nodal lines at a CI of 105 ms in the presence (black) and absence (gray) of discontinuities for the four beats above. In control, nodal lines originate at the lower boundary and move progressively upward toward the stimulus. With fixed heterogeneities the beat-to-beat variation of nodal lines is much more constrained with lines typically locked onto the structure.

Figure 4 summarizes the effects of structural barriers on APD restitution and compares this with the control case. As expected, there is no difference between the two in the region above the discontinuities (Figure 4A). Below the discontinuous region (Figure 4C), there is again little difference in the restitution relationship compared with control, although the spread of minimum DIs and slopes is wider (see insets). Within the discontinuous region (Figure 4B), there is greater variability in fitted restitution curves compared with control and this is particularly evident at low DI (see inset). There is much wider variation in minimum DI and the associated maximum restitution slopes are scattered substantially above the trend for control.

The predicted effects of patchy discontinuities on CV are shown in Figure 5, where the tortuosity of conduction pathways in the discontinuous region (Figure 5A) is related to CV distribution for a single cycle at CI=105ms (Figure 5B). As expected, tortuosity is
greatest where discontinuities are dense with dimensions distributed across a range of spatial scales. While the presence of complex conduction pathways impedes the spread of activation (see Figure 2B), it does not affect median CV. However, increased tortuosity amplifies CV variability and discordance. This increases the risk of conduction block. Figure 5 demonstrates that block occurs in several regions where tortuosity is greatest.

Figure 6, shows electrical behavior during 3 successive stimuli at a CI of 105 ms in a subregion within the discontinuous area. Conduction block is observed through much of the region with marked slowing within and below it. While beat-to-beat differences in AT distributions are relatively subtle, there are marked disparities in APD distributions for all three stimuli within and below the region. Figure 6B presents action potentials for specific areas within the region of structural discontinuities. Beat-to-beat variability in DI is least in regions of low tortuosity (o, +) and increases in regions of greater tortuosity (*, x). Overlaid APD restitution relations at these points (Figure 6C) provide some insight into the variability in Figure 6B. Restitution slope at minimum DI is highest in regions of greatest tortuosity.

The extent of discordance at the markers indicated in Figure 6 for a sequence of 20 beats at a CI of 105 ms after the initial dynamic pacing sequence is summarized in Table 1 for cell models with steep and flat APD restitution relations. APD alternans is more prevalent in the presence of structural heterogeneity and occurs even with flat APD restitution, whereas this is not observed in control. The magnitude of alternans is greater in areas of high (*, x) rather than low (o, +) tortuosity. APD and CV restitution for a region of high tortuosity (x) are shown in Figure 7. These data were obtained using a dynamic stimulus protocol with cellular kinetics adjusted to give rise to steep (Figure 5A) and flat (Figure 5B) APD restitution in the control case. CV is normalized because it was ~20% greater than control in this region at low stimulus rates. In the presence of fixed heterogeneities, APD was reduced at the lowest DI's, which was slightly greater than control. The greatest difference in APD relative to control occurred with "flat" cellular kinetics, where APD restitution slope was >1 at minimum DI. The affects of fixed heterogeneities on CV restitution relations was less marked than for APD. However, the DI's at which APDs
deviate most from control are those at which CV is also substantially reduced.

Reentry occurred on the 21st successive stimuli at a CI of 105 ms in the presence of fixed heterogeneities, whereas there was complete wavefront block in control around the center of the domain. In Figure 8A, we present AT and APD distributions in the entire discontinuous region and below for the three stimuli immediately prior to reentry. Beat-to-beat variation in AT varies substantially, with slowing and conduction block evident in all three cycles. Conduction block is near complete across the region for the final stimulus, but activation propagates slowly through the centre of the region where tortuosity is least. The relatively rapid activation in (2) gives rise to prolonged APDs below the structure that is responsible for the partial block in (3), but APD alternans is least extreme in the central region where propagation is maintained. These trends can be seen clearly in the action potentials at sites within the discontinuous region for the three beats prior to reentry and subsequently (Figure 8B). Extreme alternans gives rise to 2:1 block in regions of high tortuosity (\(\ast, \times\)), but not in more uniform areas (\(o, +\)). Figure 8C illustrates the progress of the reentrant wavefront. Initially, it proceeds rapidly from the stimulus site (1), slows as it follows a convoluted path through the discontinuous region (2), and blocks in areas of high tortuosity. Propagation also blocks along the edges of the heterogeneous region as the activation wavefront approaches the preceding waveback. However, it progresses slowly through the centre of the discontinuous region where tortuosity is least (3), tracks up the sides of the structure (4), slows almost to the point of block (5), but eventually reenters the domain above the heterogeneous region (6).

**Discussion**

This study elucidates the mechanisms by which structural discontinuities (fixed heterogeneities) generate conduction block and reentry at rapid stimulus rates. Tissue regions that contain patchy, nonuniformly distributed barriers to excitation provide a substrate for tortuous conduction. We have demonstrated that this results in large spatial and temporal fluctuations in AT with rapid stimulation, increasing the probability of discordant alternans. This instability modulates restitution relations and can give rise to
extensive wavefront block interspersed with regions of slow conduction that initiate re-entrant excitation.

Substrates for reentry and arrhythmia

The idea that tissue heterogeneity may cause conduction disturbances, reentry and wavebreak is long established. Experimental and modeling studies provide insight into factors that may initiate reentry in this context. These include branching of cell tracts that gives rise to source-sink mismatches, tortuous, slow-conducting pathways, rapid changes in myofiber orientation and heterogeneous cellular coupling. Each of these can result in discontinuous propagation which, under the right conditions, may give rise to conduction block and reentry. Related computer modeling studies also suggest that the risk of wavebreak is exacerbated by structural discontinuities that spread across a range of spatial scales and that interstitial fibrosis is more likely to give rise to reentry and fibrillation if it is patchy and nonuniformly distributed.

Dynamic factors linked with the ion channel kinetics or altered intracellular Ca\(^{2+}\) homeostasis also provide a substrate for reentrant arrhythmia. The phenomenon of APD alternans provides an example of this. Beat-to-beat alternation of APD is observed at high stimulus rates in animals and humans. Initially, alternans is spatially concordant, but discordant alternans occurs as stimulus rate is increased further. Clinically, this electrical instability is associated with T wave and QRS alternans and both are linked with increased probability of sudden death. While alternans rhythm can be induced in structurally normal tissue, there are marked differences in APD restitution and alternans dynamics in patients with structural heart disease. Nash, Taggart and coworkers have also demonstrated spatially heterogeneous restitution in patients undergoing surgery for coronary artery disease and aortic valve disease. In both cases, differences in restitution dynamics were attributed to spatial variation in cellular electrical properties. Finally, computer modeling studies show that spatially non-uniform APD restitution can initiate re-entry and fibrillation.

The extent to which fixed heterogeneities or unstable cell dynamics provide the dominant
contribution to wave break and VF remains unclear. It is difficult to resolve this issue through in vivo investigation alone, because structural and electrical remodeling occur together in many forms of cardiac disease. However, there is accumulating evidence from computer modeling studies and patterned cell culture experiments that APD and CV restitution relations are modified by structural heterogeneity. It has been shown that structural barriers increase alternans magnitude and APD dispersion, alter CV restitution, and can provide a substrate for reentry at high stimulation rates when arranged asymmetrically. A possible limitation of these studies is that the structural barriers were macroscopic and relatively ordered in their arrangement.

In this work, we have sought to address these issues using a computer model that incorporates anatomic heterogeneities typical of those observed in patchy fibrosis, but in which cellular electrical properties are uniform.

Rate dependent effects of structural discontinuities on activation

We are able to dissect the rate-dependent effects of structural heterogeneity on electrical activation by comparing the results of numerical experiments in a uniform, continuous domain with those obtained in the presence of regional discontinuities. The control data are entirely consistent with the findings of comparable modeling studies. We observe discordant APD alternans in control simulations that is a result of spatial variation in CV. Relative to control, discontinuities have little qualitative impact on electrical activation at low stimulus rates (Figure 2). At high rates, however, there are significant perturbations to the spread of activation as well as in the spatial distributions of repolarization and APD. Most striking is the extent of dynamic instability that occurs in the presence of tortuous conduction pathways (see Table 1). Alternans occurs at lower stimulus rates and the variation in both DI and APD is substantially greater. Discordant alternans is seen with flat as well as steep APD restitution relations and the nodal lines that indicate the loci of zero phase shift are locked to the structure (Figure 3). At high stimulus rates, wavefront block occurs in control and in the presence of structural discontinuities. The critical difference is that local variability in DI and APD can give rise to nonuniform block within and below the heterogeneous region. This allows activation to propagate.
slowly through it, setting up conditions for reentry (Figure 8).

While APD and CV alternans in these numeric experiments are the result of membrane ion channel kinetics, the nature and extent of local dynamic instability observed does not reflect regional variation in cellular electric properties, but is due to structural heterogeneity alone. The extrinsic mechanisms through which structural discontinuity and nonuniform electrical coupling can influence activation and repolarization times are straightforward. Activation delays may occur because of tortuous “zig-zag” conduction or as a result of increased current load due to branching and/or dilatation of conduction pathways. In this study, median CV was not affected by tortuosity (Figure 5) indicating that activation delays were principally due to greater conduction pathlength. However, increased tortuosity amplified local CV variability potentiating the risk of regional conduction block. The delays will be magnified and the probability of local conduction block increased at high stimulus rates. It is also likely that electrotonic coupling, which acts to smooth repolarization gradients in normal myocardium, is impeded by structural discontinuity. Structure-related factors therefore amplify dynamic instability causing more extreme spatial and temporal variation in AT, CV, DI and APD. High gradients of repolarization form because of this instability and structure-related conduction changes near the interface between the region of discontinuities and normal tissue (Figure 3).

**Implications**

The most important feature of this work is that it shows how fixed heterogeneities associated with SHD could provide a substrate for rate-dependent block and reentrant electrical activation. It suggests that patchy, nonuniformly distributed regions of inexcitable tissue modulate and magnify local dynamic instability at high stimulus rates, giving rise to partial conduction block. The results presented here are similar to those reported recently for patients with SHD using comparable stimulus protocols. Koller and co-workers presumed that the altered APD restitution properties they reported were due to changes in cellular electrophysiology. In contrast, we show that this result could equally well be the result of fixed heterogeneities. In our studies, APD alternans occurred
at high stimulus rates in the presence of structural discontinuities (but not in control) when electrophysiological cell properties were uniform and intrinsic APD restitution was relatively flat (Figure 8). In the presence of fixed heterogeneities, APD restitution slopes were >1 at the lowest DIs for “flat” as well as “steep” cell parameters. This reflects the fact that the relationship between APD and DI is affected not only by cellular kinetics, but also by electrical coupling between cells, spatial nonuniformity of AT and the history of wavefront propagation. Thus, variations in local estimates of APD or CV restitution do not necessarily reflect changes in underlying cellular electrophysiology and these measures therefore need to be interpreted with circumspection.

These results challenge the view that fixed and dynamic heterogeneities are necessarily separate substrates for reentry and fibrillation. They also complement clinical and experimental observations from human hearts with SHD. Saumarez et al. characterized the relationship between ventricular AT and CI for a stimulus protocol in which CI was progressively decremented, and found consistent delays for patients with noncoronary heart disease. They argued that this activation delay was likely due to multiple tortuous conduction pathways through the myocardium and that it therefore provides a means of stratifying risk of ventricular fibrillation. Kawara and coworkers showed that epicardial activation delays for explanted hearts in end-stage heart failure were most marked in regions of dense patchy fibrosis with long fibrotic strands. Our work provides a mechanistic framework which links these observations. We have demonstrated that structural heterogeneity increases the probability of dynamic instability and confirmed that the risk of regional block and re-entry is greatest when the barriers to conduction are patchy and nonuniform. Therefore, clinical measures that quantify the extent of tortuous ventricular conduction and/or structural heterogeneity may aid stratification of risk of sudden cardiac death.

With SHD and for heart failure, in particular, both structural and electrical remodeling are present and likely combine to magnify risk. In the border zone of healed infarcts, interstitial fibrosis appears to disrupt transverse gap junctions reducing side-to-side electrical coupling between adjacent cells, but not longitudinal coupling. Our computer
model replicates this reduction in lateral electrical coupling and the resultant regional anisotropy. Kostin and coworkers reported that ventricular connexin 43 expression is markedly reduced during the progression to decompensated heart failure in patients with pressure-overload and argued that this would lead to slowed conduction. The development of interstitial fibrosis is associated with proliferation of myofibroblasts and in vitro studies suggest that this could slow cardiac conduction, due to electrotonic loading. The implication of our study is that slowed conduction, whatever its origin, would potentiate the spatial variation of activation within fixed heterogeneities increasing the probability of electrical instability.

Finally, this work demonstrates the potential of structure-based computer modeling for dissecting factors that may be responsible for the initiation and maintenance of arrhythmia and fibrillation. The approach used in this and other similar computer modeling studies may be viewed as complementary to the patterned cell culture experiments that have also been used to study the effects of structural heterogeneity on electrical propagation in monolayers of cardiac cells. Both approaches are 2D model systems, however, in silico models have the advantage that the influence of more realistic structural heterogeneity can be studied systematically at high spatial resolution. Also, cell culture studies are currently limited to using rat neonatal or chick embryo cardiomyocytes, whose electrical behaviour may be dissimilar to mature cardiac cells.

Limitations
For reasons of computational tractability, a 2D computer model has been used to study the effects of nonuniform structural discontinuities on cardiac electrical dynamics. The model captures key features of the regional heterogeneity seen in SHD, but cannot reproduce the more complex electrical properties of the 3D heart. In particular, 2D models overstate the extent of electrical decoupling introduced by structural discontinuities, because coupling in the the third dimension is not accounted for. Three dimensional models also better incorporate the inherent anisotropy of ventricular myocardium. Kawara and coworkers have demonstrated that the progression of activation through a region of patchy fibrosis depends on the direction of propagation and
this will certainly be affected by the 3D microstructure of the surrounding myocardium. The LR1 activation model used in this study does not provide a detailed representation of intracellular Ca\(^{2+}\) dynamics. Beat-to-beat alternation of intracellular Ca\(^{2+}\) transients are thought to contribute to dynamic instability by magnifying and, in some cases, driving electrical alternans. Finally, in order to address the central hypothesis of this study, we have assumed that cellular electrical properties are uniform. This is not the case in SHD where there may be substantial regional electrical remodeling, but we argue that this would magnify the dynamic instability reported here. While the issues outlined above will certainly impact quantitatively on the results presented here, they will not alter the key finding that fixed heterogeneities within a uniformly connected tissue region can modify CV and APD restitution and increase the probability of electrical instability.

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**Conflict of Interest Disclosures:** None.

**References**


Table 1: Mean difference (+ standard deviation) in beat-to-beat APD (ms) at specific locations during a train of 20 stimuli at CI = 105 ms in the absence and presence of structural discontinuities. The kinetics of the activation model has been adjusted to produce flat and steep cellular APD restitution. For cells marked #, data are given for the first 5 cycles only, because 2:1 block occurred subsequently. The points o, +, * , x, are located within the region of discontinuity (see Figure 6A). Tortuosity is given in parenthesis for each location.

<table>
<thead>
<tr>
<th>Location (Tortuosity)</th>
<th>Control Flat</th>
<th>Control Steep</th>
<th>Structural discontinuities Flat</th>
<th>Structural discontinuities Steep</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ (0.62)</td>
<td>0.36 ± 0.50</td>
<td>15.4 ± 8.1</td>
<td>1.8 ± 0.83</td>
<td>16.8 ± 9.3</td>
</tr>
<tr>
<td>o (0.43)</td>
<td>0.41 ± 0.52</td>
<td>15.9 ± 7.9</td>
<td>8.8 ± 4.2</td>
<td>12.6 ± 6.0</td>
</tr>
<tr>
<td>* (0.89)</td>
<td>0.42 ± 0.57</td>
<td>16.7 ± 9.0</td>
<td>27.8 ± 8.8*</td>
<td>27.0 ± 10.2</td>
</tr>
<tr>
<td>x (0.85)</td>
<td>0.45 ± 0.60</td>
<td>16.8 ± 10.2</td>
<td>55.0 ± 3.8*</td>
<td>44.8 ± 15.2</td>
</tr>
</tbody>
</table>

Figure Legends:

Figure 1. Cardiac activation and propagation through an electrically uniform and isotropic 14x14 mm² domain with a 10 x 4 mm² region containing patchy inexcitable voids. Transmembrane potentials are shown 1 and 30 ms after the first bipolar stimulus: anode A, cathode C. Action potentials from a typical pacing protocol are shown for points above (1), within (2) and below (3) the heterogeneous region.

Figure 2. AT, RT, and APD in ms in the absence and presence of structural discontinuities (shown as voids). A CI = 500 ms. B CI = 110 ms.

Figure 3. Spatial distributions of APD for 4 sequential cycles of pacing at a CI of 105 ms. Both the fully continuous control (A) and discontinuous cases (B) are shown. Structural discontinuities give rise to marked APD variability within the highlighted region. The voids indicate regions where there is no
conduction. C shows the distribution of nodal lines for the given beats in the control (grey) and with structure (black). The location of the discontinuities is indicated.

**Figure 4.** APD restitution relationships in the presence (black) and absence (grey) of structural discontinuities for (A) upper, (B) mid, and (C) lower thirds of the domain. An exponential function was fitted at each point across the domain to APD and DI data obtained with a pacing protocol in which CI was reduced progressively from 500 to 105 ms and then followed by 20 beats at CI = 105 ms. Inset panels show the slope of the fitted restitution curves at the shortest DI.

**Figure 5.** A Tortuosity across heterogeneous region. B Boxplot of CV distribution with tortuosity for the first beat at CI = 105 ms. Number of points considered for each range of tortuosity, from smallest to largest are: 63956, 4079, 4600, 3644, and 482 While the median CV is relatively constant, the spread of CVs is proportional to increasing tortuosity.

**Figure 6.** Activation characteristics for lower right region of discontinuities. A Spatial distributions of AT (above) and APD (below) in ms for first 3 cycles at CI = 105 ms. B Action potentials at the points indicated in A with APD given in ms. C Restitution curves constructed at +, o, *, x, are rendered in black and run from top to bottom, respectively. The corresponding control curves (green) were constructed for the same points in the absence of structural discontinuities. The inset zooms in on these fitted restitution curves over a narrow range comprising the minimum DIs.

**Figure 7.** Dynamic APD (A) and normalized CV (B) restitution relationships in the presence (+) and absence (o) of structural discontinuities. Cell kinetics adjusted to give rise to steep (left) and flat (right) APD restitution dynamics.
Figure 8. Activation characteristics in region of discontinuity before and during reentry. 
A Spatial distributions of AT (above) and APD (below) in ms for 3 cycles immediately before reentry at CI = 105 ms. B Action potentials at the points indicated in A (same positions as in Figure 6) with APD given in ms. The first 3 cycles correspond to those in A above. Arrows show continuous conduction and the flat line indicates the conduction block that occurs during the 3rd cycle in which reentry is initiated. C Spread of activation during reentry. Contours 1 to 6 represent activation times of 20-25, 50-55, 70-75, 100-105, 120-125, and 140-145 ms, respectively.
A

B

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CV (mm/ms)

<0.2  0.2-0.4  0.4-0.6  0.6-0.8  >0.8

Tortuosity
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SUPPLEMENTAL MATERIAL

“Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution”

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Supplemental Introduction

This supplemental document details methods used to construct, solve and analyze the two-dimensional computer models presented in the parent paper: “Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution.”

Supplemental Methods

\textit{Heterogeneous Discontinuous Myocardial Tissue Model}

A two-dimensional description of heterogeneous myocardial discontinuities was derived from a long axis section of rat LV. The tissue was stained with picrosirius red, which binds nonsterically to collagen, and imaged using confocal microscopy at 1 µm voxel dimension\cite{1}. The original tissue sample was 13.5x5.5 mm\textsuperscript{2} (Fig. 1).

\textbf{Figure 1} Long axis section of rat LV stained with picrosirius red.

Processing of this tissue section naturally expanded septae between adjacent muscle layers. These discontinuities provide a cascade of scale features that are similar to those found in patchy fibrosis and structural heart disease (SHD) \cite{2}. In order to dissect out these discontinuities, the image was subsampled to 10 µm and thresholded on intensity, to give a mask for myocardial tissue and non-myocardium (Fig. 2).
A 10x4 mm$^2$ subregion (green box in Fig. 2) was cropped and resampled to a resolution of 50 µm using a median filter to preserve geometry (Fig. 3A). This coarsening step had the additional effect of digitally enhancing the discontinuities. Vessels and exceptionally large discontinuities were filled. Diffuse features were conglomerated for pathways <100 µm, since electrical diffusion in the computational model was not possible at this scale, producing an effective structure mask with much larger and contiguous discontinuities (Fig. 3B).

**Local Tissue Conduction Pathway Tortuosity Measure**

The local tissue conduction pathway tortuosity is a measure of the connected myocardial environment surrounding a point. The measure is constructed over a 1.0x1.0 mm$^2$ window centered on each pixel in the solution domain. It is defined from the ratio of the normalized center-line path length from an excitable tissue skeletonization to the normalized total area of excitable tissue in that window. Example values for five sample locations are shown in Fig. 4. Normalizations are made with respect to the continuous tissue (♦) which has normalized area and distance values of 1. The ratio is shifted and normalized to have a maximum of 1 (most tortuous region) and a minimum of zero (least tortuous region, i.e. continuous tissue). In the examples shown in Fig. 4, point ● has a higher pathway tortuosity measure than ×, and * has a higher pathway tortuosity measure than +. Fig. 5 shows the spatial distribution of the normalized local conduction pathway tortuosity measure. The normalized measure is greatest where the pathways are more tortuous and occupy a lesser volume of the sample region. A region of lower tortuosity exists through the middle of the block and is highlighted.
**Figure 4.** A. Effective structure in center of 14x14 mm² solution domain. Structural tortuosity was considered for each point using a surround window measuring 1x1 mm². B. Normalized area of excitable tissue for points indicated in A. C. Normalized pathway lengths for the points indicated in A.

**Figure 5.** Spatial distribution of the local conduction pathway tortuosity measure. Reproduced from Fig. 1 of “Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution.” The region of lower pathway tortuosity through the middle of the structural discontinuities is highlighted with an arrow.

**Computer Modeling of Electrical Activation**

Electrical activity was represented by the biodomain model, with intracellular (myocardial) and extracellular domains communicating through capacitance and the membrane ionic current, $I_{\text{ion}}$. The dependent variables are the transmembrane potential, $V_m$, and the extracellular potential, $\phi_e$.

$$
\frac{\partial V_m}{\partial t} + \nabla \cdot (\sigma_i \nabla V_m) = \nabla \cdot (\sigma_e \nabla \phi_e) - A_m \frac{\partial}{\partial t} I_{\text{ion}}
$$

$$
\nabla \cdot ((\sigma_e + \sigma_i) \nabla \phi_e) = -\nabla \cdot (\sigma_e \nabla V_m) - i_e
$$

Here $A_m$ is the surface to volume ratio of the representative cell membrane between the domains, $C_m$ is the specific capacitance of the membrane, $\sigma_i$ and $\sigma_e$ are the intra- and extra-cellular conductivity tensors and $i_e$ is a current injection per unit volume into the extracellular space. In this work, the ionic current is determined using an LR1 model.

Assuming isolated tissue, these equations are subject to the no-flux current boundary conditions:
\[ \nabla (V_m + \phi_e) \cdot (\sigma \mathbf{n}) = 0 \quad \text{on } \Gamma_o \text{ and } \Gamma_c \]
\[ \nabla (\phi_e) \cdot (\sigma_e \mathbf{n}) = 0 \quad \text{on } \Gamma_o \]

\( \Gamma_o \) are the exterior boundaries and \( \Gamma_c \) are the internal boundaries in the intracellular domain, i.e. discontinuities. The transmembrane potential, \( V_m \), and extracellular potential, \( \phi_e \), were sequentially solved on bilinear finite element meshes using an operator splitting method for advancing through time[3].

Bilinear finite element meshes with element dimensions of 0.05x0.05 mm\(^2\) were constructed of the 14x14 mm\(^2\) solution domain shown in Fig. 4A. The mesh of the intracellular domain accounts for the myocardial discontinuities shown in Fig. 3 by preventing intracellular current flux across the discontinuities, whereas the extracellular mesh is continuous and covers the entire domain. This is shown in Fig. 6. A finite element discretization of the governing bidomain equations naturally enforces no current flux boundary conditions along the discontinuities if the intracellular domain elements highlighted in Fig. 3A are not explicitly constructed.

Transmembrane and extracellular potentials were solved at 78,961 nodes for 0.02ms time steps. Bipolar extracellular stimulation was applied via an anode and cathode located 2 and 4 mm respectively from the upper boundary of the domain (Fig. 6C). Each electrode had dimensions 400x400 µm\(^2\) and the stimulus strength was 2x diastolic threshold (~200 mA/m\(^2\)). The models were solved at various temporal and spatial resolutions to ensure solution convergence had been reached. Dynamic restitution was characterized with a pacing program of 17 beats, in which coupling interval (CI) was progressively reduced from 500 to 105 ms in increments from 100 to 5 ms. Electrical steady state was examined by repeated stimulation at a CI.
Cell membrane electrophysiology was represented using the LR1 model of ionic currents. The CellML description from the online repository (www.cellml.org) was modified by reducing the conductivity of the slow inward current, $G_{Si}$, to 0.06 mS/cm$^2$. Further modifications following Qu et al.\cite{4} provided models with both steep and flat action potential duration (APD) restitution relationships for isolated cell models. Steep APD restitution was obtained by increasing the time constant $\tau_i$, of the slow inactivation variable of $I_{Na}$ five-fold. Conversely, flat restitution was achieved by decreasing the calcium current time constants, $\tau_d$ and $\tau_f$, by factors of 0.3.

Activation was denoted as the time that the transmembrane potential crossed from below to above the 50% threshold of -35 mV and a repolarization time was marked as the potential crossed from above to below a threshold of -75 mV. Transmembrane potential distributions for 3 beats at different CIs are shown in Fig. 7. A video of the entire beat depicted in Fig. 7A is available as a supplemental video file BCL_500.avi. The beats in Fig. 7B&C are available in the supplemental video file BCL_120_110.avi.

**Figure 7.** Transmembrane potential distributions at 25ms intervals subsequent to stimulation at CIs of A. 500ms, B. 120 ms and C. 110ms. These beats are part of the preconditioning train of 17 beats in which CI was progressively reduced from 500 to 105ms in steps ranging from 100 to 5ms. B and C are consecutive beats.
**Conduction Velocity Calculations**

The discontinuous domain was accounted for in the calculation of conduction velocity (CV). Subdomains of size $5 \times 5$ nodes are centered on each node of the $281 \times 281$ node domain. Specialized local derivative templates were constructed for each of these subdomains based on the discontinuity mask. Fig. 8 shows local details of activation times and discontinuities which dictate the derivative mask that will be constructed.

![Figure 8](image_url)

**Figure 8.** Sample activation time field and choice of connected points for derivative calculations. A. Complete discontinuity mask. B. Activation times in $31 \times 31$ node region with mask overlaid. C. Nodal activation times in a $5 \times 5$ mask used to compute a local derivative.

The point of interest for a template is at the center of the $(2n+1) \times (2n+1)$ subregion (in Fig. 8C, $n=2$, i.e. $2n+1=5$). To determine which points should be included in the derivative template construction, discontinuous, $D$, and continuous, $C$, Manhattan distance maps of the subregion are constructed in layers, $k$, moving out from the center of the template, $(i,j)=(0,0)$ (see Fig. 9A). The algorithm is:

\[
\text{do } k = 1 \rightarrow n \\
\quad \text{do } j = -k \rightarrow k \\
\quad \quad \text{construct } d_j \\
\quad \quad s = 2k - (2k - 1)|\text{trunc}(j/k)| \\
\quad \quad \text{do } i = -k \rightarrow k, \text{ step } s \\
\quad \quad \quad \text{construct } d_i \\
\quad \quad \quad \text{construct } D_{i,j} \text{ and } C_{i,j} \\
\quad \text{end} \\
\text{end}
\]

The $k=0$ layer does not need to be constructed as the distance map is trivially 0 at the point of interest. The distance templates are functions of the unit step directions, $d_i$ and $d_j$, which take values -1,0 or 1. They are given by:

\[
d_i = \begin{cases} 
0 & \text{if } i = 0 \\
-\frac{i}{|i|} & \text{otherwise}
\end{cases} \quad \quad \text{and} \quad \quad d_j = \begin{cases} 
0 & \text{if } j = 0 \\
-\frac{j}{|j|} & \text{otherwise}
\end{cases}
\]

Given a mask, $M (M_{i,j} = 1 \text{ for myocardium and } 0 \text{ for voids})$, the discontinuous Manhattan distance map, $D$, is given by:
The map, $D$, is compared with the equivalent size continuous Manhattan distance map, $C$:

$$C_{i,j} = \min(D_{i+di,j+dj} + |di| + |dj|)$$

and only the points where $C_{i,j} = D_{i,j}$ are used for constructing the activation time derivative template weights. An example of the discontinuous and continuous Manhattan distance maps for the discontinuity mask of Figure 8C, are shown in Figures 9B and 9C.

**Figure 9.** Distance maps used to determine which nodes are to be included in a specialized derivative template. A. Distance map construction layers. Layer 0 is not explicitly constructed because its distance map is trivially zero. B. Distance map for a continuous subdomain. C. Distance map for a subdomain with discontinuity. This subdomain is equivalent to that shown in Fig. 8C.

When the points supporting the node of interest are identified on the 5x5 subregion, a generalized finite difference approximation template is constructed using methods described in detail elsewhere [5]. These templates are used to compute the local spatial gradient of activation time, $\nabla AT$. The magnitude of this gradient, $|\nabla AT|$, is inverted to give the local scalar conduction velocity. Figure 10 shows an example of the computed derivative template weights and conduction velocity. Subregions of size 3x3 and 7x7 nodes were also investigated. The 5x5 subregion and derivative approximation template provided the best balance of a smooth derivative measure while retaining the essential impact of the discontinuous domain.

**Figure 10.** Derivative weights and conduction velocity vector for example from Fig. 8C. A. $x$-derivative weights. B. $y$-derivative weights. The weights are calculated using the model mesh spacing of 0.05 mm. The conduction velocity is 0.19 m/s for this example.

Conduction velocities calculated using a 5x5 subregion centered on each of the 78,961 nodes in the domain are shown in Fig. 11 for slow (1) and fast (2 and 3) pacing rates. As expected, CVs are greatest at slow pacing rates. Spatial discordance and alternans of CVs manifests at fast rates both in the presence and absence of structural discontinuities. This mirrors the spatial and temporal behavior of APDs (Figure 3 in the article “Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic
Instability and Altered Restitution.”). CVs in the region of discontinuities are spatially heterogeneous at all pacing rates.

The CVs shown in Fig. 11 are fast in the vicinity of the current source (cathode) and, conversely, slow in the vicinity of the current sink (anode). The boundary of the solution space also constrains current flow resulting in greater load and thus a faster CV. The transition between regions of fast and slow propagation in Fig. 11 appears speckled. This is the result of the gradient CV measure amplifying two aspects of the numerical solution. Firstly, solutions to the reaction-diffusion equations were computed at finite temporal and spatial resolutions of 0.2 ms and 0.05mm, respectively. Secondly, activation times were recorded to 5 digits of precision in order to conserve memory and reduce output bottlenecks. While these finite approximation errors are negligible in maps of AT and APD (Figures 2,3,6, 7 in the article “Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution”), they manifest in the calculation of CV because of the enhanced sensitivity of that gradient measure to small fluctuations of AT.

Figure 11. Conduction velocity magnitudes corresponding to pacing at a CI of (1) 500ms and repeated pacing (2 and 3) at a CI of 105ms.

APD Restitution Relationship

APD restitution relations were characterized by fitting APD and diastolic interval (DI) data to a three parameter mono-exponential function [6]:

$$APD = APD_{ss} - \alpha_1 e^{-\alpha_2 \cdot DI}$$

using the nonlinear least squares function \textit{lsqnonlin} available through Matlab Release 12 2007b©. In this equation the unknown parameter \(APD_{ss}\) approximated steady state APD at a very slow pacing rate (CI of 500ms). Fitted curves were excluded from analysis when the normalized best-fit residual error was > 0.1. Such poor fits occurred as a result of the development of stable or unstable conduction block within a region. Maximum slopes were determined at the minimum DI. Restitution slopes were derived from the analytical derivative of the mono-exponential function. Because of the nature of the exponential fit, maximum slopes always occurred at minimum DI.

Figure 12 shows the distributions of maximum APD restitution slopes at the associated minimum DI for control and discontinuous paradigms using an activation model with a steep APD restitution
relationship. This map illustrates the distribution used to derive the slope-DI inserts in Figure 5 of the article “Structural Heterogeneity Alone is a Sufficient Substrate for Dynamic Instability and Altered Restitution.”

**Figure 12.** Maximum slope and associated DI in the absence (A) and presence (B) of discontinuities.

**Supplemental Video Legends**

*BCL_500.avi:* Transmembrane potential distributions for a beat with a CI of 500 ms.

*BCL_120_110.avi:* Transmembrane potential distributions for two beats are shown. The first beat has a CI of 120 ms and is succeeded by a stimulus with a CI of 110 ms. This sequence is part of a stimulus train with progressively reduced CIs from 500 ms. Simulated time of the pacing series is given.

**Supplemental References**


