Electric shock, the only effective treatment for ventricular fibrillation, was originally thought to be effective by temporarily suppressing cardiac electrical function, resulting in cessation of any activity including fibrillation for seconds until excitability is recovered. An alternative theory originally proposed by Gurvich and Yuniev suggested that a shock abolishes fibrillation by "synchronization of separate heart elements." However, Gurvich and Yuniev acknowledged that defibrillation had a suppressive effect on cardiac function, which may be injurious and thus necessitated limiting the magnitude of shock current.

Clinical evidence suggests the existence of both excitatory and suppressive effects of shocks on the myocardium. Stimulation effects range from the induction of transient ectopy to tachycardia to the induction of ventricular fibrillation. Such stimulating effects also take place during defibrillation in intact heart models. Virtual electrode polarization may be one of the underlying mechanisms of these effects.

There is also evidence for depression of cardiac electrical and mechanical function after the delivery of shocks. Bradycardia, complete heart block, and increased pacing thresholds are known to occur, particularly at high energies. In addition, mechanical dysfunction (stunning) has been demonstrated in both the atria and ventricles and appeared to be directly related to the strength of shocks. Thus, both stimulating and inhibiting effects of electric shocks are believed to participate in defibrillation. However, their relative contribution to both proarrhythmic and antiarrhythmic effects remains controversial.

Electroporation is the disruption of the lipid matrix and the creation of aqueous pathways (electropores) in the cell membrane, resulting from the delivery of high-voltage electrical pulses. That the depressive effect of a strong electric shock via electroporation lasts for seconds has been demonstrated in both isolated cells and cardiac tissue preparations. However, the localization and spatial extent of electroporation in intact hearts remains unknown.

Electroporation is more likely to develop in sites with maximal shock-induced transmembrane polarization. The occurrence and amplitude of electroporation have been related to the external field gradient. Heterogeneity of myocardial structure has also been implicated in development of strong shock-induced transmembrane polarization. Thus, electroporation may develop in sites with maximal external field gradient as well as in sites with maximal structural heterogeneity.

We hypothesized that in normal heart the trabeculated endocardium might be most susceptible to electroporation as a result of its heterogeneous structure. In this study we used optical mapping techniques to assess and compare the endocardial and epicardial cellular responses to high-energy
shocks so that we might delineate the relationship between myocardial structure and electroporation and evaluate its effect on electrical activity.

Materials and Methods

Experimental Preparation

Experiments were performed in vitro on rabbit coronary-perfused cardiac preparations \((n=27)\). The techniques have been described previously\(^{10,31}\) and will be restated briefly. Rabbits were anesthetized by sodium pentobarbital \((50 \text{ mg/kg})\). The heart was removed and Langendorff-perfused. Average heart weight was \(7.5\pm0.7 \text{ g}\). The temperature and \(pH\) in the chamber were maintained at \(37\pm0.5^\circ\text{C}\) and \(7.30\pm0.05\), respectively. The chamber was filled with Tyrode’s solution to cover the heart and the active areas of the defibrillation electrodes. Two types of electrodes were used to deliver shocks. Coil defibrillation electrodes \(10 \text{ mm} \times 2 \text{ mm}\) in diameter were used to deliver electroporation shocks. A pair of mesh \(35\times95\text{ mm}\) electrodes positioned at both sides of the heart were used to investigate the vulnerability.

The experimental preparation was stained by bolus injection of \(350 \mu\text{L}\) of a \(2 \text{ mmol/L}\) solution of di-4-ANEPPS (Molecular Probes) in DMSO (Fisher Scientific). Movement artifacts were suppressed by \(15 \text{ mmol/L}\) 2,3-butanedione monoxime \((\text{BDM, Sigma})\) added to perfusate. In 6 experiments, measurements were conducted before and after administration of BDM.

Experimental Protocol

In the first group \((n=10)\), measurements were performed first in intact hearts at the epicardium and then after dissection at the endocardium. The field of view ranged from \(4\times4 \text{ to } 12\times12 \text{ mm}\). In the second group \((n=9)\), the measurements were focused on the conduction in papillary muscles and septum and thus were performed in dissected preparations only \((n=10 \text{ papillary muscles})\). In the third group \((n=8)\), vulnerability was evaluated in intact hearts.

The dissection procedure in the second group was performed after staining and perfusion with BDM. The right ventricular free wall was dissected along its septal border, sparing its basal septal attachments to protect the right coronary blood flow. The free portion was then lifted up to expose the right side of the interventricular septum. At least \(1 \text{ septal papillary muscle } 1.1\pm0.3 \text{ mm}\) in diameter was selected for mapping. An area of \(4\times4 \text{ or } 5\times5 \text{ mm}\) was mapped so that the base and most of the papillary muscles constituted at least a third of the mapped surface, along with an area of the septum on 1 or either side of it. An active fixation bipolar pacing electrode \((\text{Medtronic, Inc})\) was fixed to the basal septum. The heart was paced at a cycle length of \(300 \text{ ms}\).

One of the shocking leads was placed in the right or left ventricular cavity. The other lead was placed in the bath on either side of the heart with an approximate interelectrode distance of \(2 \text{ cm}\). Monophasic shocks were applied \(50 \text{ and } 100 \text{ ms}\) after the onset of the last basic beat stimulus by a defibrillator \((\text{VHS-02, Ventritex})\). Truncated exponential monophasic shocks were \(8 \text{ ms}\) in duration. A total of \(6 \text{ to } 10 \text{ monophasic shocks starting with } 50 \text{ V and incrementing by } 50 \text{ V (up to a maximum of } 500 \text{ V), both anodal and cathodal and at both coupling intervals, were applied. A period of } 3 \text{ minutes or more was allowed between successive shocks. We measured impedance for different distances between electrodes and different locations of electrodes. An increase of distance between the } 2 \text{ electrodes placed in the bath outside of the heart from } 0.5 \text{ to } 4 \text{ cm resulted in an increase of impedance from } 45 \text{ to } 70 \Omega. \text{ Similar measurements conducted with } 1 \text{ electrode inside the right and left ventricles and with the reference electrode outside of the heart resulted in a range of impedances of } 60 \text{ to } 85 \left(70 \text{ to } 85 \Omega, \text{ respectively, for distances } 0.5 \text{ to } 4 \text{ cm.}

Optical Mapping Techniques

A previously described\(^{32}\) imaging system was used in our experiments. The magnification was adjusted to focus on an area from \(250\times250 \text{ to } 312\times312 \mu\text{m}\) per diode. The entire field of view ranged from \(4\times4 \text{ to } 5\times5 \text{ mm}\) depending on the size of the papillary muscle. Mapping of intact hearts was performed from a field of view up to \(12\times12 \text{ mm}\).

Each scan contained 1 to 3 seconds of data sampled at 1 or 2 kHz. When electroporation was observed, longer scans that allowed observation of recovery were performed at a lower frame rate.

Endpoint Definition

Electroporation was considered to have occurred if at least 10% reduction of one of the following parameters was observed in the first postshock beat compared with the beat preceding the shock, in any of the recorded 256 voltage signals: (1) resting membrane potential, (2) action potential amplitude, and (3) rate of rise \((dV/dt)\left(V\text{-V}\right)\) of the upstroke of the action potential.

Results

Evidence of Electroporation

Figure 1 shows a representative trace recorded during the delivery of a 500-V shock. As in microelectrode data of Moore and Spear,\(^{33}\) one can see a reduction of postshock optical action potential \((\text{OAP})\) amplitude and an upward shift in optical resting membrane potential. The lower trace shows postshock reduction of \((dV/dt)\text{max}\). All of these 3 effects were voltage dependent (see Figure 2). Such observations are consistent with previously reported effects observed in cardiac cells during strong electric shocks and associated with electroporation.\(^{14,16,33,34}\) Thus, we identified these observations as electroporation and will refer to them as such throughout the article.

Difference Between the Epicardium and Endocardium

A comparison of epicardial and endocardial electroporation revealed significant difference. Electroporation was detected in both the epicardium and the endocardium and was shock voltage dependent. However, endocardial electroporation was detectable at significantly lower voltages. Figure 2 shows...
typical examples of responses with maximal electroporation recorded from the endocardium and epicardium of the same heart. This observation was consistent in all 10 hearts in which we conducted measurements at both the epicardium and the endocardium. Electroporation thresholds were 229 ± 68 and 318 ± 84 V (P = 0.01) for the endocardium and the epicardium, respectively. It is interesting to note the lack of effect of shock polarity. The same figure shows that both anodal and cathodal shocks produced similar effects on postshock electrical activity.

In addition, the epicardial and endocardial surfaces had different spatial distributions of areas of electroporation. When the lead was in the right ventricle, epicardial electroporation was first evident near the shocking electrode. In contrast, endocardial electroporation was observed throughout the entire endocardium, with most of the effect at the bundles and papillary muscles, independent of the position of the electrode within the left ventricle or outside of the heart (see online Figure 1; data supplement available at http://www.circresaha.org).

This finding led us to hypothesize that endocardial trabeculae; small papillary muscles; and other bundles, including the bundle branches and the Purkinje network, might be most vulnerable to electroporation during defibrillation shocks. Thus, electrical activity of these structures might be selectively suppressed for seconds to minutes after the shock. To test this hypothesis, we conducted the next phase of the study with higher spatial resolution, concentrating on single papillary muscles with a field of view of 4 × 4 or 5 × 5 mm.

**Evidence of Shock-Induced Block in Papillary Muscles**

Using the techniques described above, we investigated the effect of electroporation on postshock electrical activity.

Figure 5 shows representative data recorded during a 300-V shock. All upstrokes recorded at the papillary muscle except the first postshock response had 2 components (Fig-
ures 3 and 5B). The left pair of maps in Figure 5C illustrates spread of activation in the right ventricular septum (upper map) followed by the activation of the papillary muscle (lower map). Application of the shock resulted in electroporation, as is evident from the depolarization of the optical resting membrane potential. In addition, the first postshock response had dramatically reduced amplitude as a result of the loss of its second component, which then recovered by the next beat (compare with Figure 5). The slow recovery of the amplitude of the first component of the upstroke illustrates recovery from electroporation at the septum. The lack of a second component and the all-or-none behavior can be explained by transient conduction block in the papillary muscle caused by selective electroporation of the papillary muscle. The second pair of maps in Figure 5C shows the activation pattern during the first postshock beat, which was present only at the septum. Conduction was restored in the papillary muscle during the second postshock beat (Figure 5C).

The online data supplement illustrates shock-induced block in the papillary muscle in greater details (see online movie 2 and online Figures 4 and 5, available at http://www.circresaha.org). Such a block was observed in all 10 papillary muscles. The duration of conduction block was voltage dependent. Table 1 summarizes the data.

An important finding of this study is the lack of any evidence of spontaneous arrhythmias (reentrant or focal) associated with electroporation of the endocardium or the papillary muscles.

**Antiarrhythmic Preconditioning With High-Energy Shocks**

The importance of the involvement of different bundles, including the Purkinje system, in the maintenance of arrhythmias is generally accepted.37,38 Our results suggest that the temporary incapacitation of such bundles after shock may suppress the genesis and maintenance of postshock arrhythm-
mias. We conducted a separate series of 8 experiments in intact hearts to test this hypothesis. In these experiments, we evaluated the hypothesis that strong electric shocks that are known to cause electroporation and temporary incapacitation of bundles would reduce vulnerability to arrhythmias provoked by a shock applied during the period of electroporation.

The protocol included uninterrupted pacing at a coupling interval of 300 ms. Initially, the lower limit of vulnerability, upper limit of vulnerability, and the defibrillation threshold (DFT) were determined using an up-down protocol. Determination of the lower limit of vulnerability was limited by the minimum output of the defibrillator, which was 50 V. Two shocks were applied 100 ms after the action potential upstroke. The first “preconditioning” strong shock was applied from the pair of coil electrodes. These anodal shocks were 1/3, 2/3, and in one experiment 3/3 DFT measured in each heart separately (DFT = 181.3 ± 45.8 V [n = 8]). Test shocks were applied from a separate pair of mesh electrodes using the average of the low and upper limits of vulnerability. Average voltage of test shocks was 72.9 ± 20.0 V (n = 105). Coupling interval between the 2 shocks was 1200 and 1500 ms.

Figure 6 shows that test shock without preconditioning resulted in arrhythmia in 94.8%. Most of these arrhythmias were sustained. Preconditioning with 1/3 DFT and 2/3 DFT shock reduced arrhythmia inducibility to 70% and 42.9%, respectively. Preconditioning with 3/3 DFT completely abolished the ability of the test shock to induce arrhythmia. Online Figure 6 (data supplement available at http://www.circresaha.org) provides representative optical recordings obtained during this study. Table 2 summarizes these findings. As is evident from these data, increment in preconditioning shock intensity resulted in a decrease in vulnerability to arrhythmias. It is interesting that such a decrease in vulnerability associated with 1/3 DFT and 2/3 DFT preconditioning is accompanied by an increase in nonsustained arrhythmias compared with the control. Such an increase may indicate that electroporation reduces the probability of shock-induced scroll wave to degenerate into sustained fibrillation, perhaps by reducing the mass of tissue participating in the conduction.

### Effect of BDM

Use of the excitation-contraction decoupler BDM made it possible to optically image the effects of shocks on myocardial

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**TABLE 1. Thresholds of Electroporation and Block in Papillary Muscles**

<table>
<thead>
<tr>
<th>PM No.</th>
<th>EP Threshold Voltage, V</th>
<th>PM Block Threshold Voltage, V</th>
<th>No. of Beats Blocked</th>
<th>Maximum Applied Voltage, V</th>
<th>No. of Beats Blocked</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>400</td>
<td>1</td>
<td>500</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>300</td>
<td>1</td>
<td>500</td>
<td>25</td>
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<td>3</td>
<td>350</td>
<td>470</td>
<td>1</td>
<td>530</td>
<td>6</td>
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<td>260</td>
<td>1</td>
<td>320</td>
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<td>500</td>
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<tr>
<td>10</td>
<td>250</td>
<td>300</td>
<td>1</td>
<td>400</td>
<td>8</td>
</tr>
</tbody>
</table>

EP indicates electroporation; PM, papillary muscle.
TABLE 2. Preconditioning With High-Intensity Shocks Reduced Vulnerability to Ventricular Fibrillation

<table>
<thead>
<tr>
<th>Relative Shock Intensity (× DFT)</th>
<th>Postshock Rhythm</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PR</td>
<td>NSVF</td>
<td>VF</td>
<td>Total</td>
</tr>
<tr>
<td>0</td>
<td>5.3</td>
<td>14.3</td>
<td>26.7</td>
<td>100.0</td>
</tr>
<tr>
<td>% within relative shock intensity</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% within postshock rhythm</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% of total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>×1</td>
<td>30.0</td>
<td>66.7</td>
<td>36.1</td>
<td>41.1</td>
</tr>
<tr>
<td>% within relative shock intensity</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% within postshock rhythm</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% of total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>×2</td>
<td>12.0</td>
<td>36.1</td>
<td>17.8</td>
<td>41.1</td>
</tr>
<tr>
<td>% within relative shock intensity</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% within postshock rhythm</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% of total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>×3</td>
<td>9.0</td>
<td>11.0</td>
<td>23.3</td>
<td>60.0</td>
</tr>
<tr>
<td>% within relative shock intensity</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% within postshock rhythm</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>% of total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Count indicates number of measurements from all 8 hearts in the third group; PR, paced rhythm; NSVF, nonsustained ventricular fibrillation; and VF, ventricular fibrillation.

Factors Contributing to Shock-Induced Transmembrane Polarization

The external electric field is capable of stimulating the myocardium through its ability to induce transmembrane polarization. Until recently, the electric field gradient has been considered the principal factor contributing to this effect. Yet recently, the electric field gradient has been considered the principal factor contributing to shock-induced polarization. The theoretical concept of a generalized activating function provides conceptual unification of both myocardial structure and the field gradient. Myocardial structure actively interacts with the field, producing local sources of current, known as the secondary sources, and resulting in virtual electrode polarization during point stimulation and during defibrillation shocks.

The seminal work of Weidmann demonstrated that a discontinuity in current flow arising at the boundary between myocardium and the bath results in strong transmembrane polarization, which exponentially decays with spatial scale known as the space constant. This effect is important during defibrillation, resulting in significant transmembrane polarization produced by shocks at the surface of the myocardium. Yet this effect does not affect the bulk of the myocardium, where fiber curvature or other syncytial heterogeneities are the likely causes of transmembrane polarization. Nevertheless, in the far field, Weidmann’s effect appears to produce transmembrane polarization of significantly stronger amplitude compared with that resulting from alternative mechanisms. Furthermore, the curvature of tissue surface may be an important factor modulating the effect demonstrated by Wiedmann. Theory predicts that smaller bundles with higher surface curvature are subject to stronger polarization by far-field stimulation when compared with their less-curved counterparts. Curvature and branching of such bundles is likely to further contribute to their polarization and electroproportion in the far field.

Implications of the Postshock Block of Electrical Activity in Bundles for Defibrillation: Is Electroproportion a Proarrhythmic or Antiarrhythmic Phenomenon?

The relative volume affected by the effect of Weidmann is not significant in large hearts, because it is confined proximal to the surfaces of myocardium. Yet because of the important role of some surface structures such as the Purkinje network in the genesis and maintenance of arrhythmias, this effect might still play an important role in defibrillation. Selective disruption and depolarization of these structures might have both proarhythmic and antiarrhythmic effects. Sustained depolarization of a bundle electrically coupled with the normal excitable myocardium can hypothetically result in abnormal repolarization and arrhythmogenesis via a focal mechanism. Alternatively, impairment of electrical activity in the Purkinje system and other conductive bundles might exclude them from participation in reentrant circuits. Berenfeld and Jalife recently suggested that the Purkinje system is an important factor in the maintenance of ventricular fibrillation. We demonstrated earlier that subthreshold stimulation applied at the Purkinje system terminates arrhythmic activity in trabeculated endocardial structures; selectivity transient impairment of electrical activity in endocardial bundles is caused by electroproportion; and electroproportion might transiently reduce myocardial vulnerability to arrhythmias, as is evident from the preconditioning effect of supra-DFT shocks on postshock vulnerability.
rhythms in the guinea pig heart. Thus, exclusion of such bundles may contribute to the antiarrhythmic effects of shocks. Which of the 2 effects plays the major role in clinical defibrillation remains to be determined. Our data support the latter rather than the former.

Role of Electroporation in Defibrillation

The success or failure of defibrillation therapy has usually been attributed to 1 or both of the following mechanisms: (1) success in extinguishing ongoing fibrillatory activity and (2) failure to reinitiate a new arrhythmia. Our data suggest that electroporation may be actively involved in both of these processes. Transient impairment of the conduction system may facilitate termination of ongoing fibrillation and reduce probability of virtual electrode-induced reentry to degenerate into sustained fibrillation. Demonstrated preconditioning with supra-DFT shocks indicates that such impairment may indeed be acutely antifibrillatory.

On the other hand, high-energy shocks are known to produce a permanent damage, perhaps associated with electroporation. This effect of electroporation may provide the substrate for arrhythmogenesis.

Limitations

Our model of defibrillation cannot be directly scaled to the human heart. Block of conduction was evident in all of the papillary muscles in the rabbit heart, yet the larger size of human papillary muscles might prevent this effect from occurring to the same extent. It appears that the space constant is a good estimate of the size of the affected papillary muscles and bundles, which could exhibit transient block after shock. If the thickness of the fiber is comparable to the spatial depth of electroporation, then the failure of conduction is more likely to occur in thinner, compared with thicker, bundles. The latter might be electroporated less and only at the surface while the large functional core is preserved where electroporation cannot reach. Thus, we suggest that the right and left bundles, as well as the entire Purkinje system in humans, are likely to be affected by the selective blockade associated with electroporation. Clinical evidence of post-shock bradycardia, asystole, and widening of QRS is consistent with such a hypothesis. Thus, despite the scale limitation, our model predicts that a similar mechanism may play an important antifibrillary role in clinical defibrillation.

As a result of limited resolution of experimental techniques, we are unable to provide direct evidence that pores were actually formed in cellular membranes during the shock, which would prove electroporation. It appears that such explanation to the detector. Because of this limitation, we did not attempt to quantify absolute levels of transmembrane voltage at which electroporation does occur.

Similarly, recordings of electrical activity from trabeculated structures at the endocardium are likely to pick signals from opposite sides of papillary muscles or fibers, which would undergo opposite polarization. The total signal will represent an average of opposite signals. Yet this average is unlikely to be 0, because shock-induced polarization is known to be highly polarity asymmetric with positive polarization = 1.5 to 2 times stronger than positive polarization for the same field amplitudes. Thus, an average of the 2 opposite polarizations is likely to be biased toward the negative polarization for both shock polarities as seen in Figure 2.

Accurate assessment of slow recovery of the resting potential after the shock was also unattainable as a result of low-frequency noise and AC coupling (τ = 30 seconds) of our instrumentation. In addition, electroporation might be irreversible in a part of the myocardium, which is also likely to contribute to the irreversible shift in the optical “resting potential.”

Acknowledgments

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The Role of Electroporation in Defibrillation

(AI-Khadra: Electroporation in defibrillation)

by

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Department of Cardiology, Cleveland Clinic Foundation

and

Department of Biomedical Engineering, Case Western Reserve University
Supplement figure and movie legends

**Online Figure 1.** Spatial distribution of electroporation produced by a 300-V shock in the proximity to a papillary muscle. See movie 1 illustrating the same data.

A. Raw traces recorded at the smooth septal surface (the upper trace) and the papillary muscle (the lower trace). Arrows show a time point at which the resting potential shown in panel B was measure.

B. Distribution of post-shock resting membrane potential. As evident from the map, electroporation was observed everywhere, but the maximal effect was at the single papillary muscle seen in the field of view. The same observation was reproduced in all 19 hearts of groups I and II (see methods).

C. The diagram of the preparation with a single large papillary muscle in the 8x8 mm field of view. Notice spatial correlation between the maximum of electroporation and location of the papillary muscle.

**Online Figure 2.** Two-layered conduction pattern in the proximity to a papillary muscle.

A. Raw optical recordings of action potential upstrokes from a 5x5 mm field of view containing a single papillary muscle dividing the field in halves in the middle. Dual-humped recordings mark the location of the bundle.

B. Activation isochronal maps (1ms) of the septum (the upper map) and of the papillary muscle (the lower map) are shown. Septal map was reconstructed using the only upstrokes in recordings from the septum (upper trace) and the first upstroke out of the two in the papillary recording (lower trace). Papillary muscle map was reconstructed using only the second upstroke in dual-humped recordings (lower trace).
**Online Figure 3.** Evidence of correlation between optical signal morphology and the direction of the activation. Mapping was conducted in a 5x5 mm field of view with a single papillary muscle located in the right hand side of it. Maps illustrate that the direction of impulse propagation across the septum with respect to that in the papillary muscle influences the morphology of OAP.

A. Activation isochrone map (4 ms) reconstructed during apical pacing. No dual-component morphology is evident at the papillary muscle (upper trace in panel B). Thus, no separate bundle map could be reconstructed.

B. Signal morphology in a single papillary muscle recording site during apical (the upper trace) and basal (the lower trace).

C. Septal activation map (4 ms) during basal pacing.

D. Papillary activation map (4 ms) continued from septal activation shown in panel C. Black color corresponds to recordings sites which produced only a single upstroke.

**Online Figure 4.** Dynamics of wavefront propagation during normal conduction and shock-induced block in the field of view shown in figure 5 of the manuscript. Each map shows a distribution of dV/dt recorded during a single 528 µs sample. Every 5th acquired frame is shown without averaging, such that there is 2.64 ms time difference between subsequent frames. Movie 2 shows all frames without skipping. Each frame shows dV/dt relative to (dV/dt)\text{max} recorded during the preceding normal beat. Thus, these maps visualize the upstrokes of propagating OAPs representing wavefronts.

A. Conduction of a normal action potential wavefront. The wavefront originates at the lower-left edge of the field of view. The wavefront then propagates under the papillary muscle, which can be inferred by its shadow in the upper row of frames and spreads across the RV septum, reaching the right edge of the field of view. At this point in time the base of the papillary muscle is activated (lower row of frames). Muscle branching near the base (see photograph...
of the field of view in figure 3 of the manuscript) caused a slowing of conduction near the point of bifurcation at the lower-left corner of the field of view.

B. Conduction of the 1st post shock beat: wavefront propagated across the septum, but is blocked in the papillary muscle. Shock application resulted in a decrease in dV/dt amplitude in the RV septum as evident from direct comparison of the last three frames in the upper row of panels A and B. More dramatic changes are evident in the papillary muscle. Comparison of the lower row of panels A and B shows that the bundle was not activated.

C. Recovery of conduction in the papillary muscle in the subsequent beat.

**Online Figure 5.** Prolonged block of conduction in the papillary muscle induced by a 500V shock.

A. Photograph of the preparation is shown. The field of view is selected with a box.

B. Optical traces recorded from the papillary muscle (the upper trace) and the septum (lower trace). Notice intermittent block of conduction in the upper trace post-shock.

A. Activation isochronal maps (1 ms) reconstructed from the two components of optical recordings (see text for detail).

**Online Figure 6.** Preconditioning with a strong electric shock reduced myocardial vulnerability to a pro-arrhythmic test shock. Traces were collected from the same site during "pre-conditioning" shock followed by a test-shock, which was pro-arrhythmic under control conditions (upper trace). Preconditioning reduced (1x DFT and 2xDFT) and eliminated (3 x DFT) pro-arrhythmic effect of the test shock.

**Online Movie 1.** Spread of activation in the papillary muscle. Animation of the data shown in figure 1. Optical action potentials were differentiated in order to show the spread of wavefronts of action potentials. Frame number is shown in the lower-right corner. Frames were acquired 528 µsec apart.
Online Movie 2. Spread of activation in the papillary muscle before and after the shock. Three beats are illustrated: pre-shock beat, 1st post-shock beat with block of conduction in the pupillary muscle, 2nd post-shock beat with recovery of conduction. Frame number is shown in the lower-right corner. Numbering starts from pacing pulse. Frames were acquired 528 µsec apart.
A. Normal activation

B. Papillary muscle block (first post-shock beat)

C. Recovery of conduction (second post-shock block)
Online Figure 6

No Shock

x1 DFT

x2 DFT

Test shock 300 msec

Preconditioning shock