Response to Research Commentary

Effects of Cardiac Microstructure on Propagating Electrical Waveforms

Madison S. Spach, Roger C. Barr

**Abstract**—Electrical waveforms measured during propagation at microscopic level are considerably affected by normal variations in cardiac microstructure as well as by the superfusing fluid. On the basis of evidence we present in this article, we argue that the anisotropic waveform variations discussed here are explained primarily by the associated variations in different microstructural components of myocardial architecture rather than by the effects of the perfusing bath. The results suggest that different components of myocardial architecture have preferential effects on $V_{\text{max}}$ and on the shape of the foot of the transmembrane action potential ($V_{\text{m foot}}$). Resistive discontinuities primarily affect $V_{\text{max}}$ and an additional capacitive component in the local circuit due to the capillaries in interstitial space primarily affects $V_{\text{m foot}}$. Resistive discontinuities also have an important influence on cardiac conduction. These discontinuities include spatial variations in the size of interstitial space (interstitial resistive discontinuities) and the role of cellular scaling (effects of cell size) when changes occur in the cellular and multicellular distribution of gap junctions during remodeling of normal mature myocardium to proarrhythmic structural substrates. The full text of this article is available at http://www.circresaha.org. (*Circ Res.* 2000;86:e23–e28.)

**Key Words:** discontinuous conduction ♦ anisotropy ♦ action potential foot ♦ capillaries ♦ interstitial discontinuities

On the basis of well-known bidomain model studies,1–10 Roth11 notes in his commentary about our 1981 and 1998 papers12,13 that directional differences in the shape of the foot of the transmembrane potential ($V_{\text{m}}$) and in $V_{\text{max}}$ cannot be taken as definitive evidence of discontinuous propagation or capillary effects. Specifically, he concludes8,11 that the directional differences in $V_{\text{max}}$ and $V_{\text{m}}$ foot that we reported12,13 result from the way the tissue was perfused and the interaction of current flow in the tissue with that in the surrounding fluid.

For purposes of clarity, we limit our response to uniform anisotropic myocardium and begin by noting the following about our recent (1998) article.13 We considered our experimental tests (and “2-domain” model results) in this paper to provide strong support for the following hypothesis: interstitial electrical field interactions between the electrically passive capillaries and active myocytes produce an additional interstitial component in the local electrical circuit of propagating excitation waves in working myocardium. This extra capacitive component depends on the direction of propagation in relation to the anisotropic distribution of the capillaries, and it also depends on the density of the capillaries and the size of interstitial space. The extra capacitive component due to normal capillaries primarily affects $V_{\text{m foot}}$, with much less effect on $V_{\text{max}}$. This mechanism represents an extension of the general model of 1-dimensional cardiac fiber interactions of Medvinskii and Pertsov.14 To formalize our new “capillary” hypothesis, we developed a 2-domain model of cardiac muscle represented as 2 separate but interconnected spaces, anisotropic intracellular space with or without 2-dimensional discrete ventricular cells15 and anisotropic interstitial space containing the anisotropic distribution of capillaries. The electrical model was based on the morphology of working myocardium. When the documented variations in microstructure (cell geometry, density of capillaries, and dimensions of interstitial space) were approximated in the model, its predictions for $V_{\text{m foot}}$ and $V_{\text{max}}$ were in good agreement with the experimental data.13

During longitudinal (LP) and transverse propagation (TP), there was considerable variability in the general pattern of $V_{\text{max}}$ and in the phase-plane trajectories of $V_{\text{m foot}}$; eg, there were variable deviations from an exponential rise of $V_{\text{m foot}}$. Our hypothesis and model did not include the perfusing bath effects that have been demonstrated in the bidomain model studies of Roth and others,1–11 because it focused on the tissue at a microscopic size scale. As detailed in an excellent review by Henriquez,10 bidomain models of bath effects have considered propagation at the larger macroscopic level where the stochastic effects of normal cardiac microstructure are averaged.15 We think these differences in size scale are highly important when considering our results12,13 in relation to the effects of the perfusing bath shown by bidomain models.1–11

We wish to address the following 3 specific points in response to Roth’s commentary: (1) matters on which we agree with Roth; (2) ways in which our results are not accounted for by bidomain model results of the bath effect;...
(3) our conclusion, which we consider to emphasize the need to develop more comprehensive models with the salient characteristic of incorporating features of actual cardiac architecture at the microscopic level.\textsuperscript{13,15,16}

**Points of Agreement Between Experimental Data and Results of Perfusate Effect**

We agree that Roth’s results in his accompanying paper,\textsuperscript{11} as well as the results of other bidomain model studies,\textsuperscript{10} clearly demonstrate the same general changes in the shape of $V_m$ foot and in $V_{max}$ that we presented in our 1981 initial report of discontinuous conduction.\textsuperscript{12} That is, the rise of the action potential foot is slower (greater $\tau_{foot}$) and $V_{max}$ is less during fast LP (low-resistance direction) than slow TP (high-resistance direction). If these general features, consistent with those of Plonsey and Barr,\textsuperscript{1,2} were the only consideration, we would agree that one cannot distinguish between a mechanism due to the discrete structure of cardiac muscle\textsuperscript{12,1,13} and the effects of the perfusing bath described in bidomain model studies.\textsuperscript{1–11}

In retrospect, we also agree with Roth that comparison of the shape of $V_m$ foot in mature myocardium with action potentials measured in single layers of cultured neonatal cells\textsuperscript{17} (no capillaries) does not distinguish between a capillary mechanism and the superfusate effect. On the other hand, our analysis of the optical action potentials of Fast and Kleber\textsuperscript{1} provided a necessary first step before proceeding to the major experimental test of the capillary hypothesis, ie, comparison of the phase-plane trajectory of $V_m$ foot in neonatal versus mature ventricular muscle.\textsuperscript{13} At that time there were no available data about the shape of $V_m$ foot in neonatal preparations, including the elegant data of Fast and Kleber\textsuperscript{17} in neonatal cell cultures. We therefore considered that their published optical action potentials provided an excellent opportunity for an initial experimental test of our hypothesis. Our analysis of their data also provided a rigorous test of the phase-plane analysis method to ensure that the deviations from an exponential rise of $V_m$ foot we found in working myocardium were not the result of experimental artifact. That is, there were concave deviations from a linear (exponential) phase-plane trajectory of $V_m$ foot during LP in ventricular muscle, and these deviations should not (and did not\textsuperscript{13}) occur in the optical action potentials that Fast and Kleber\textsuperscript{17} had recorded in neonatal cell cultures.

Roth\textsuperscript{11} suggests that a good experiment would be to remove the superfusing fluid in the bath. We attempted that experiment but could not achieve successful measurements. Although we were unable to cover the preparations with oil in our large tissue bath (15 cm in diameter), we lowered the level of the superfusate to the surface of the tissue. When there was no fluid covering the surface, beat-to-beat changes in the intracellular ($\Phi_i$) and extracellular ($\Phi_e$) potential waveforms occurred, which prevented stable measurements.

**Perfusate Effects**

The choice of the depth of perfusate in the volume conductor produces conflicting considerations in the study of anisotropic conduction events when measuring intracellular and extracellular potentials.\textsuperscript{18–21} In our first study of anisotropic conduction in uniform anisotropic muscle,\textsuperscript{18} we found systematic differences in the general shape and amplitude of $\Phi_e$ waveforms when conduction progressed in different directions from a single stimulus site. The directionally dependent changes in $\Phi_e$ at the tissue surface were accounted for by a “continuous” anisotropic model of directional differences in effective axial resistance, which ignored interstitial anisotropy in a preparation exposed to a large volume conductor.\textsuperscript{18} We then collaborated with David Geselowitz\textsuperscript{22} to evaluate the effects of the volume conductor on $\Phi_e$ at the surface of tissue with and without interstitial anisotropy. The model of Geselowitz et al.\textsuperscript{22} and the associated experimental measurements, showed that as long as the level of the perfusate exceeded 1 mm above the tissue surface, there was good agreement with the measured $\Phi_e$ waveforms and the bidomain model predictions; ie, interstitial anisotropy was not necessary to explain the $\Phi_e$ waveforms.\textsuperscript{22} However, when the fluid level decreased to $<$1 mm above the surface, interstitial anisotropy became important; ie, the general $\Phi_e$ wave shape was altered and the amplitude of the waveforms increased considerably.\textsuperscript{22} Consequently, to achieve extracellular measurements at the surface, we have maintained an adequate volume conductor in our tissue bath experiments.

**Waveform Variations Not Accounted for by Bath Effect**

**Discontinuous Propagation Evident in Extracellular Waveforms**

We subsequently found that the most straightforward evidence for discontinuous propagation in uniform anisotropic myocardium lies in the analysis of extracellular waveforms.\textsuperscript{20} Superimposed on the smooth-appearing $\Phi_e$ waveforms, which are accounted for by a continuous anisotropic medium,\textsuperscript{18} there is notching of the $\Phi_e$ first and second derivative waveforms, and the notches become more frequent as the wavefront shifts from LP to TP.\textsuperscript{20} Recent results with a 2-dimensional discrete cellular model\textsuperscript{15} of uniform anisotropic ventricular muscle produced good agreement with the experimental $\Phi_e$, $d\Phi_e/dt$, and $d^2\Phi_e/dt^2$ waveforms during LP, oblique conduction, and TP.\textsuperscript{23} The $\Phi_e$ notching was found to be associated with more prominent conduction delays between cells and groups of cells during TP than LP in mature myocardium,\textsuperscript{15,23} ie, discontinuous propagation.

At this point, therefore, it is important to separate the question as to whether discontinuous propagation does or does not occur at a microscopic level from Roth’s\textsuperscript{11} additional important question as to how one can distinguish between the effects of cardiac microstructure and the effects of the perfusing bath shown by bidomain models on propagating waveforms. We have not questioned the validity of the bidomain model predictions of the bath effects.\textsuperscript{1–11} Rather, we found that the experimental variations in $V_{max}$ and in the time course of $V_m$ foot “… were not explained by computer simulations based on bidomain models,”\textsuperscript{7,13} (p 1145) at least to the present time.

$V_{max}$

On the basis of measurements in trabecula and papillary muscles from ferret hearts, in 1985 Suenson\textsuperscript{24} reported that
differences in resistance of the medium to which the tissue surface was exposed produces curved propagating wavefronts beneath the surface. He noted that when a wavefront deviates from a plane wave, the leading cells will be loaded electrically by the lagging neighboring cells. He also extended the interpretation of his results about curvature beneath the surface (along the z axis) to the x-y plane of the tissue surface by noting that “the effects of a curved wavefront may represent an alternative explanation of the findings [directional differences in \(V_{\text{max}}\) and \(V_{\text{foot}}\)] of Spach et al. (1981) who introduced the theory of discontinuous propagation in dog myocardium...” (p 89).

Suenson was correct, given that, at that time, we had not considered the effects of the curvature of wavefronts in the x-y plane (along the surface) at a macroscopic size scale. Therefore, we challenged our 1981 data by performing detailed measurements of \(V_{\text{max}}\) for conditions under which there should be no x-y plane curvature effects. We produced “4-way conduction” of macroscopic plane waves at each microelectrode impalement site; ie, propagation occurred in both directions along the long axis of the fibers and in both directions along the transverse axis. The results showed that mean TP \(V_{\text{max}}\) was significantly greater than mean LP \(V_{\text{max}}\), as we had originally found. We concluded, therefore, that our \(V_{\text{max}}\) results were not explained by curvature of wavefronts.

The chronological appearance of “new” information based on bidomain models now becomes pertinent. Although in 1978 Tung formalized in a bidomain model the mathematical representation of the separate intracellular and interstitial spaces as a single interpenetrating domain, the powerful applications using mathematical representations of this model to in vitro measurements (eg, virtual cathode) did not appear until after our 1981 paper. Since then, a number of model results have demonstrated the effects of the perfusing bath on propagating depolarization. All of the bidomain models that we know of predict the following fixed relationships between \(V_{\text{max}}\) and \(V_{\text{foot}}\): (1) at the surface, or at a specific depth below the surface, \(V_{\text{max}}\) and the shape of \(V_{\text{foot}}\) are constant during propagation along any given axis of conduction, and (2) when \(V_{\text{max}}\) decreases, the rate of rise of \(V_{\text{foot}}\) decreases (and vice versa).

In contrast to the bidomain model predictions of fixed values of \(V_{\text{max}}\) along a given axis of propagation, our 4-way conduction analysis showed that the values of \(V_{\text{max}}\) were different from cell to cell during LP (93 to 139 V/s) and TP (110 to 181 V/s), and at a few sites TP \(V_{\text{max}}\) was less than LP \(V_{\text{max}}\). That these considerable variations along the same axis of conduction were not measurement artifact, or due to the perfusing bath, was indicated by fact that the absolute values of LP \(V_{\text{max}}\) and TP \(V_{\text{max}}\) at the same impalement site varied independently of each other (some of the lowest values of LP \(V_{\text{max}}\) occurred at the same site as the highest TP \(V_{\text{max}}\) values). On the other hand, one might argue that the considerable cell-to-cell \(V_{\text{max}}\) variation along the same axis of conduction was influenced by the tip of the microelectrode penetrating to different depths beneath the surface at different sites, which could produce different \(V_{\text{max}}\) values according to bidomain model predictions. However, when we maintained the microelectrode tip in a fixed position (ie, no change in its depth), \(V_{\text{max}}\) changed considerably at the same site when the direction of conduction was reversed 180 degrees during LP. Similarly, prominent \(V_{\text{max}}\) changes occurred at the same site when conduction was reversed 180 degrees during TP.

Thus, undulations values of \(V_{\text{max}}\) occur in any given direction of propagation, and it is the average \(V_{\text{max}}\) value that is larger during TP than LP. This anisotropic feature of \(V_{\text{max}}\) is explained by variations in cellular loading that generate variations in \(V_{\text{max}}\) that are dependent on the complex distribution of \(r_{i}\) discontinuities produced by the gap junctions and cellular boundaries in the path of an advancing excitation wave.

**V_{\text{max}}**

Because there had been no detailed experimental analysis of the time course of \(V_{\text{max}}\) during anisotropic propagation, we performed a similar 4-way conduction analysis of \(V_{\text{max}}\) in which we used phase-plane analysis. Although the average values of \(V_{\text{max}}\) were greater during LP than TP, as found originally, different sites demonstrated variable deviations from a linear (exponential) \(V_{\text{foot}}\) trajectory during both LP and TP. Quite importantly, \(V_{\text{max}}\) and \(V_{\text{foot}}\) varied independently of one another. We therefore considered the major point established by the experimental results to be that “... electrical loading due the microstructure of working myocardium can independently alter \(V_{\text{max}}\) or the foot of the action potential.” (p 1159). This experimental result is different from the predictions of continuous medium theory, as well as the bidomain model predictions for the effects of the perfusing bath as done to date.

To explain these results, several resistive models were evaluated but discarded. None of the models of resistive discontinuities produced deviations from a linear phase-plane trajectory of \(V_{\text{max}}\) at any site for all directions of conduction. On the basis of the measurements by Oleson and Olesen and Oleson and Crone of the passive properties of cerebral capillaries, we then considered that the capillaries provided a structure in interstitial space for electrotonic interactions with the active myocytes. This structural arrangement is similar to that in the model of Medvinskii and Pertsov of electrical field interactions between active and inactive cardiac fibers. Thus, we hypothesized that the microstructure that could produce an effect as observed in the data was that of the capillaries.

Because the perfusing bath conditions were the same for all of our experiments, we consider the following to provide experimental support for the capillary hypothesis, rather than for the effects of the perfusing bath, as the explanation of our experimental \(V_{\text{max}}\) foot results.

1. The magnitude of the concave deviations from linearity in the phase-plane trajectory of \(V_{\text{max}}\) foot varied considerably at different sites during LP. During TP, 30% of the impalement sites demonstrated an initial slur in the trajectory of \(V_{\text{max}}\) foot, whereas 70% of the sites had a linear \(V_{\text{max}}\) foot trajectory. These directionally different variations were accounted for in our 2-domain model results by known variations in capillary density and the variations we measured in the size of interstitial space (see Fig. 9 in Reference 13). That is, the
greater the density of capillaries and the smaller the size of interstitial space, the greater the deviation from a linear \( V_m \) foot trajectory.\textsuperscript{13}

(2) In adult canine working myocardium, the concave deviations from linearity during LP were greater in ventricular than in atrial muscle (see Table 1 in Reference 13). Our subsequent analysis has shown that the density of capillaries is significantly greater (\( P<0.001 \)) in adult canine ventricular muscle than in atrial muscle bundles (ie, number of capillaries adjacent to each myocyte). This morphological result is consistent with that of Ludwig.\textsuperscript{34}

(3) Neonatal ventricular muscle produced the most prominent concave deviations from a linear trajectory of \( V_m \) foot that we encountered; ie, the maximum difference from linearity during \( V_m \) foot was greater in neonatal than in adult ventricular muscle (\( P<0.02 \)).\textsuperscript{13} Neonatal ventricular myocardium has the highest density of capillaries that occurs during any time interval from early life to adulthood.\textsuperscript{35,36}

(4) The relationship between the average values of \( \dot{V}_{\text{max}} \) during LP and TP are different in neonatal than adult canine ventricular muscle. That is, in contrast to adult ventricular muscle, in neonatal ventricular muscle the mean value of LP \( \dot{V}_{\text{max}} \) is not significantly different from mean TP \( \dot{V}_{\text{max}} \).\textsuperscript{13,16} However, at a macroscopic level the LP/TP velocity ratios are the same in neonatal and adult canine ventricular muscle.\textsuperscript{16} It is difficult to understand how the same bath effects could produce different adult and neonatal patterns in \( \dot{V}_{\text{max}} \), in addition to the differences in \( V_m \) foot, in preparations that are normal with similar uniform anisotropic properties and the same LP/TP velocity ratio at a macroscopic level. On the other hand, our recent results show that growth effects with associated increases in cell size, along with remodeling of the cellular distribution of gap junctions from birth to maturity, can explain the different neonatal and adult anisotropic \( V_m \max \) patterns at a microscopic level in the presence of the same neonatal-adult LP/TP velocity ratios at a macroscopic level.\textsuperscript{16}

(5) We encountered other trajectories with atypical phase-plane trajectories of \( V_m \) foot that are described, but not illustrated, in our 1998 article.\textsuperscript{13} Figure 1 shows an example of the unusual \( V_m \) foot shape changes we encountered at a single site when LP conduction was reversed along the long axis of the fibers. In a left-to-right direction (Figure 1, left), there was the usual concave deviation from a linear trajectory of \( V_m \) foot. However, when the direction of conduction was reversed along the same axis, the trajectory of \( V_m \) foot changed to a convex shape (Figure 1, right).

We have not been able to account for this behavior of \( V_m \) foot by branching,\textsuperscript{28} by any model of internal resistance (\( r_i \)) discontinuities,\textsuperscript{15,17,29–31} or by an effect of the perfusing bath.\textsuperscript{1–11} However, in our morphological studies of serial sections viewed rapidly (in motion) with computer displays, abrupt changes (discontinuities) in the size of interstitial space, the greater the deviation from a linear \( V_m \) foot trajectory.\textsuperscript{13}

Two-domain capillary model demonstration of the effect of a discontinuity of interstitial resistance (abrupt change in interstitial volume) on the trajectory of \( V_m \) foot during LP. Results were obtained with the 2-domain capillary model using the same parameters detailed in Table 2 of Reference 13. The drawings illustrate spatial differences along the long axis of the fibers in the interstitial resistance (\( r_i \)) and the discontinuity of interstitial resistance produced by the abrupt change in \( r_i \). For purposes of focusing on the effects of the interstitial discontinuity, the anisotropic active layer was continuous with a homogeneous longitudinal internal resistance (\( r_i \)) of 133 \( \text{M} \Omega \text{cm} \). The same maximum density of capillaries was present in all interstitial space. However, in the left side of the model, \( r_i \) was assigned a value of 236 \( \text{M} \Omega \text{cm} \) (high \( r_i \)) to represent an average interstitial width of 2.1 \( \mu \text{m} \); in the right side of the model, \( r_i \) was assigned a value of 118 \( \text{M} \Omega \text{cm} \) (low \( r_i \)) to represent an average interstitial width of 4.2 \( \mu \text{m} \). (The dimensions of interstitial space were obtained from morphological measurements.\textsuperscript{15}) A, During left-to-right LP, the concave deviation of the trajectory of \( V_m \) foot was more prominent in the region of high \( r_i \) than low \( r_i \), as expected.\textsuperscript{13} B, When the direction of propagation was reversed to occur from the low to the high \( r_i \) region, the trajectory of \( V_m \) foot changed to a convex shape (*) in same high \( r_i \) area that had produced a prominent concave deviation in panel A. That is, over a distance of ~1 mm in the high \( r_i \) region adjacent to the \( r_i \) discontinuity, the trajectory of \( V_m \) foot changed to a convex shape when the direction of conduction was reversed. However, as propagation continued in the high \( r_i \) area, at sites farther away from the \( r_i \) discontinuity the prominent concave deviation from linearity in the trajectory of \( V_m \) foot returned to that shown for the high \( r_i \) region in panel A. When the capillaries were removed, the trajectory of \( V_m \) foot was linear at all sites for both directions of conduction.
space became apparent without apparent changes in the distribution of the gap junctions or density of the capillaries. When we included an abrupt change in the size of interstitial space (ie, an abrupt change in interstitial resistance) in our 2-domain model with capillaries, the model results (Figure 2) were in good agreement with the experimental results shown in Figure 1. Contrariwise, when the capillaries were removed from the 2-domain model, both the concave and convex shapes of the trajectory of $V_{\text{m}}$ foot disappeared and the $V_{\text{m}}$ foot trajectory was linear at all sites during conduction across the $r_s$ discontinuity.

Conclusions

We agree with Roth that the depolarization potential waveforms can be affected by the superfusing volume conductor, consistent with his publications and also our own. We do not agree, however, that such effects are sufficient or the most significant in accounting for the range of detailed changes that we observed in our studies. Our understanding of Roth’s article is that he does not question whether discontinuous conduction occurs in cardiac muscle, a phenomenon that is supported by several converging lines of investigation since 1981. Rather, he points out that care should be exerted when interpreting experimental data that may include the effects of the tissue bath as described by bidomain models. Here, we have attempted to point out the detailed anisotropic variations in $V_{\text{max}}$ and the shape of $V_{\text{m}}$ foot that thus far have been explained only by specific features of the documented natural architecture of cardiac bundles. However, because of the relative paucity of available information about the effects of different components of cardiac microstructure, both Roth’s admonition and our results emphasize the need for future study of variations in the depolarization shape of cardiac action potentials and their cause.

The results presented here provide an evolving picture that suggests that resistive discontinuities primarily affect $V_{\text{max}}$, and an additional capacitive component due to capillaries in interstitial space primarily affects $V_{\text{m}}$ foot. Therefore, the results emphasize that there are yet important, unexplored resistive discontinuities at a microscopic level. These include spatial variations in the size of interstitial space and the role of cellular scaling (effects of cell size) when changes occur in the cellular and multicellular distribution of gap junctions during remodeling of mature myocardium into structural substrates that are arrhythmogenic.

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References


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