Spatial Changes in the Transmembrane Potential During Extracellular Electric Stimulation

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Abstract—The purpose of this study was to determine the spatial changes in the transmembrane potential caused by extracellular electric field stimulation. The transmembrane potential was recorded in 10 guinea pig papillary muscles in a tissue bath using a double-barrel microelectrode. After 20 S1 stimuli, a 10-ms square wave S2 shock field with a 30-ms S1–S2 coupling interval was given via patch shock electrodes 1 cm on either side of the tissue during the action potential plateau. Two shock strengths (2.1±0.2 and 6.5±0.6 V/cm) were tested with both shock polarities. The recording site was moved across the tissue along fibers with either 200 μm (macroscopic group [n=5], 12 consecutive recording sites over a 2.2-mm tissue length in each muscle) or 20 μm (microscopic group [n=5], 21 consecutive recording sites over a 0.4-mm tissue length in each muscle) between adjacent recording sites. In the macroscopic group, the portion of the tissue toward the anode was hyperpolarized, whereas the portion toward the cathode was depolarized, with 1 zero-potential crossing from hyperpolarization to depolarization present near the center of the tissue. In the microscopic group, only 1 zero-potential crossing was observed in the center region of the tissue, whereas, away from the center, only hyperpolarization was observed toward the anode and depolarization toward the cathode. Although these results are consistent with predictions from field stimulation of continuous representations of myocardial structure, ie, the bidomain and cable equation models, they are not consistent with the prediction of depolarization-hyperpolarization oscillation from representations based on cellular-level resistive discontinuities associated with gap junctions, ie, the sawtooth model. (Circ Res. 1998;83:1003-1014.)

Key Words: hyperpolarization • defibrillation • simulation

To understand the mechanism of ventricular defibrillation, it is important to acquire knowledge of the transmembrane potential changes (ΔV_m) caused by the shock in addition to the action potential changes after the shock. This is because the cellular excitation and alteration in action potentials that occur after a defibrillation shock are thought to result from the changes in the transmembrane potential caused by the shock.1,2 Several studies have been performed to obtain such information. Some studies used optical recording techniques to evaluate the ΔV_m caused by the shock field in either isolated myocytes1,3–5 or perfused hearts.2,6–10 With a few exceptions,1,3,4,11 the optical recording represents the weighted average of the ΔV_m changes in many cells.2,7,9 Recordings of the transmembrane potential by a double-barrel microelectrode can supply information about the ΔV_m caused by the shock from an individual cell within the myocardial syncytium.12,13

A range of modeling studies completed over the past 15 years suggests that shock-induced ΔV_m can change markedly over the length of an individual cell.14–17 These studies are based on the hypothesis that highly resistive gap junctions located at the cell ends create a sawtooth pattern of depolarization-hyperpolarization oscillation in the spatial distributions of V_m. Experimentally, the sawtooth pattern has been observed during shocks applied to isolated myocytes,1 with the cell end closest to the cathode depolarized and the end closest to the anode hyperpolarized. However, because the gap-junction resistance is considerably less than the membrane resistance at the end of an isolated myocyte, the possibility exists that the sawtooth pattern is never established within the myocardial syncytium or that its magnitude is so small that it can be neglected. Either possibility might explain the fact that the sawtooth pattern, while evident in modeling studies assuming resistive discontinuities of intracellular coupling in both monodomain14–16 and bidomain17 representations of tissue structure, has not been observed experimentally.2,4 For example, a recent study by Gillis et al18 used optical recording techniques to record the ΔV_m caused by electric shocks in individual cells of monolayers and strands of cultured neonatal rat myocytes. In that study, a local hyperpolarization and depolarization of the sawtooth pattern was not observed at the borders between cells when

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electric field stimulation was applied across the entire strand of cells.

We undertook the present study to examine whether the sawtooth pattern arises in shock-induced $\Delta V_m$ in syncytial tissue. In an attempt to exclude possible contributions of fiber curvature associated with a bidomain representation of tissue structure in the shock-induced $\Delta V_m$, we used isolated guinea pig papillary muscles. Histological characteristics of this preparation are well documented; ie, fibers are oriented parallel to one another through the preparation.\(^{18}\) Using a double-barrel microelectrode recording technique and fine spatial resolution that included multiple impalements along the longitudinal axis of the papillary muscle, we were able to examine whether the sawtooth pattern arose during shocks of different strengths and polarities.

Materials and Methods

Details about the use of the double-barrel microelectrode to record the transmembrane potential before, during, and after a shock from a papillary muscle in a tissue bath have been previously reported.\(^{12,13}\)

Tissue Preparation

Ten guinea pig papillary muscles were used. Guinea pigs weighing approximately 300 g were injected with sodium pentobarbital (Nembutal, 75 mg in 1.5 mL via the abdomen). The hearts were rapidly excised through a median sternotomy and immersed in cold Tyrode’s solution. The Tyrode’s solution was of the following formula (in mmOL/L): NaCl 129, CaCl\(_2\) 1.8, MgCl\(_2\) 1.1, KCl 4.5, Na\(_2\)HPO\(_4\) 1, NaHCO\(_3\) 20, and glucose 11. The left ventricular anterior papillary muscle, 4.5±0.4 mm long and 1.5±0.1 mm wide, was removed and pinned on silicon rubber in the center of a 2×2-cm tissue bath. The base of the papillary muscle faced the right side of the tissue bath, and the apex faced the left side of the bath (Figure 1). The tissue was continuously superfused with Tyrode’s solution bubbled with a mixture of 95% O\(_2\) and 5% CO\(_2\) (pH 7.35 to 7.40). The solution temperature was maintained in the range of 35 to 36°C. Two mesh patch platinum shock electrodes were placed on each side of the tissue bath and immersed in the Tyrode’s solution to generate a constant electric field across the tissue bath. In this way, the shock potential gradient was oriented along the long axis of the tissue.\(^{12}\) The cardiac tissue was paced at one end via 2 extracellular (0.2-mm diameter) pacing electrodes with a stimulator controlled by a Macintosh II computer. Two extracellular recording electrodes, which were fixed on the silicon rubber, were just beside the tissue to record the extracellular potentials generated by the shock. The distance between these 2 extracellular recording electrodes was about 2 mm, measured with a dissecting microscope. These 2 extracellular electrodes were aligned so that the line between them was perpendicular to the mesh shocking electrodes. This line was assumed to be parallel to the shock potential gradient. The potential between the two electrodes was recorded differentially with a data acquisition system.\(^{13}\) The potential gradient generated by the shock was obtained by dividing the potential difference between the 2 extracellular electrodes generated by the shock by the distance between them.

Signal Recordings

To make the double-barrel microelectrode, 2 single glass capillaries (WPI Glass 1BBL W/FIL 1.0 mm) were glued together, except in the region where the tips were to be formed, and were pulled by a horizontal micropipette puller (Industrial Science Associates, Inc). The capillary tubes were pulled to have an impedance of \(>10 \text{ M} \Omega\) for both tips when filled with 3 mol/L KCl. The distance between the 2 microelectrode tips measured under a light microscope was about 30 \(\mu\text{m}\). The double-barrel microelectrode was mounted on a motorized micromanipulator (WPI DC5001) that could move in 3 dimensions with 0.5-\(\mu\text{m}\) resolution. Each barrel of the double-barrel microelectrode was connected to a differential preamplifier (WPI Duo 773 Dual Microprobe System) with an Ag-AgCl wire. Capacitor compensation within the preamplifier was used to eliminate capacitive coupling between the 2 tips when needed. The signals were recorded differentially as a voltage between the 2 tips just below the tissue represent the extracellular recording electrodes. B. Schematic of the model used for computer simulation. In the bidomain, the intracellular space (tissue) was coupled to the interstitial and extracellular space (tissue and bath) through the cell membrane. Membrane properties were prescribed from the LRd equations. Anisotropic conductivities were assigned in the tissue part of the model. Isotropic conductivity was assigned in the bath.

Experimental Protocol

The 10 guinea pig papillary muscles were divided into 2 groups, with 5 experiments in each group. Group 1 was studied to determine how the $\Delta V_m$ caused by a shock varied over the length of the papillary muscle. This was called the macroscopic group. Group 2 was studied to determine how the $\Delta V_m$ caused by a shock varied over a much shorter length. This was called the microscopic group. In the macroscopic group, there were 12 consecutive recording sites in each muscle, with 200 \(\mu\text{m}\) between adjacent recording sites. In the microscopic group, there were 21 consecutive recording sites in each muscle, with 20 \(\mu\text{m}\)
between adjacent sites. The total recorded tissue length was 0.4 mm, a distance that spanned at least 2 recording sites in the macroscopic group (Figure 1). In each papillary muscle of both groups, the double-barrel microelectrode was moved from the first recording site to the last recording site in the same direction along the longitudinal axis of the papillary muscle. If an action potential was not obtained at the intended recording site, the double-barrel microelectrode was moved <3 μm to the left or right of that recording site to obtain the action potential recording. The next recording site was still at the originally intended site. Because of the high spatial resolution in the macroscopic group, the tissue was treated with 10 mmol/L diacetyl monoxime to eliminate motion of the tissue caused by contraction.

The total length of the papillary muscle was determined with a motorized micromanipulator. The total recording length in the macroscopic group was predetermined to be in the midportion of the tissue so that the recordings were not performed at either end of the tissue (Figure 1). The recording sites in the macroscopic group were made within the center 2 mm of the tissue and so were within the region in which recordings were made for the macroscopic group. Because the mean length of the tissue was 4.5 ±0.4 mm long, recordings were usually not made within 1 mm of either end of the tissue. After the recording location was determined, the double-barrel microelectrode was slowly lowered into the Tyrode’s solution just above the tissue with a motorized micromanipulator. It was then rotated slightly until the potential difference between the 2 tips was almost undetectable during shocks that created a shock field of approximately 6 V/cm, as reported previously.12 The double-barrel microelectrode was then lowered into the tissue until an action potential was seen in the differential recording between the 2 barrels shown on the monitoring oscilloscope. An S1–S2 stimuli–shock protocol was then performed as described below. After recording the ΔVm caused by the shock at one site, the double-barrel microelectrode was withdrawn just above the tissue and moved to the next recording site until recordings were made from all sites. All recordings from the same papillary muscle were made from the superficial layer of the tissue and with the same double-barrel microelectrode.

After 20 S1 stimuli consisting of 2-ms square waves with a 300-ms S1–S1 pacing interval at twice the diastolic threshold, a 10-ms S2 shock field consisting of a square waveform was applied along the papillary muscle through the pair of mesh shock electrodes. The onset of the S2 shock was given with a 30-ms S1–S2 coupling interval so that it was delivered during the plateau of the 20th S1 action potential. This S1–S2 interval was chosen to give shocks that created 10-ms S2 shock field consisting of a square waveform was applied to the superficial layer of the tissue and with the same double-barrel microelectrode. During the shock than just before the shock, whereas hyperpolarization was defined as a more negative membrane potential during than just before the shock.

The paired t test and analysis of variance were used for statistical analysis of the data as presented in Results. A P value <0.05 was considered significant. Data are given as mean ± SD.

### Computer Simulations

The electric activity within the guinea pig papillary muscles was modeled using a bidomain representation of tissue structure based on a standard formulation:

\[ I_m = \nabla \cdot (\sigma (V_m + \phi_i)) = -\nabla \cdot (\sigma \nabla \phi_i), \]

where \( \sigma \) was the specific intracellular conductivity, \( \sigma_e \) the specific extracellular conductivity, \( V_m \) the transmembrane potential, and \( \phi_i \) the interstitial potential, and \( I_m \) the transmembrane current density. \( I_m \) was further specified in terms of membrane sources as follows:

\[ I_m = A_m (C_m \frac{dV_m}{dt} + I_m). \]

where \( C_m \) was the specific membrane capacitance, \( I_m \) the ion current source density, and \( A_m \) the ratio of membrane surface to intracellular volume. By Equation 2, we determined \( V_m \) from the following equation:

\[ g_e / A_m (d^2V_m/dx^2 + d^2\phi_i/dx^2) + g_o / A_m (d^2V_m/dy^2 + d^2\phi_i/dy^2) = -g_{m} \cdot dV_m/dt + I_m, \]

where \( g_e \) was the intracellular bidomain conductivity along fiber axis and \( g_o \) was the intracellular bidomain conductivity across fiber axis. In solving Equation 3, we assumed sealed-end boundary conditions at the edges of the tissue. The tissue portion of the model measured 4 mm in length by 50 μm in width. We then determined the interstitial and extracellular potential distributions from the transmembrane potential distribution using a rewritten form of Equation 1:

\[ g_s / A_m (d^2V_m/dx^2 + d^2\phi_i/dx^2) + g_o / A_m (d^2V_m/dy^2 + d^2\phi_i/dy^2) = -g_{m} \cdot dV_m/dx + g_{m} \cdot dV_m/dy, \]

where \( g_s \) was the interstitial bidomain conductivity along the fiber axis and \( g_{m} \) was the interstitial bidomain conductivity across fiber axis. To allow numerical solutions on a single grid containing interstitial and extracellular nodes, we generalized Equation 4 to the following:

\[ g_s (\delta^2\phi_i/\delta x^2 + \delta^2\phi_i/\delta y^2) = 0, \]

by assigning \( g_s = g_{m} = 0 \) and \( g_s = g_o = g_{m} \) at nodes within a surrounding tissue bath, where \( g_{m} \) was the bath conductivity. The bath was then simply a continuation of the interstitial region within the tissue.

A schematic of the model is shown in Figure 1B. All conductivity parameters for the simulation were selected to approximate ones used by Roth.19 The full size for the combined interstitial and extracellular region measured 4 mm in length by 100 μm in width. Sealed-end boundary conditions were imposed on all edges, and matching conditions to ensure that the continuity of the interstitial and extracellular potential distribution were imposed at the interface between the tissue and the bath.

The numerical solution scheme was similar to that reported previously.13 Ordinary differential equations defining the gating variables for the individual ionic currents of \( I_{m} \) in Equation 2 were integrated numerically in time using an analytic method. We prescribed Luo-Rudy (LRd)22,23 membrane equations at all tissue nodes. The parabolic partial differential equation in Equation 3 was discretized in space over those nodes using a 5-point finite difference stencil. Discretization in time used a semi-implicit averaging scheme analogous to the Crank-Nicholson method in 1 space dimension. The resulting matrix for the linear system was sparse. As a result, efficient solutions for \( V_m \) were achieved with a preconditioned conjugate gradient scheme (DITSO-LCPG from the Digital Equipment Corp Digital Extended Math Library, dmxl). Integration time steps were dynamically adjusted during the calculations between minimum (\( dt_{\text{min}} \)) and maximum (\( dt_{\text{max}} \)) values to take advantage of
slowly changing characteristics of \( V_m \) after the depolarization wave-front had spread through the model. Adjustment followed a variation of the method of Rush and Larsen.\(^{3,26} \) Equations 4 and 5 were also discretized in space over the full model using a 5-point finite difference stencil. Matching conditions at the interface between the tissue and the bath were embedded in the difference equations. During the parts of each simulation at which we applied "shocks," we modified the difference equations for nodes on the left and right edges of the model to fix the extracellular potentials on each edge. The resulting sparse linear system for \( \phi_r \) and \( \phi_t \) was solved using the same preconditioned conjugate gradient method as in solutions for \( V_m \).

To represent the electrophysiological state within the papillary muscle preparations, we simulated a train of 10 action potentials initiated with 200 \( \mu A/cm^2 \) strength transmembrane current pulses of 2.0-ms duration applied at the leftmost tissue nodes. The S1–S1 interval measured 300 ms. Action potential waveform shapes and durations were constant after the fourth beat. Because the bulk tissue and the bath were embedded in the difference equations, the duration of the zero-potential crossing, only hyperpolarization in the direction of the zero-potential crossing, near the center of the tissue, as shown in Figure 2. Two shock strengths, 2.2 V/cm (panel A) and 6.8 V/cm (panel B), with both shock polarities were applied to the papillary muscle. When the shock polarity was cathodal on the left side of the tissue and anodal on the right side of the tissue (upper tracings in each panel), the left side of the tissue was depolarized and the right side was hyperpolarized with 1 zero-potential crossing present at the site 100 \( \mu m \) to the right side of the center of the tissue. \( \Delta V_m \) in response to the 6.8-V/cm shock underwent a polarization shift from 54 mV of depolarization to \(-111 mV \) of hyperpolarization (total 165 mV) across the tissue length of 2.2 mm (upper tracing in panel A). When the shock polarity was reversed with the anode on the left side of the tissue and the cathode on the right side of the tissue (lower tracing in each panel), the direction of \( \Delta V_m \) was also reversed, with hyperpolarization on the left side of the tissue and depolarization on the right side of the tissue. \( \Delta V_m \) in response to the 6.8 V/cm shock underwent a polarization shift from \(-102 mV \) of hyperpolarization to 64 mV of depolarization (total 166 mV) across the tissue length of 2.2 mm (lower tracing in panel B). The magnitude of the \( \Delta V_m \) over space in response to the 2.2 V/cm shock underwent a polarization shift from \(-54 mV \) of hyperpolarization to 49 mV of depolarization (total 103 mV) across the tissue length of 2.2 mm (lower tracing in panel A).

Results

\( \Delta V_m \) Caused by Shocks in the Macroscopic Group

In the macroscopic group, which had adjacent recording sites spaced 200 \( \mu m \) apart, the portion of the tissue toward the anode was hyperpolarized, whereas the portion toward the cathode was depolarized. There was only 1 zero-potential crossing near the center of the tissue, as shown in Figure 2. Two shock strengths, 2.2 V/cm (panel A) and 6.8 V/cm (panel B), with both shock polarities were applied to the papillary muscle. When the shock polarity was cathodal on the left side of the tissue and anodal on the right side of the tissue (upper tracings in each panel), the left side of the tissue was depolarized and the right side was hyperpolarized with 1 zero-potential crossing present at the site 100 \( \mu m \) to the right side of the center of the tissue. \( \Delta V_m \) in response to the 6.8-V/cm shock underwent a polarization shift from 54 mV of depolarization to \(-111 mV \) of hyperpolarization (total 165 mV) across the tissue length of 2.2 mm (upper tracing in panel A). When the shock polarity was reversed with the anode on the left side of the tissue and the cathode on the right side of the tissue (lower tracing in each panel), the direction of \( \Delta V_m \) was also reversed, with hyperpolarization on the left side of the tissue and depolarization on the right side of the tissue. \( \Delta V_m \) in response to the 6.8 V/cm shock underwent a polarization shift from \(-102 mV \) of hyperpolarization to 64 mV of depolarization (total 166 mV) across the tissue length of 2.2 mm (lower tracing in panel B). The magnitude of the \( \Delta V_m \) over space in response to the 2.2 V/cm shock underwent a polarization shift from \(-54 mV \) of hyperpolarization to 49 mV of depolarization (total 103 mV) across the tissue length of 2.2 mm (lower tracing in panel A).

Results in Figure 2 are consistent with results from the other 4 muscles in the macroscopic group. Figure 3 shows the magnitude of the \( \Delta V_m \) caused by the shock over space for all 5 muscles in the macroscopic group. Zero-potential crossings were observed in the center region of the tissue about 200 \( \mu m \) to the right side of the center, whereas, away from the region of the zero-potential crossing, only hyperpolarization in the portion toward the anode or depolarization toward the cathode was present. The direction of \( \Delta V_m \) changed when the shock polarity was reversed. The gradient of changes in the magnitude of \( \Delta V_m \) in response to 6.5\( \pm 0.6 \) V/cm was 166\( \pm 23 \) mV/2.2 mm for the shock polarity of L–R+ and 166\( \pm 34 \) mV/2.2 mm for the shock polarity of L+R–. The magnitude of the shock-induced hyperpolarization was significantly greater than that of depolarization at the same recording sites when the same shock strength was applied (\( P<0.05 \)). The response of the membrane to a shock field of 6.5\( \pm 0.6 \) V/cm...
was greater than that to a shock field of 2.1 ± 0.2 V/cm at all recording sites (P < 0.05). The ratio of the \( \Delta V_m \) for 6.5 V/cm to 2.2 V/cm shocks was 1.95 ± 0.56, demonstrating that tripling the shock strength did not triple the \( \Delta V_m \).

\[ \Delta V_m \] Caused by Shocks in the Microscopic Group

In the microscopic group, the transmembrane potential was recorded at 21 sites with 20 \( \mu \)m between adjacent sites in each papillary muscle. The shock strength used in this group was 6.2 ± 0.4 V/cm. Figure 4 shows an example of the transmembrane potential recordings within a space of 0.4 mm from the central region of 1 papillary muscle. During cathodal stimulation on the left side of the tissue and anodal stimulation on the right side of the tissue (upper tracings in Figure 4), the portion of the recorded tissue nearest the cathode was depolarized, whereas the portion nearest the anode was hyperpolarized. As in the macroscopic group, there was only 1 zero-potential crossing present near the recording site, 120 \( \mu \)m to the right side of the center of the tissue. Changing the shock polarity reversed the direction of the \( \Delta V_m \) and moved the zero-potential crossing near the recording site at 140 \( \mu \)m to the right side of the center of the tissue (lower tracings in Figure 4). Figure 5 shows another example of the transmembrane potential recordings within the space of 0.4 mm in the right portion of the tissue starting at the recording site 600 \( \mu \)m away from the center of the tissue. One shock polarity caused hyperpolarization at all recording sites without a zero-potential crossing present in the space of 0.4 mm (lower tracings in Figure 5).

Figure 6 shows the changes in the transmembrane potential caused by the shock over space in the microscopic group for each of the 5 papillary muscles. Recordings were made in the central regions in 3 muscles (labeled −200, −100, and −100 with respect to the center of the tissue in Figure 6), in the right portion of the tissue in 1 muscle (labeled 600 with respect to the center of the tissue in Figure 6), and in the left portion of the tissue in another muscle (label −700 with respect to the center of the tissue in Figure 6). As shown in Figure 6, no zero-potential crossing was recorded in either the left or right portion of the tissue. Only 1 zero-potential crossing was recorded in the central region of the tissue. Changing the shock polarity reversed the direction of the \( \Delta V_m \) during the shock. Although no zero-potential crossing was recorded away from the central region of the tissue, a jump in the transmembrane potential of >10 mV between 2 adjacent recording sites was sometimes observed, raising the possibility of nonuniform intracellular conductivity between adjacent recording sites.

**Computer Simulations**

The computer simulations closely mimicked the experimental preparation and stimulation protocol. Results from these simulations are presented in Figure 7. Figure 7A shows the spatial distribution of \( \Delta V_m \) after subthreshold stimuli by current injection at the left end of the model. With all tissue in the model at rest initially, \( \Lambda \) measured 362 \( \mu \)m, which was close to that reported for frog ventricular myocardium by Chapman and Fry\textsuperscript{27} at \( \approx 300 \mu m \) but much less than that reported for skinned calf trabeculae by Weidmann\textsuperscript{28} at \( \approx 880 \mu m \). During the action potential plateau, the space constant was longer than at rest.
From Figure 7A, $\lambda_p$ measured 738 $\mu$m as determined by current injection at a 30-ms S1–S2 coupling interval.

Shocks applied at an S1–S2 coupling interval of 30 ms during the action potential plateau affected the spatial distribution of $\Delta V_m$ across the model. Figure 7B shows $\Delta V_m$ for shock strengths of 0.6, 1.5, and 3.0 V/cm. Field strengths were determined by dividing the potential difference recorded at the ends of the model by the total model length. As a result, these field strengths were lower than the ones used in the experiments. Nevertheless, even at low field strengths, $\Delta V_m$ was positive at all model nodes toward the cathode and negative at all nodes toward the anode. In the central 2 mm of the model (Figure 7C), which was the sampled region referred to as the macroscopic group in the experiments, $\Delta V_m$ changed by 48 mV ($+24$ to $-24$ mV) during L+R− stimulation and during L−R+ stimulation ($-25$ to $+23$ mV) with a 1.5-V/cm shock field strength. $\Delta V_m$ was as high as 5 mV near the center of the model. From Figure 7D, a zone of 0 $\Delta V_m$ present for a distance along the tissue was not even observed at the lowest shock strength of 0.6 V/cm.

**Discussion**

This study demonstrates experimentally in the papillary muscle of the guinea pig that the response to electric field stimulation consists of hyperpolarization in the portion of the tissue facing the anode and depolarization in the portion facing the cathode with 1 zero-potential crossing near the center of the tissue.

Cardiac muscle is a syncytium, the morphology of which is anisotropic and the electrophysiological properties of which depend on the cell membrane, cell-to-cell coupling, and the properties of the extracellular and intracellular volume conductors. Therefore, the response of simple resistor-capacitor membrane models, 1-dimensional cable models, and cultured cell strand preparations to field stimulation may have limited applicability to defibrillation of the intact ventricles. For example, our previous studies have indicated that the relationship between the shock potential gradient and the $\Delta V_m$ caused by that gradient is much more complicated than the relationship predicted by mathematical models.2,12,13

The present study uses papillary muscles from the left ventricle, which have a unidirectional fiber orientation. That geometry simplified the complex relationship between the response of the membrane during a shock and fiber orientation for this study. Thus, our results approximate the $\Delta V_m$ caused by a shock in 1-dimensional tissue, which has been the focus in previous mathematical modeling studies of defibrillation.14–16,29,30 Because our results are obtained from real cardiac tissue characterized by a multicell syncytium connected by gap junctions between cells, they should offer some information, although not all information, about the $\Delta V_m$ caused by extracellular electric field stimulation in myocardium and, hence, about the mechanism of defibrillation.

Knisley et al1 found that in a single isolated ventricular cell with a length of 119±29 $\mu$m, the end of the cell that faces the...
cathode during field stimulation is depolarized, while the other end facing the anode is hyperpolarized. A zero-potential crossing is present in the middle region of the cell separating the regions of depolarization and hyperpolarization. This phenomenon is referred to as a sawtooth pattern. According to sawtooth pattern models, the depolarization-hyperpolarization oscillation at each cell is established by the intracellular periodic conductivity caused by high gap-junction resistance. Because an isolated myocyte has high resistance at the sealed ends, depolarization at one end of the cell and hyperpolarization at the other end of the cell can be established during an electric shock field as observed in experiments. But, because a single cell can also be treated as a 1-dimensional cable, the same phenomenon can be effectively modeled with the cable equation or with the bidomain model. To test whether the sawtooth pattern exists in multicellular syncytia with gap junctions between cells, Gillis et al used optical recording techniques to record the $\Delta V_m$ caused by a shock field from a monolayer of cultured cardiac cells. The length of cell strands in the study by Gillis

![Figure 4](image1.png)

**Figure 4.** Transmembrane potential recordings during the shock over space in the central region of the tissue in 1 papillary muscle from the microscopic group. L and R, Left and right sides of the recorded region with respect to the center of the tissue. Numbers represent the distance in $\mu$m between the recording site and the center (0 $\mu$m) of the tissue. L-R+ represents shock polarity, with the cathode on the left side of the tissue and the anode on the right side; L-R− represents the reversed shock polarity. The shock strength is 6.6 V/cm. The bottom 2 tracings are the continuation of the top 2 tracings. Voltage and time scales are shown at the bottom right.

![Figure 5](image2.png)

**Figure 5.** Transmembrane potential recordings during the shock over space in the right portion of the tissue in 1 papillary muscle from the microscopic group. L and R, Left and right sides of the recorded region with respect to the center of the tissue. Numbers represent the distance in $\mu$m between the recording site and the center (0 $\mu$m, not shown) of the tissue. L-R+ represents the shock polarity with cathode on the left side of the tissue and anode on the right side; L-R− represents the reversed shock polarity. The shock strength is 6.3 V/cm. The bottom 2 tracings are the continuation of the top 2 tracings. Voltage and time scales are shown at the bottom right.
et al\textsuperscript{5} was 2 to 3 mm. A local depolarization-hyperpolarization oscillation was not observed at the borders between cells in that study.\textsuperscript{5} Instead, hyperpolarization occurred in the portion of the cell strand nearest the anode and depolarization in the portion nearest the cathode, similar to the results of our study in the papillary muscle.

In the macroscopic group of the present study, recordings were made over a total length of 2.2 mm with 200 μm recording resolution in the middle portion of the papillary muscle. The recorded length in the microscopic group was 0.4 mm, with a 20 μm recording resolution in either the central or lateral portion of the papillary muscle. Because the length of a cardiac cell is about 100 μm and the long axis is parallel to the papillary muscle,\textsuperscript{18} zero-potential crossing should have been recorded in either the middle or lateral portion of the tissue in both the macroscopic and microscopic groups if the sawtooth pattern were present. Yet, only 1 zero-potential crossing was observed in the present study. This implies that either the model of the sawtooth pattern is incorrect or that each sawtooth oscillation occurs over a bundle of cells >2 mm long instead of over a single cell.\textsuperscript{32}

The model study of Plonsey and Barr\textsuperscript{14} indicated that the magnitude of the depolarization-hyperpolarization oscillation of the sawtooth pattern depends on the resistance of the gap junctions between cells in addition to the shock strength. These authors showed that the depolarization-hyperpolarization oscillation of the sawtooth pattern is largest when a large gap-junction resistance is chosen.\textsuperscript{14} Therefore, if the resistance in the gap junction of the normal syncytial tissue is not very large in the real myocardium,\textsuperscript{33,34} the sawtooth pattern may be very small and not observed or may be superimposed on other membrane responses.\textsuperscript{15} As shown in Figure 6, the curves of the ΔV_m caused by the shock were not very smooth over short distances, and sometimes an obvious voltage difference between 2 adjacent recording sites was observed. This finding might be caused by gap junctions. Thus, if a sawtooth pattern exists, its amplitude may be small and superimposed on a larger pattern of transmembrane potential changes, as observed in this study.

The following considerations also suggest that the depolarization-hyperpolarization oscillation of the sawtooth pattern is not the primary mechanism for defibrillation. (1) If the sawtooth pattern exists and the magnitude of depolarization equals that of hyperpolarization, the ΔV_m during a shock should be summed to 0 when a single averaged measurement of transmembrane potential is made from many cells over a large space. However, both depolarization and hyperpolarization have been recorded by using optical recording techniques.\textsuperscript{2,6–8} (2) Defibrillation efficacy depends on the shock polarity for some waveforms,\textsuperscript{35} suggesting that the response of ventricular myocardium is not symmetrical as predicted by the sawtooth pattern. (3) Excitation latency of the ventricles with field stimulation is much shorter for a depolarizing shock than for a hyperpolarizing shock of the same strength,\textsuperscript{2} and this difference in excitation latency (∼10 ms) is much larger than the time required for activation at one end of a cell to propagate to the other end of the cell.\textsuperscript{2} Taken together, these findings suggest a minimal contribution of gap-junction resistance to successful defibrillation.

The cable equation model and the bidomain model, which is equivalent to the cable equation when a 1-dimensional strand is used,\textsuperscript{17} both predict that the tissue beneath the anodal electrode undergoes hyperpolarization while the tissue beneath the cathodal electrode undergoes depolarization, and
that the magnitude of such exchanges in the transmembrane potential decreases with distance away from the stimulating electrode.\textsuperscript{17,31,36} The computer simulations in the present study used a bidomain model. Results were consistent with the prediction of a single zero crossing and also with the experimental results. Because the simulation included active ionic channels defined by the LRd membrane equations,\textsuperscript{22} it was possible to apply shocks during the action potential plateau, mimicking the experimental protocol. Results from this simulation demonstrated that the space constant during this part of the action potential greatly exceeded the resting space constant (Figure 7A). Therefore, membrane resistance is perhaps greater in importance than is axial resistance in predicting shock-induced $\Delta V_m$. Furthermore, the strong qualitative fit between the experimentally observed spatial distribution of $\Delta V_m$ and the simulated $\Delta V_m$ from a model with no resistive discontinuities supports our assertion that the sawtooth pattern plays a minimal role in defibrillation. Even at low shock strengths, as shown in Figure 7D, all nodes on the anodal side of the model hyperpolarized and all nodes on the cathodal side of the model depolarized.

The primary goal of the simulation reported here was to provide an explanation for the point of zero crossing in shock-induced $\Delta V_m$ that we observed experimentally. As a result, lower shock strengths were used in the simulation than in the experiments. Despite this difference, the qualitative fit between the model and experimental data was strong. However, the simulation did fail to reproduce (1) an asymmetric $\Delta V_m$ response with greater hyperpolarization than depolarization at all shock strengths and (2) the relatively sharp transition in $\Delta V_m$ at the zero-crossing point. Although these differences may simply be a consequence of the lower field strength used in the simulations, they may also be related to the membrane equations themselves. The equations are based, primarily, on data from voltage clamp experiments using isolated cells. As a result, responses of individual ion channels at the large $dV_m/dt$ associated with shocks may not be complete, suggesting the need for further study.

The limitations of the present study are the following:

1. Shock potential gradients: The spatial recordings of the transmembrane potential during the shock were obtained with a resolution as fine as $20 \mu m$, whereas the shock potentials were recorded only by a single pair of extracellular recording electrodes in the solution. Therefore, the distribution of the shock potential throughout the tissue, especially the shock potential gradients at each microelectrode recording site, was not known in the present study. However, we speculate that the shock potential distribution is fairly even in the microscopic region, because (1) the shock potential field in the present study was created by 2 large patch shock electrodes across the entire tissue bath and (2) the size of the papillary muscle was much smaller than the size of the shock electrodes and the tissue bath.

Figure 7. Simulated $\Delta V_m$ distribution. A, Normalized $\Delta V_m$ distribution after subthreshold current injection that depolarized the left end of the tissue portion of the model. The length constant was greater for the plateau than for the resting state. B, $\Delta V_m$ across the model after S2 shocks generating end-to-end fields measuring 3.0, 1.5, and 0.6 V/cm. L+R− shocks are drawn as solid lines. L−R+ shocks are drawn as dashed lines. C, $\Delta V_m$ within the $−1000$–$1000-\mu m$ region defined as “macroscopic” in the experiments. Field strengths measured 1.5 and 0.6 V/cm. D, $\Delta V_m$ within $−350$ to $350-\mu m$ of the center of the model during S2 of 0.6 V/cm. The arrow shows the center of the model. Note that a single, discrete zero crossing was present without a region of zero $\Delta V_m$ along the tissue. In A through D, horizontal units are distance in $\mu m$, and vertical units are mV. In B through D, the zero-mV line is marked with a horizontal line in the middle of each graph.
2. One- versus 3-dimensional tissue: Although the papillary muscle has a uniform fiber orientation and the results obtained from the present study are consistent with those from simulation in 1-dimensional tissue, a 4-mm-long and 1.5-mm-wide papillary muscle still has a characteristic 3-dimensional structure with respect to 20-μm cell diameters. In addition, the data of the present study were obtained when the electric field was parallel to the longitudinal axis of the papillary muscle and hence along the fiber orientation. Thus, the results are likely to represent a special case with regard to the direction of the shock field, the structure, and connective tissue distribution and may be different in more complicated 3-dimensional myocardium, which has a nonuniform fiber orientation. In addition, lateral gap junctions exist between 2 adjacent cells so that lateral averaging effect may occur to affect a local ΔVm caused by a shock. Although a double-barrel microelectrode records the end result, it may not be able to elucidate a lateral averaging effect if it exists.

3. Location of the recording sites within the cardiac cells: Although the recording resolution in the present study was 20 μm in the microscopic group, it was not known where the impalements were located, ie, near the end or near the center of a cell, or whether consecutive impalements were made within the same cell or in different cells. Therefore, the results cannot directly elucidate the phenomena occurring within each cell. However, the results of the present study still offer information for evaluation of the mathematical models. Statistically, the total recording length (0.4 mm) in the microscopic group should cover several cells. Therefore, with a resolution of 20 μm between 2 adjacent recording sites, the probability of recording depolarization during a shock should be almost the same as that of recording hyperpolarization with several zero-potential crossings if the sawtooth pattern existed. Yet, we did not observe this pattern.

4. Space constant versus the recording region: When electric stimulation is locally applied to the cardiac tissue, the stimulated region undergoes either depolarization or hyperpolarization or both. The response of the membrane is largest near the stimulating region, and the magnitude of the response decreases with distance, indicating the existence of a space constant that has been shown to be about 1 mm in cardiac tissue.28 The sawtooth pattern has been predicted to be superimposed on the local transmembrane potential changes near the stimulating sources and to become more obvious away from the sources.19 The recordings in the present study were made in the middle region, about 1 mm away from the end of the tissue. This means that the recordings were obtained in the region between 1 and 2 space constants from the sources. Thus, measurements were not made more distant from the electrodes, where the sawtooth pattern may have been more obvious. However, as shown in the Appendix, the depolarization-hyperpolarization oscillation of the sawtooth pattern is not observed in the central region of a 20-mm-long fiber, where the measurements were made far beyond several length constants.

5. Effects of diacetyl monoxide: Diacetyl monoxide has been used in the myocardium to eliminate the effect of motion on the optical recordings. Whereas several studies have documented no significant effects of 10 mmol/L diacetyl monoxide on the electrophysiological properties of cardiac tissue,37,38 other studies have shown an electric uncoupling effect of diacetyl monoxide.39 Although the present study did not investigate the effects of diacetyl monoxide on the cardiac electrophysiological properties, the sawtooth pattern would have been more easily observed if 10 mmol/L diacetyl monoxide had caused electric uncoupling in papillary muscles. Thus, the conclusion of the present study that the sawtooth pattern is small or absent is not influenced by using 10 mmol/L diacetyl monoxide.

**Appendix**

The purpose of the study described in this Appendix is to verify that the double-barrel microelectrode can record a change in the local transmembrane potential caused by a discrete, small region of increased intracellular resistance, ie, the sawtooth pattern. Recordings were made from the median giant fiber in the ventral nerve cord of the earthworm. This animal model was chosen because the axons in the median giant fiber are extremely large, so that (1) the location of each tip of the double-barrel microelectrode can be seen with a dissecting microscope to verify that one electrode is within the axon while the other is outside of it, and (2) the intracellular space of a single axon can be interrupted by constricting it at a discrete location with a suture and recording a known distance away from this constriction with a double-barrel microelectrode. The median giant fiber consists of a chain of about 100 median giant axons with electric connections by gap junctions called septa, which have an averaged resistance of 38 KΩ.40

**Materials and Methods**

Adult Lumbricus terrestris earthworms (Carolina Biological Supply, Burlington, NC) were anesthetized by immersion for 15 minutes in 0.2% (wt/vol) chlorobutanol (Chloroone). A 20- to 30-mm-long ventral nerve cord was carefully isolated from the earthworm; pinned, ventral side down, on a silicon rubber within a chamber 10 mm wide, 40 mm long, and 8 mm deep; and filled with earthworm saline (in mmol/L) NaCl 100, KCl 1.6, CaCl2 1.8, and NaHCO3 1.2, pH 7.3 to 7.4). The earthworm saline also contained 50 to 100 μmol/L carbachol to stop contractions of the ventral nerve cord. Two patch shocking electrodes (8×10 mm) were placed on opposite sides of the chamber so that the shock field vector was parallel to the longitudinal axis of the ventral nerve cord (Figure 8). Under the dissecting microscope with ×80 magnification and a view field 2.5 mm in diameter (Leeds Microscope, Leeds Precision Instruments, Inc.), the median giant fiber was clearly seen with 2 lateral giant fibers on each side. The median giant axon could be identified approximately by their branches.40 The median giant fiber was penetrated by a double-barrel microelectrode with a 100- to 150-μm intertip distance. Because the diameter of the median giant axons is approximately 5 times greater than that of myocardial fibers, the intertip distance was also increased by a factor of 5 to perform a comparable test of the ability of the double-barrel microelectrode to record local changes in the transmembrane potential. The recording technique was the same as that described in Materials and Methods of the main text. In 2 earthworm experiments, optical recordings were used to detect the ΔVm during a shock simultaneously from 8 sites along a 2-mm region of median giant fiber stained with di-4-ANEPPS. The diameter of each laser spot was approximately 40 μm, and the sampling rate was 8 KHz. The optical recording techniques are the same as those reported previously.8

The double-barrel microelectrode was slowly lowered into the saline just above the tissue with a motorized micromanipulator. It was then rotated slightly until the potential difference between the 2 tips was almost undetectable on the monitoring oscilloscope during a 10-V shock. The double-barrel microelectrode was next lowered into the tissue until an action potential was seen. The transmembrane potential was recorded from a total of at least 6 sites within a 2-mm region of the central portion of a median giant fiber (Figure 8). At
least 2 recording sites were made within a single median giant axon. A total of 8 ventral nerve cords were used, 2 in each protocol. In protocol 1, one end of the 2-mm region was tied by a 0.05-mm silk thread with the other end open (Figure 8A). In protocol 2, both ends of the 2-mm region were tied (Figure 8B). In protocol 3 (consisting of protocol 3a, using double-barrel microelectrode recordings, and protocol 3b, using optical recordings), both ends of the 2-mm region were first left open followed by a tie at the center of the 2-mm region. (Figure 8C and 8D). In the electric recording experiments, a square wave shock with a 50-ms duration and at a strength of 1 V below the excitation threshold was applied for all recordings from all sites. In the optical experiments, a 35-mA square wave shock of 30-ms duration was applied during all recordings.

Results and Discussion

The length between 2 adjacent branches, which is approximately the length of a single axon, was 0.79 ± 0.06 mm (n = 13), and the width was 0.064 ± 0.01 mm (n = 8). In protocol 1 (Figure 8A), the ΔV_m caused by a 5-V shock decreased with distance from the tied site. The results demonstrate that the polarization at the tied site of the median giant fiber caused by an electric shock can be recorded with the double-barrel microelectrode. In protocol 2 (Figure 8B), in which a 6-V shock was given, a small hyperpolarization was recorded near the tied site facing the anode (tracing a in panel B), and a small depolarization was recorded near the tied site facing the cathode (tracing f in panel B). One zero-potential crossing was observed in the middle region.

In protocol 3 (Figure 8C and 8D), no ΔV_m during a 4-V shock with electric recordings (tracings a through f in Figure 8C) or during a 35-mA shock with optical recordings (tracings a through h in Figure 8D) was observed in the central 2-mm region. After the center of this 2-mm region was tied (dotted crossing lines in panels C and D), depolarization was observed to the left of the tie (tracings L1 and L2 for electric recordings and tracings a through c for optical recordings), whereas hyperpolarization was observed to the right (tracings R1 and R2 for electric recordings and tracings d through h for optical recordings).

Each protocol was performed twice under the same conditions in 2 different ventral nerve cords. The results were similar for both ventral nerve cords, suggesting that the protocol is repeatable.

Conclusion

The results are consistent with the predictions of mathematical models of secondary sources created by interruptions of the intracellular space in a core conductor model. These predictions include (1) depolarization on the side of the intracellular interruption closer to the anode and hyperpolarization on the side closer to the cathode, (2) a decrease in ΔV_m with distance away from the interruption, and (3) a decrease in ΔV_m caused by an intercellular interruption when another intracellular interruption is made nearby. The results from
the earthworm study demonstrate the double-barrel microelectrode as an effective tool for detecting such changes in transmembrane potential within a single cell.

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