Cardiac Response to Low-Energy Field Pacing Challenges the Standard Theory of Defibrillation

Bryan J. Caldwell, PhD; Mark L. Trew, PhD; Arkady M. Pertsov, PhD

Background—The electric response of myocardial tissue to periodic field stimuli has attracted significant attention as the basis for low-energy antifibrillation pacing, potentially more effective than traditional single high-energy shocks. In conventional models, an electric field produces a highly nonuniform response of the myocardial wall, with discrete excitations, or hot spots (HS), occurring at cathodal tissue surfaces or large coronary vessels. We test this prediction using novel 3-dimensional tomographic optical imaging.

Methods and Results—Experiments were performed in isolated coronary perfused pig ventricular wall preparations stained with near-infrared voltage-sensitive fluorescent dye DI-4-ANBDQBS. The 3-dimensional coordinates of HS were determined using alternating transillumination. To relate HS formation with myocardial structures, we used ultradeep confocal imaging (interrogation depths, >4 mm). The peak HS distribution is located deep inside the heart wall, and the depth is not significantly affected by field polarity. We did not observe the strong colocalization of HS with major coronary vessels anticipated from theory. Yet, we observed considerable lateral displacement of HS with field polarity reversal. Models that de-emphasized lateral intracellular coupling and accounted for resistive heterogeneity in the extracellular space showed similar HS distributions to the experimental observations.

Conclusions—The HS distributions within the myocardial wall and the significant lateral displacements with field polarity reversal are inconsistent with standard theories of defibrillation. Extended theories based on enhanced descriptions of cellular scale electric mechanisms may be necessary. The considerable lateral displacement of HS with field polarity reversal supports the hypothesis of biphasic stimuli in low-energy antifibrillation pacing being advantageous.

Key Words: computer models ▪ electrical stimulation ▪ optical imaging

The response of heart tissue to low-energy electric field stimuli has attracted significant interest in the search for alternative, more efficient therapies against life-threatening cardiac arrhythmias.1,2 Although conventional high-energy shocks restore orderly cardiac contractions by resetting irregular electric activity of cardiac myocytes, low-energy shocks can also be used to achieve a similar result. Commonly referred to as low-energy antifibrillation pacing, these produce multiple simultaneously firing excitation centers, or hot spots (HS), which gradually take control over the entire heart until it beats normally.3,4

Understanding the nature of HS and their connection with the anatomic organization of myocardial tissue is critical for understanding the defibrillation mechanisms and efficacy of low-energy antifibrillation pacing. There is a consensus that HS generated by low-energy electric field stimuli represent regions with maximum membrane depolarization.3 According to theory,4 the majority of HS should emerge at the tissue surface nearest the negative field electrode (cathode) and migrate to the opposite surface with changing field polarity.3,5–9 Alternative localizations of HS predicted by models are the cathodal boundaries of major coronary vessels,1,3,10 with migration to the opposite side of the vessel with changing field polarity.

Here, we test these predictions in the intact pig ventricular wall using a novel tomographic optical imaging technique, enabling 3-dimensional (3D) localization of HS during field stimulations while preserving tissue integrity. Near-threshold field pacing of quiescent tissue is used to ensure a small number of solitary HS form that do not coalesce into regional activation, masking the onset and location of local activation. In this lowest energy field, HS will form only on the largest coronary vessels.

The Data Supplement is available at http://circcep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.114.002661/-/DC1. DOI: 10.1161/CIRCEP.114.002661

Circ Arrhythm Electrophysiol is available at http://circcep.ahajournals.org

© 2015 American Heart Association, Inc.
WHAT IS KNOWN

• High-energy electric field shocks effectively terminate arrhythmias but are painful and potentially damaging.
• Low-energy antifibrillation pacing is being developed as a method to terminate arrhythmias without pain and injury of high-energy shocks.
• Low-energy antifibrillation pacing generates discrete sites of electric activation which spread, coalesce, and gradually establish orderly electric rhythm.

WHAT THE STUDY ADDS

• An experimental characterization of the 3-dimensional distribution of field-induced activation sites (hot spots) through the ventricular wall was performed.
• The study advanced understanding of the mechanisms of hot spot formation, in particular the role of lateral intercellular coupling and extracellular resistive heterogeneities.
• The findings provide mechanistic justification for the use of biphasic stimuli in low-energy antifibrillation pacing.

Methods

All data are expressed as mean±SD.

Optical Fluorescence Imaging

All experimental protocols conformed to institutional and National Institutes of Health guidelines. Seven right ventricular (RV) and 3 left ventricular pig preparations were used in this study. The preparations were perfused through respective coronary arteries, mounted between mesh field electrodes, and stained with the near-infrared dye DI-4-ANBDQBS (40 μmol/L) as described previously.11 Figure 1A shows a schematic of the experimental setup. A pulsed electric field was applied transmurally from planar mesh electrodes, sufficiently sparse for passage of both illumination light and voltage-sensitive fluorescence signals. The field intensity was 3% to 5% above the excitation capture threshold (0.4±0.31 V/cm, 5 ms duration). The preparation was continually paced in the range 358 to 508 ms base cycle length, with both positive and negative field orientations. This produced 1 to 3 discrete HS in the optical viewing frame, commencing soon after pulse termination. Higher field strengths make HS localizations ambiguous because the HS form at a range of discontinuity scales and they coalesce into regional activations. Control point stimulation pacing was applied to the surfaces of 2 RV and 1 left ventricular preparation. The 3D optical imaging system has been described in full previously.12

To localize the HS in 3D, we used alternating transillumination with rapid switching of the illumination from the epicardium (P) to endocardium (N) and synchronous recording using 2 high-speed CCD cameras, labeled P and N, respectively. Camera recordings were demultiplexed into 4 quasi-simultaneous movies: PP, PN, NP, and NN. (The letters indicate the position of the light source and the camera, respectively.) The HS depths from the epicardium (Z) were determined by comparing integral intensities in PP, PN, NP, and NN images over the areas containing the respective HS.

Structural Imaging

After HS detection experiments, 4 RV preparations (40x40x10 mm) were subjected to ultradepth confocal imaging using an optical clearing technique.13 Confocal imaging (Zeiss LSM 510 system, ×10 objective) used the fluorescent properties of DI-4-ANBDQBS, which remained during the clearing process. The dye was excited at 633 nm and recorded using a 650 to 715 nm IR bandpass filter. Voxel resolution was isotropic (32 or 83 μm).

Data Analysis

The coordinates of the earliest activation HS were determined using a previously described method.14 To account for simultaneous multiple sites of activation, we adapted the original algorithm by subdividing the optical recording images into smaller regions containing only 1 source. Optical recordings were registered with 2-dimensional surface images and confocal images by matching structural landmarks, such as vessel branching and trabeculae. Vessels were segmented from confocal images in LabVIEW® (National Instruments Inc) and colocalized with the sites of early activation in V oxx (Indiana University). The mean radius R of the 3 largest coronary vessels was estimated by taking an axial line through 3 straight segments (=2 mm length) of each vessel and sectioning the vessel normal to this line. R was estimated from the area of an ellipse fitted to each cross section using ImageJ and averaged across vessel cross sections. The minimum distance (d) of the HS to the surface of the 3 largest coronary vessels was calculated using MATLAB (Mathworks).
Modeling

Computer modeling was used to interpret our experimental observations. We constructed a 15×10 mm² bidomain model of electric activity in a cross section of cardiac tissue surrounded on 2 sides by a 5 mm bath. The electric conductivities varied spatially in the tissue. Discrete structural features at multiple scales were included in the model and are shown in Figure 2. Key features distinct from previous models of field stimulation are discrete cell-scale lateral discontinuities and boundary layers on extracellular conductivity transitions at myocardial edges.

The experimental electric field stimulus was orthogonal to the epicardium (Figure 1A) and primarily oriented across the short axes of myocytes. Consequently, we assumed the experimental problem could be modeled as a 2-dimensional curvilinear surface of cell cross sections orthogonal to the transmurally rotating myocyte long axis. This also mitigated the computational complexity of incorporating anisotropic intracellular electric conductivity and variable cell topology.

The transmural dimension of our 2-dimensional model was divided into 3 structural regions based on a visual interpretation of the work of Pope et al: subepicardium (25%), midwall (50%), and subendocardium (25%). Laminar clefts in the subepicardial and subendocardial regions are orthogonal to the epicardium, whereas those in the midwall region are at an angle of 70° to the epicardium (Figure 2). The lengths of the laminar clefts were varied randomly. Lengths in the subepicardium were 0.16±0.016 mm, in the midwall 0.54±0.23 mm, and the subendocardium 0.11±0.022 mm. The ratio of laminar cleft length:end-to-end distance between the clefts is 1:2 in the subepicardium and subendocardium regions and 1:3 in the midwall region. Two vessels of diameter 1.2 mm, consistent with expected sizes, were placed in the subepicardium. The vessels have a wall thickness of 0.04 mm. The model was discretized into finite volumes of edge length 20 μm and solved using previously described techniques.

To construct cell-scale cross sections around the intramural features, transmural strips 60 μm apart and 1 finite volume wide were populated with a cell height varying randomly between 40 and 80 μm. The cell strips were then grown volume-by-volume left and right until they abutted with cells from adjacent strips (Figure 2, inset). Single finite volume holes between the cells and the laminar clefts were assigned characteristics of connective tissue. The randomly generated cell cross sections were 3215.7±1160.6 μm² (8±3 finite volumes); an approximation of actual cardiac ventricular cell shapes.

Membrane currents were modeled using a dynamic Lao and Rudy model with Rush–Larsen approximations. The membrane capacitance was set to 0.01 μF/mm² and the cell surface:volume ratio for a typical problem varied spatially with cell discretization as 223±12 mm⁻¹. Isotropic intra- and extracellular conductivities were based on previous studies and were 0.15 and 0.05 mS/mm, respectively. The bath and perfused vessel conductivities were assigned a typical value for Tyrodes solution of 2 mS/mm, connective tissue of 1 mS/mm (including the interlaminar clefts), and the vessel lumen of 0.01 mS/mm. Following Krassowska and Neu, a cell-dimension boundary layer or transition in extracellular conductivity was applied at myocardium to nonmyocardium (bath, vessels, and laminar clefts) boundaries. A smooth extracellular conductivity transition distance of 0.1 mm was used at the tissue–bath interface (Figure 2, inset), and a transition distance of 0.04 mm was used at the tissue–laminar cleft interface. Consequently, the extracellular conductivities through the myocardial region were highly heterogeneous.

Lateral cell-to-cell connectivity was modeled for the following cases: (1) fully connected cells (standard model), (2) fully disconnected cells, (3) cells disconnected in the direction of the electric field, and (4) cells disconnected in the direction orthogonal to the electric field. The fully disconnected cells are influenced by the extracellular potential but do not directly interact with each other.

The model was stimulated on the top and bottom edges using a 5 ms oriented field stimulus (capture at 0.72 V/cm). Six structural model variations with differing discontinuities were investigated. Three had different random distributions of cell-level cross-sectional areas and shapes (models 1–3), 2 had different proportional thickness of subendocardial region (model 4 is 20% and model 5 is 30%), and 1 had an alternative random distribution of midwall laminar cleft lengths (model 6).
Results

Our experiments show that HS do not cluster at surfaces, as predicted by models, but are distributed across the thickness of the heart wall. Figure 1B illustrates HS in a RV preparation 22 ms after field pulse termination with an endocardial cathode. The average wall thickness (L) of this preparation was 8.6±0.9 mm. Three HS (1–3) are apparent in NN and NP images with 1 and 2 bright in NN and faint in NP images. This is consistent with HS1 (Z=5.5 mm) and HS2 (Z=5.2 mm) being closer to the endocardial (N) than the epicardial (P) surface. HS3 (Z=4.3 mm) is clearly visible in all 4 images indicating a midwall location. Figure 1C and 1D shows the localization of HS with respect to coronary vessels and the endocardial surface for opposing field polarities in a different RV preparation. With field reversal, the HS depth and lateral locations changed, but not toward the surfaces.

Figure 3A shows the depth distribution of HS in left and RV wall preparations (n=10) for opposing field polarities. In all but 3 experiments (4/37 HS) excitation emerged >3.2 mm from the cathodal surface; 2 SDs greater than the detection algorithm error. To ensure that the total lack of cathodal surface activation was not an artifact of our depth detection algorithm, we applied a point stimulus directly onto the surfaces using a unipolar electrode (Figure 3A, control). In these experiments, our algorithm consistently detected the origin of excitation immediately under the stimulated surface (Z=1.2±0.6 mm; Z/L=11±3%), providing a positive control.

For opposing field polarities, the distribution of HS depths (Figure 3B) remains largely unchanged with the maximum around Z/L=50% to 70%. This is dramatically different from predictions by a conventional bidomain model where cells are fully coupled as a functional syncytium (Figure 3C) and HS always form adjacent to the cathodal surface.

We also found, contrary to recent predictions, that the HS did not colocalize with the largest coronary vessels. Models suggest that the relatively large spatial scale of coronary vessels should make them a main mediator of HS formation. However, this was not the case in our experiments. Figure 1C and 1D shows HS mapped onto 3D images of the vascular tree for both field polarities in 1 experiment. Reversed field polarity changes the location of the HS across the heart wall, but they are not colocalized with major vessels in either case.

To correlate the location of the HS with the coronary vessels in different experiments, we assessed the minimum distance of each HS from the 3 largest vessels (Figure 4). The radii of the largest coronary vessels ranged in size from 0.2 to 1.5 mm. Figure 4 shows the results of this analysis in 4 preparations with 3D vessel reconstructions. (A total of 21 HS for 2 field polarities were evaluated). The majority of HS were located far away from the largest vessels, with an average distance of 4.9±1.8 mm. Those few HS with a minimum distance similar or smaller than the lateral error (3 mm) of the periodic wave localization error were still detached from the vessel surface when the depth error bounds (1.3 mm) were taken into account as shown in the projections of minimum distance, d, in Figure 4. This is further corroborated by significant shifts of HS in the plane orthogonal to the field, after polarity reversal (Figure 5). On average, the minimal lateral shift was 12.7±10.7 mm. If vessels were a primary origin of the HS, we would have observed a shift in the field direction only with practically no lateral displacement. Hence, it is unlikely that the large vessels have a significant effect on promoting HS formation. Although the 3D colocalizations in Figure 4 show HS located near small vessels, the projections of minimum distance, d, suggest that this is not a causal link for the same reasons as the major vessels.

The data of Figures 3 to 5 show significant discrepancies between our experimental observations and the predictions of conventional bidomain models (Figure 3C) for the depth distribution of the HS and the role of major coronary vessels. These discrepancies can be reconciled by accounting for the discontinuity at the cellular length scales. Such discontinuities are absent in the conventional models of cardiac excitation and electric defibrillation where cells are highly coupled electrically and function as a unit rather than individually.

We developed a more detailed bidomain model, which included discontinuities at various spatial scales (Figure 2): (1) cell-scale discontinuities, (2) laminar clefts of both varying length and increased extracellular conductivity, which were oriented according to transmural location, and (3) cell-scale extracellular conductivity boundary layers across transitions between the myocardium and the nonmyocardium. These features, except for laminar clefts, have typically not been incorporated in conventional activation models.

Figure 6A shows simulation results for geometric model 1, where we assumed full lateral coupling of cells (a standard defibrillation model or control). In this case, HS formed exclusively under the cathodal surface and transitioned from one surface to another with the change of field polarity. The situation changed dramatically when we included cell-scale uncoupling in the transmural (field) direction (Figure 6B). Similar to the experimental studies, HS are now mostly distributed within the tissue. A change in field polarity produced a different distribution of HS, which is consistent with the experimental observations. Finally, like in the experiments, HS do not colocalize with large coronary vessels (large circles) and show significant lateral displacements. A similar result was obtained when we completely eliminated lateral coupling in the intracellular domain (Figure 6C). Note that the field activation threshold increased moving from the fully coupled model (0.35 V/cm) to lateral uncoupling in the field direction (0.47 V/cm) to the fully uncoupled model (0.72 V/cm). For completeness, we also considered the case of lateral uncoupling orthogonal to the electric field (Figure 6D). This case shared HS distribution characteristics of both fully coupled and fully uncoupled cells, with HS forming both in the midwall and on cathodal tissue and vessel surfaces (Figure 6D). The results shown in Figure 6 suggest that at a minimum, lateral uncoupling in the direction of the electric field is a required feature for model consistency with the experimental observations.

Figure 7 summarizes the HS distributions predicted from the 6 geometric perturbations from the base model, both with cell-scale lateral uncoupling in the field direction (Figure 7A–7C) and lateral uncoupling in both directions (Figure 7D–7F). The combined HS distributions for both field orientations are shown in Figure 7A and 7D. Predictions of HS distributions for the individual models are shown in the Data Supplement.
For all models, most HS form in the midwall. The variation of HS with relative transmural distance from the epicardium is shown in Figure 7B and 7E. These figures are directly comparable with Figure 3A. Likewise, the histograms of depth distributions in Figure 7C and 7F are directly comparable with Figure 3B. These data are consistent with the experimental observations. Also noteworthy, Figure 7B (and the Data Supplement) clearly shows that different HS distributions are excited for each field polarity. This is also consistent with the experimental observations summarized in Figure 5.

The physical mechanisms that made aspects of these models consistent with the experimental observations were as follows. Lateral cell uncoupling emphasized cell-scale discontinuities. This shifted the length scales driving the intra- and extracellular current redistributions responsible for HS formation away from the tissue surfaces and the laminar clefts. Under this condition, any intramural region is as likely as any other to be a HS.

The simulations with lateral cell uncoupling alone (not shown) gave unrealistically high excitation thresholds.
The introduction of the extracellular conductivity boundary layers on the myocardium to nonmyocardium interfaces removes sudden depolarization-inducing jumps in extracellular conductivity and mitigates HS formation on the extracellular surfaces when the cathode is located at the endocardium. This component ensures the midwall distribution of HS remains independent of field polarity.

The assumption of lateral cell-scale uncoupling in our modified models, which is sufficient for replicating the HS distribution at the time of field application, compromises lateral propagation after cardiac excitation. There is no conduction velocity when the cells are fully uncoupled, and the conduction velocity is ≈50% of the fully coupled case when the cells are uncoupled in the field direction (Data Supplement).

**Discussion**

For the first time, depth distributions of HS through the heart wall after weak electric field stimuli have been measured noninvasively using a novel tomographic imaging technique developed in our laboratory. Our most striking findings were that at near-threshold field strengths, HS were scattered across the wall and not clustered near the cathodal surface as predicted by models. Also, contrary to recent predictions, the HS location is not determined by coronary vessels.

According to Luther et al., vessels are substrates for maximum polarization and the largest vessels should generate the largest polarizations and become sites for earliest excitation. To test this, we used near-threshold field strengths to isolate the sites of maximal polarization, which manifest as sources for discrete HS. If the largest vessels were the source of HS, then reversing the field direction could have little effect either on HS location or the HS would shift to a similar size vessel. However, in the preparations where 1 vessel was larger than the other 2, we still found lateralization away from the largest vessel, which is contradictory. In the preparations where there were 3 large vessels of similar size, lateralization also occurred but the new location was not adjacent to any of the other vessels. Furthermore, the lateral displacement in Figure 5 also precludes the contribution of major laminar clefts to HS formation because if they were the source, HS should only perturb the short distance across the cleft with a reversal of field orientation. Discrepancies between experimentally measured surface polarizations and conventional model predictions have been noted before, where the experimentally measured surface polarization was significantly lower than theoretical predictions. These discrepancies were attributed to the depth averaging effects of optical mapping, which underestimated the actual degree of surface polarization. However, other experiments at near-threshold field strengths reported the lack of activation on the myocardial surface under the cathode, which suggested that surface polarization was indeed small and could not be explained by existing models. Nevertheless, these findings failed to attract attention to this significant inconsistency. A major criticism of previous experimental reports questioning the validity of the existing models was that the observations were likely experimental artifacts caused by tissue damage. Importantly, our experimental techniques enable us to preserve the integrity of the myocardial tissue, which makes such criticism less

**Figure 5.** Lateral displacement of hot spots (HS). The lateral displacement of HS in the epicardial plane after reversal of field polarity (n=10). Diamonds show the minimal displacement in a given experiment. The cross indicates zero displacement. The solid line is the mean radial shift (12.7±10.7 mm).

**Figure 6.** Formation of hot spots (HS) based on cell-scale uncoupling in model 1. A. Cells are fully coupled. The HS form on tissue surfaces at a threshold field strength of 0.35 V/cm. These data are the foundation of Figure 3C. B. Cells are uncoupled along the field direction. HS form in the midwall at a threshold field strength of 0.47 V/cm. The solitary endocardial HS (red) forms late in the stimulus and at a different location to the endocardial HS in A, which is located in the endocardial valley. C, Cells are uncoupled in both directions. HS form in the midwall at a threshold field strength of 0.72 V/cm. D, Cells uncoupled across the field direction. In addition to the midwall, HS form on the epicardial and endocardial surfaces and at the vessels for a threshold field strength of 0.47 V/cm.

because the imposed field gradient had to depolarize membranes of cell-scale dimension. The incorporation of extracellular resistive heterogeneities increased localized potential gradients and reduced the field threshold for HS formation in the model to 0.47 V/cm for lateral uncoupling in the field direction and 0.72 V/cm for lateral uncoupling in both directions. Both these are comparable with the mean threshold of 0.4±0.31 V/cm measured experimentally. In our model, the intramural laminar clefts functioned as low-resistance extracellular pathways and were a mechanism for local perturbations of the electric field at their end points (this is illustrated in the Data Supplement). This was critical for achieving a realistic excitation threshold.
relevant and indicates that the problems may not be with the experiment, but with the theory itself.

The analysis of this study suggests that the theory can be reconciled with the experimental observation by including in the model more accurate descriptions of electric heterogeneities at the cell-scale. By introducing lateral cell-scale uncoupling in the intracellular domain together with heterogeneities of extracellular resistivities into a conventional bidomain model, we were able to correctly reproduce the experimental observations. This was not possible in standard models, which do not include these features.

Our experimental and model findings shed new light onto the mechanism of electric field excitation. In particular, they expose the possible effects of macroscopic and cellular scale heterogeneities on the distributions of potential throughout the heart wall during weak electric field stimuli. This understanding is central to the development of effective low-energy defibrillation therapies. Although designed to elucidate mechanisms of electric defibrillation, our study has broader implications for understanding the biophysics of impulse propagation in the heart. This combined experimental and theoretical analysis challenges the functional syncytium paradigm and may signal the need for fundamental changes to our current mechanistic understanding of defibrillation and impulse propagation in the heart.

There are 2 principal translational implications from our study. First, any models developed for informing device design and clinical practice of low-energy antifibrillation pacing will need to account for nonsyncytial electric activation and propagation. Second, it has been speculated that the use of biphasic stimuli in low-energy antifibrillation pacing could be advantageous.2 Our study supports this concept because our experimental data show that HS translate throughout the myocardial wall with field reversal, indicating that with biphasic stimuli more HS could be formed leading to more effective antifibrillatory pacing.

Limitations
There is a systematic bias of our depth detection method that moves HS away from the surface toward inside the tissue.14 However, the bias is too small to mask the cathodal surface
activation if the latter were consistently present. In Figure 3A, we show the depth distributions of surface pacing. Although the HS produced by surface pacing are not placed by our algorithm directly on the surface, they are distinctly closer to the surface than the majority of HS initiated by field pacing. The important result is that HS during field pacing are found throughout the myocardial wall.

We chose to limit our modeling analysis to a 2-dimensional model of transmural cell cross sections. This assumption will have an effect on current loading and ideally a 3D model based on actual tissue reconstructions would be constructed. However, resolving structures at the cellular level in 3D models is computationally challenging and limited in the extent of tissue that can be modeled. We are not aware of any that fully capture the transmural dimension of cardiac tissue,22–29 which would be required to address the problem in question.

Although reproducing the intramural HS distribution during field stimulus well, our model does not accurately reproduce the transmural conduction velocity29 and will require further development. One of the logical approaches would be to analyze it in the range of low but nonzero lateral coupling.31 The other approach would be to account for the ephaptic coupling mechanism,32–36 which could become essential in the absence of, or at reduced, gap junctional coupling. Ephaptic coupling is common in the central nervous system33 and most effective where cells are densely packed. The discussion of ephaptic current in the range of low but nonzero lateral coupling.31 The other approach would be to account for the ephaptic coupling mechanism,32–36 which could become essential in the absence of, or at reduced, gap junctional coupling. Ephaptic coupling is common in the central nervous system33 and most effective where cells are densely packed. The discussion of ephaptic coupling in cardiac propagation, which started in the early six-

R01HL07162 and R03TW008039 (Dr Pertsov).

This work was supported by National Institutes of Health grants: R01HL07162 and R03TW008039 (Dr Pertsov).

Acknowledgments
Dr Caldwell designed, performed, and analyzed all experiments, as well as cowrote the article. Dr Trew designed and implemented the model and cowrote the article. Dr Pertsov conceived the project, analyzed the data, and cowrote the article.

Sources of Funding
This work was supported by National Institutes of Health grants: R01HL07162 and R03TW008039 (Dr Pertsov).

Disclosures
None.

References
16. LeGrice IJ, Smaill BH, Chai LZ, Edgar SG, Gavin JB, Hunter PJ, Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog. Am J Physiol. 1995;269(2 Pt 2):H571–H582.
29. Stinstra J, MacLeod R, Henriquez C. Incorporating histology into a 3D microscopic computer model of myocardium to study propagation...


Cardiac Response to Low-Energy Field Pacing Challenges the Standard Theory of Defibrillation

Bryan J. Caldwell, Mark L. Trew and Arkady M. Pertsov

Circ Arrhythm Electrophysiol. 2015;8:685-693; originally published online March 15, 2015;
doi: 10.1161/CIRCEP.114.002661

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circpecifications.org/content/8/3/685

Data Supplement (unedited) at:
http://circpecifications.org/content/suppl/2015/03/15/CIRCEP.114.002661.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circpecifications.org//subscriptions/
SUPPLEMENTAL MATERIAL

S1. Introduction

This supplemental material presents additional modeling results showing: (a) variation in the number and distribution of HS for specific geometric models with lateral cell-scale uncoupling in the field direction and in both directions; (b) the intramural variability in extracellular potential field that drives HS formation; and (c) conduction velocity distributions for different coupling scenarios.

S3. Results

Five perturbations on the basic model were considered, with each varying a structural feature of the base model. The HS distributions for the resulting six models together with lateral uncoupling in the direction of the applied field are shown in Figure S1. The equivalent results, but for total lateral uncoupling are shown in Figure S2 and the results for lateral uncoupling in the direction orthogonal to the applied field are shown in Figure S3. In both these uncoupling cases, varying the distribution and geometry of cell-scale uncoupling has some impact on the number of HS and their distribution (models 1-3). However, the greatest impact on HS formation and distribution comes from varying the thickness of the sub-endocardial region (models 4-5) and alterations to the midwall clefts (Model 6). This second group of three models alter structures that contribute to heterogeneities in the local extracellular potential field, such as those highlighted for Model 1 with complete cell-scale uncoupling in Figure S4. In all cases, reversing the stimulus field results in different numbers and distributions of HS formation.

**Figure S1.** HS formation in the six different modeling experiments used in this work. Uncoupling is in the field direction. Models 1-3 used different random generations of cell-scale geometries. Models 4 and 5 varied the thickness of the sub-endocardial region from 25% of the wall thickness down to 20% and up to 30%. Model 6 randomly altered the lengths of the midwall clefts. In all cases, the field strengths were 0.47 V/cm.
Laminar clefts function as preferential pathways if they have a geometric component in the direction of the applied electrical field. When this happens, large extracellular potential gradients form at the end points of the cleft space. These gradients drive local membrane depolarisations and in concert with local cell membrane area are responsible for driving HS formation. This is shown for one field stimulus in S4.
Supplemental Material  Cardiac response to electric field pacing

Figure S4. Extracellular potential gradients and HS formation. 

a. Intramural HS formation for 0.72 V/cm electric field stimulation in both directions. Six HS are highlighted for the yellow field stimulus. 

b. Transmural potential field at 5 ms for the yellow field stimulus from A. The same six HS are highlighted in the exploded and magnified view in the right-hand-side panel. 

c. Extracellular potential field at 5 ms for the yellow field stimulus from A. The six highlighted HS form where the potential gradient is high.

Figure S5 shows the equivalent data to Figure 7, but for the case where cell-scale lateral uncoupling is in the direction orthogonal to the applied stimulus field.

Figure S5. Model HS distributions for uncoupling orthogonal to the applied field direction. 

a, Intramural distribution of HS for six different numerical experiments (field strength 0.47 V/cm) and both field polarities. Red – epicardial cathode; yellow – endocardial cathode. 

b, Distribution of HS with distance from the epicardial surface (Z) as a proportion of local wall thickness (L), for each numerical experiment and both field polarities. 

c, Frequency distribution of model HS with normalized depth, for all numerical experiments (n=6) and both field polarities.

Conduction velocities (CV) were determined for the four model scenarios shown in Figure 6 at the activation field threshold stimuli. The CV distributions are shown in Figure S6. In the case where cells are uncoupled and the tissue is continuous at that scale, the mean CV is 0.45±0.12 m/s (Figure S6a). There is no conduction when the cells are uncoupled in both directions. When cells are uncoupled in the direction of the applied field the CV is 0.21±0.13 m/s (Figure S6c) and when uncoupling is orthogonal to the field direction the CV is 0.34±0.13 m/s (Figure S6d). For each scenario different numbers of HS or areas of the model were activated at threshold and this is reflected in the frequency count. Consider the case where cells are uncoupled in the field direction (Figure 6c and S6c). If the activation time gradient components parallel and orthogonal to the applied stimulus field are inverted separately, then the mean CV parallel to the applied field is 0.25±0.18 m/s and the mean CV orthogonal to the applied field is 0.44±0.21 m/s.
Figure S6. Conduction velocity (CV) distributions for the models shown in Figure 6. 

a. CV when cells are fully coupled in both directions (standard model).

b. There is no conduction and no CV when cells are uncoupled in both directions.

c. CV when cells are uncoupled in the direction of the applied electric field.

d. CV when cells are uncoupled in the direction orthogonal to the applied electric field.