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Experimental Methods

Ethical Approval

All experiments were approved by the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan, and the Department of Laboratory Animal Resources. All euthanasia was performed following the recommendations of UCUCA and DLAR-CHUA. All mother rats were either donated to the university “rat recycling program” for use by other investigators, or euthanized by placing the rat in a carbon dioxide filled chamber, followed by removal of a vital organ (e.g., heart). The neonatal rats were euthanized by quick decapitation.

Myocyte Isolation and Culture

Neonatal rat ventricular myocyte (NRVM) monolayers were created as previously described (Hou *et al.*, 2010), 23 litters were used in these experiments. Ventricles from 1-2 day old neonatal Sprague-Dawley rats (Charles River, Wilmington, MA) were aseptically removed then minced in calcium- and magnesium-free Hanks’ Balanced Salt Solution (HBSS). Sequential digestion in 0.06% trypsin (Roche Applied Science) and 0.15% pancreatin (Sigma) in HBSS at 36°C was used to further isolate cells. A two hour differential preplating period was used to reduce the presence of non-cardiomyocytes. Medium M199 (Cambrex) containing 10% fetal bovine serum (FBS) (Cellgro), 20 U/mL penicillin, 20 µg/mL streptomycin and 100 µmol/L bromodeoxyuridine to inhibit fibroblast proliferation (Sigma) was used to suspend and plate cells. Cells were plated on human collagen type IV (Sigma) coated 35 mm tissue culture dishes at a density of 1×10^6 cells per monolayer. Cells were kept at 37ºC, 5% CO_2. Media was changed after 24 and 48 hours using the same 5% FBS media. Magnetofection was performed on day 2 in culture. Experiments were conducted at an additional 48 hours, allowing for protein expression.

Magnetofection

Monolayers were infected using either an Ad-hERG-GFP created by Dr. Zhengfeng Zhou (Oregon Health and Science University) containing fused cDNA sequence of human ether-a-go-go-related gene (hERG) with green fluorescent protein (GFP) sequence or an Ad-GFP virus (Zhou *et al.*, 1998; Hou *et al.*, 2010). Only the α-subunit of the I_{Kr} channel was included in the virus. Each type of virus was first complexed to ViroMag Magnetofection nanoparticles (Boca Scientific, FL); a product normally used for increasing infection efficiency

(Gliddon *et al.,* 2008; Kadota *et al.,* 2005). 0.5 µl of ViroMag was complexed with $1x10^8$ viral particles in 200 µl HBSS (Sigma) for 15 minutes then raised to a final transduction volume of 2 ml per monolayer. As illustrated in Supplementary Figure S1A, each monolayer was exposed to a Super Magnetic Plate (Boca Scientific, FL) for 1.5±0.5 minutes in the presence of the magnetofection solution. Whole infected monolayers were placed entirely over the magnet while regionally infected monolayers were created by exposing only half of the lower surface area of the dish containing the monolayer to the magnet. The magnetofection solution was then removed and monolayers were washed twice with and returned to 5% FBS media and given an additional 48 hours to express protein. Uninfected controls were treated similarly using HBSS for 1.5±0.5 minutes. Determination of the ViroMag and virus concentrations as well as the duration of exposure to magnetic field were determined to be optimal when uninfected regions did not express GFP fluorescence while maintaining infection within infected region.

Optical Mapping

Monolayers were stained with potentiometric dye di-8-ANEPPS (40 µmol/L; Molecular Probes) for 15-25 minutes and then placed on a heating chamber connected to a temperature controller maintained at 37±1°C and continuously superfused with HBSS without bicarbonate (Sigma) containing (in mmol/L): 1.6 $CaCl_2$, 5.4 KCl, 0.8 $MgSO_4$, 0.4 KH_2PO_4, 4.2 $NaHCO_3$, 136.9 NaCl, 0.3 $NaHPO_4$, 5.5 D-Glucose, and 10 HEPES; pH 7.4 (NaOH). Quiescent monolayers received repetitive stimuli (duration, 5 ms; strength, twice diastolic threshold) applied by a thin extracellular bipolar electrode at increasing frequencies, starting at 1 Hz, until loss of 1:1 capture or initiation of sustained reentry. Five-second movies were obtained at 200 frames/s (LabWindows Acquisition) using an 80x80 pixel CCD camera (SciMeasure Analytical Systems; Decatur, GA). Signals were amplified, filtered, and digitized for offline analysis. No electromechanical uncouplers were used.

Optical Data Analysis

Dominant frequency (DF) maps were constructed by taking the Fast Fourier Transformation of the fluorescence signal at each pixel (Zaitsev *et al.*, 2000; Kalifa *et al.*, 2006). APD and conduction velocity (CV) measurements used average signals obtained from individual 5 second optical movies recorded during repetitive pacing at varying frequencies. Movies were filtered as previously described (Hou *et al.*, 2010). APD color maps were constructed by measuring the time between the upstroke and 50 and 75% of repolarization in each pixel. For CV measurements, activation times were calculated for each pixel, and local conduction vectors

were determined as described (Hou *et al.*, 2010). The activation ratio (AR; dubbed n:m ratio) is a ratio between the number of activations in the uninfected to the infected regions. Thus n:m was calculated as the number of activations in the distal or uninfected region of the monolayer (n) divided by the number of activations at the proximal or infected region (m). The number of activations in a given region was determined by three 1 second time-space plots (TSP) taken along parallel lines at ¼, ½ and ¾ of the way within each dish perpendicular to the gradient. Supplemental Figure S2C shows examples of TSPs taken at the ½ location. During rotor analysis in the wholly uninfected and infected monolayers, distal and proximal regions were arbitrarily assigned; in regional infections distal refers to uninfected region and proximal refers to infected regions. Average DF and RI and their respective standard error maps were created in Matlab (Mathworks, Natick MA).

Fluorescence Microscopy

Monolayers were imaged on day 4 in culture before optical mapping using a Nikon Eclipse Ti confocal microscope; images were taken and analyzed using NIS Elements AR. Composite images were taken over an area of up to 20 by 9 mm, the length perpendicular to the gradient. Images were analyzed in Matlab version R2009B and best fits were determined using Origin 7.0.

Computer Simulations

Numerical simulations were performed using an existing mathematical model for the neonatal rat ventricular myocyte adapted to experimental data from our laboratory and previously described in detail (Hou *et al.*, 2010). AP morphology was already validated by patch clamp data previously (Hou *et al.*, 2010). Minor modifications in some ionic currents were done to better adjust the APD, conduction velocities and frequency gradients observed experimentally in our experimental model of regional I_{Kr} heterogeneity. Kinetics were left unchanged from the previous model. Table 1 summarizes those changes and Table 2 shows a comparison of the electrophysiological properties in the experiments (optical mapping) and the model. A 2D disk-shaped sheet used a Cartesian mesh of 352 x 352 nodes with a spatial resolution of 100 µm that contains a 35 mm diameter disk-shaped active domain in its center, with a non-diffusive outer domain boundary was used to simulate a NRVM monolayer of tissue. The area outside of the monolayer (the black peripheral region) was implemented with passive and non-diffusive nodes for ease of integration. The membrane model at each node was solved by the finite volume method with a time-step of 10 µs and no-flux boundary conditions, as described

previously by Zlochiver *et al*. (Zlochiver *et al.*, 2008). This model was employed to study and provide mechanistic understanding to the effects of regional hERG overexpression on reentry dynamics, impulse propagation and wavebreak formation in tissue.

This model was constructed with a resolution of 100 µm per computational cell and solved using the traditional monodomain formulation for the reaction-diffusion equation describing changes in transmembrane voltage for every computational cell (equation 1). Discretization and linearization of the differential equation were performed using the finite volume method as previously described (Abboud *et al.*, 1994).

$$\frac{\partial V_m}{\partial t} = \frac{1}{C_m}(I_{stim} - I_m) + \nabla \cdot (D \nabla V_m) \quad (1)$$

G_{Kr} maximal conductance was either set as homogeneous (whole G_{Kr}, 1xG_{Kr}–uninfected or 5xG_{Kr}–infected) or varied spatially across the model (regional G_{Kr} model). To simulate the spatial gradient in G_{Kr} expression along the model we implemented the Boltzmann best fit of the hERG-GFP fluorescence data obtained in the experiments for all the groups (Figure 1B and Supplemental Figure S4). A native level of G_{Kr} was used in the 'uninfected' region (1x). G_{Kr} was increased over a range of 1 to 20 times basal levels within 'infected region' of the model. The following equation represents the Boltzmann distribution applied to the maximal conductance (G_{Kr}) from the distal and proximal sides of the disk-shaped model (Equation 2). Parameters of the best fit are summarized in Table 3.

$$G_{I_{Kr}}(x) = G_{I_{Kr,distal}} + \frac{G_{I_{Kr,proximal}} - G_{I_{Kr,distal}}}{1 + e^{\left(\frac{X - X_{50}}{\Delta x}\right)}} \quad (2)$$

Parameter measurements in numerical simulations

All the methods used for the parameter measurements from the numerical simulations have been described in detail before. Dominant frequency maps, DF spatial profiles and proximal to distal (n:m) activation ratios were built from the simulations as previously described, matching the experimental methods of analysis (Berenfeld *et al.*, 2002). To plot APD, DF and MDP spatial profiles we averaged those parameters first in time for every pixel in the last second of the respective simulation and also in space in a band of 20 pixels width in the center. Local conduction velocity (CV) was obtained from activation times for each pixel as described previously in detail (Mironov *et al.*, 2008; Korhonen *et al.*, 2009; Hou *et al.*, 2010). Briefly, distribution of activation times for a spatial neighborhood of 5×5 pixels were fitted into a plane, and gradients of activation times in each direction were calculated for each plane constructing

both magnitude and directionality (angle) maps. Computer-assisted algorithms were used to build the phase maps and for the detection of singularity points. The instantaneous phase of the action potential at each pixel was determined by obtaining the inverse tangent of the ratio of the Hilbert transform signals and the original signals so that the angle, with values in between [–π,π], was color coded to represent the different phases of the AP (Warren *et al.*, 2003) . Singularity points (SP) were defined as a point where all the phases converged (Iyer & Gray, 2001), neighboring sites around exhibit a continuous progression of phase of approximately 2π.

During functional reentry both APD and CV change gradually as a function of the distance from the center of rotation (core). To verify the properties of the rotors obtained in the different scenarios explored (homogeneous and heterogeneous conditions) and closely fit previously reported experimental observations from our laboratory (Hou *et al.*, 2010), we measured CV as function of radial distance from the core and WL as function of distance along the curvature of the wavefront, as described elsewhere (Hou *et al.*, 2010; Munoz *et al.*, 2007).

Fitting experimental observations

Both pacing and reentry were induced in homogeneous and heterogeneous models. Reentry was induced via S1-S2 cross-field stimulation protocol (Leon *et al.*, 1994). Pacing in the homogeneous models showed similar APD restitution properties as compared with experimental optical data. Table 2 shows how APD and CV properties were in good agreement with our experiments. Supplemental Figure S4A shows on the left the Boltzman distribution of G_{Kr} between 5 and 1x. On the right is the same distribution in 2D used to simulate the experimental conditions of regional Ad-hERG infection. Panel B shows the resulting APD_{50} map with individual action potentials at locations 1-3 during pacing at 1 Hz from the 5xG_{Kr} region (white asterisk on map).

Rotors were initially generated in the homogeneous whole 1xG_{Kr} (uninfected) and 5xG_{Kr} (infected) models. The level of 5xG_{Kr} similarly matched the average DF of I_{Kr} whole experimental infections at 15.0 Hz vs. 16.3±0.51 Hz (Table 3) and was the value used as reference. Rotors in the ‘wholly infected’ model showed a frequency of 15.0 Hz as compared to 11.7 Hz in the ‘wholly uninfected’. We induced rotors in a range of frequencies by increasing the level of I_{Kr} upregulation within the infected region from 1-20xG_{Kr}. In good agreement with experimental data the frequency of rotation increased as a result of the APD abbreviation within that region secondary to I_{Kr} upregulation.

When rotors where initiated in the infected half of the I_{Kr} regional model a spatial gradient in frequency was observed along the I_{Kr} gradient direction (from 10.7 to 14.6 Hz) while uniform

profile appear otherwise (11.7 Hz). Supplementary Figure S5D shows a comparison of DF spatial profiles as function of distance (along the I_{Kr} gradient for regional models) among the 3 models explored showing similar frequency gradients as in experiments (Summary in Table 3, Figure 4 and Supplemental Figure S5).

In Supplementary Figure S5 we analyze simulation data for three different conditions as illustrated by the snapshots presented in panel A. In each case, a rotor on the left half of the monolayer generated waves that propagated toward the right. On the top, G_{Kr} was increased to 5x uniformly in the entire monolayer. Waves propagated uniformly and no wavebreaks were observed. In the middle, the left half of the monolayer had 5 times more G_{Kr} than the right half. In this case, wavebreaks appeared readily at the border zone. For the simulation below, G_{Kr} was increased 5x uniformly but a MDP gradient of ~2 mV was imposed between the left and right halves. However, no wavebreaks were observed and wave propagation proceeded unimpaired. Analysis of panels B and C shows that when the whole monolayer had $5xG_{Kr}$ (blue), both MDP and APD_{80} were uniformly distributed which explains the absence of wavebreaks. Also, when the whole monolayer had $5xG_{Kr}$ but a MDP gradient of ~2 mV was imposed (black), APD_{80} in the more depolarized was shortened by about 1.5 ms. Yet no wavebreaks were observed despite the voltage non-uniformity. (Rotors initiated in the more depolarized region did not generate wavebreak, data not shown). For comparison, the superimposed APD_{80} profile for the unclamped $5xG_{Kr}$-$1xG_{Kr}$ heterogeneous model (red) demonstrates a much broader APD_{80} gradient of about 30 ms, which resulted in wavebreaks and fibrillatory conduction. In panel D the DF profiles across the three models clearly show that only the case of the $5xG_{Kr}$-$1xG_{Kr}$ heterogeneous model displayed a DF gradient of about 5 Hz between the two regions. Taken together, the numerical results of Figures 7 and 8 demonstrate that for a range of MDP values and gradients as large as the difference between those seen in the $5xG_{Kr}$ and the $1xG_{Kr}$ models, the fibrillatory activity is driven by the APD gradient. Therefore, although during local reentry MDP gradients across the monolayer result in APD dispersion, the effect is minor compared to the APD dispersion imposed directly by the G_{Kr} gradient.

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Tables and Figures

Table 1. Parameters adjusted and their values in the numerical NRVM model.

Parameter	Description	Model
K_{IK1}	Scaling factor for I_{K1}	1.7
G_{Kr}	Maximal I_{Kr} conductance (mS/μF)	0.0123602
G_{CaL}	Maximal I_{CaL} conductance (dm^3/F ms)	0.000031185
G_{Na}	Maximal I_{Na} conductance (mS/μF)	5.3550
D	Diffusion coefficient in 2D simulations (cm^2/ms)	0.0065
$\boldsymbol{\beta_m}$	Scaling factor β_m	$\beta_m = 0.06 \cdot e^{-V/11}$

Table 2. Comparison of the optical AP characteristics from experimental NRVM monolayers with the AP obtained in the model. (DF values in the model are for 5x)

Parameter		Experiments	Model
Uninfected APD_{50} (ms)	**1 Hz**	58.9 ± 2.32 ms	102.503 ms
Uninfected APD_{75} (ms)	**1 Hz**	117.3 ± 2.39 ms	117.568 ms
DF_{max} Uninfected Whole		9.5 ± 0.57 Hz	11.7 Hz
DF_{max} I_{Kr} Infected Whole		16.4 ± 0.51 Hz	15.0 Hz
DF_{max} Regional I_{Kr}: Uninfected Region		11.1 ± 0.55 Hz	11.7 Hz
DF_{max} Regional I_{Kr}: Infected Regional		18.0 ± 0.54 Hz	14.6 Hz
CV_{1Hz} Uninfected Whole		23.0 ± 0.97 cm/s	20.861 cm/s
CV_{1Hz} Infected Whole		20.4 ± 0.24 cm/s	20.528 cm/s

Table 3. Parameters of the Boltzmann best fitting used to model the regional change in I_{Kr} across the dish.

Parameter	Description	Experiments	Model
x_{50}	Midpoint Location	~17.5 mm	17.6 mm
Δx	Slope of the Boltzmann Distribution	0.94 ± 0.078 mm	0.98676 mm

Table 4. Spontaneous Rotor Activity

Monolayer Group	**Percentage of Spontaneous Rotor Activity**	**Dominant Rotor location**			
		Uninfected/ Distal Region	**Gradient Region**	**Infected/ Proximal Region**	**Multiple Rotors Uniform DF**
Uninfected	(12/31) 38.7%	(4/12) 33.3%	(3/12) 25%	(4/12) 33.3%	(1/12) 8.3%
Ad-GFP Whole	(4/16) 25%	(1/4) 25%	(1/4) 25%	(1/4) 25%	(1/4) 25%
Ad-GFP Regional	(7/20) 35%	(3/7) 42.9%	(2/7) 28.6%	(2/7) 28.6%	
Ad-hERG Whole	(45/61) 73.8%	(8/45) 17.8%	(10/45) 22.2%	(15/45) 33.3%	(12/45) 26.7%
Ad-hERG Regional	(64/76) 84.2%	(15/64) 23.4%†	(12/64) 18.8%†	(37/64) 57.8%†	

† Significantly different from one another $p<0.0001$; χ^2 analysis

Supplementary Figure Legends

Supplemental Figure S1. A. Schematic of Regional Magnetofection. ViroMag magnetic nanoparticles are first complexed with adenovirus as per product instructions. Right: A whole monolayer infection is generated by adding the complexed ViroMag and virus to the NRVM monolayer placed entirely over the super magnetic plate. Left: A regional monolayer infection is generated by adding the same complexed ViroMag and virus to a monolayer which is only regionally exposed to the magnet. In both instances the magnetic nanoparticles tug the virus to the area of cells which is placed over the magnet. B. Magnetic field strength of the magnetic plate (right) as it decays moving a distance perpendicularly from the edge of the magnet (left).

Supplemental Figure S2. A. Quantification of Optical Action Potentials. Maximum upstroke velocity was significantly faster than the maximal repolarization velocity of optical action potentials taken from the hERG infected region as well as in the uninfected region (* $p<0.02$). There was not statistical difference between upstroke or repolarization velocities between infected and uninfected groups. B. Regional Conduction Velocity. During 1 Hz pacing from the proximal half (infected half for regional infections), regional CV between all groups was found to be non-significant (uninfected n=11, GFP whole n=8, GFP Regional n=9, hERG whole n=8 and regional hERG n=3, $p>0.05$). B. Top left. I_{Kr} whole infection paced at 15 Hz. Top mid: time space plot (TSP) (taken at the green line) shows the lines activity over time on the x-axis (total 2 sec). Top Right: DF Map. Bottom left. I_{Kr} regional infection paced at 15 Hz. Mid: TSP showing block, 70% of waves pass into the uninfected region. In all homogeneous dishes (infected with I_{Kr} (n=9), uninfected (n=14), GFP (n=2)), no block was observed. In all I_{Kr} heterogeneously infected dishes (n=9) block and/or wavebreak was seen when pacing was ≥10 Hz from the infected region. Regions of block occurred along the interface between the APD regions.

Supplemental Figure S3. Average DF Maps and DF Profiles A. Average DF maps and corresponding standard error maps of spontaneous rotors in uninfected half of regionally infected Ad-hERG monolayers (upper maps, n=15) and wholly Ad-hERG infected monolayers (lower maps, n=45) both showing uninform average DF maps. B. DF profiles of I_{Kr} regional infections when rotors are localized in the uninfected region (red, n=15), the gradient region (purple, n=12) and I_{Kr} infected region (green, n=37). The later has a significantly higher frequency than the gradient region group until 16.5 mm from the edge of the infected region, and until 19.5 mm comparing to the uninfected region group. C. Differences in DF profiles between cases of singular rotors or multiple rotors are localized within the I_{Kr} overexpressing region. While not significantly different from one another, the multiple rotor group (blue, n=17) has significantly higher frequencies over 20.5 mm while the singular rotor group (green, n=9) is significant only to 17.5 mm compared to the uninfected group.

Supplemental Figure S4. Fitting I_{Kr} Fluorescence Gradient to a Boltzmann Distribution. A. Left: Fluorescence gradient for I_{Kr} regional infection fitted to a Boltzmann distribution ($\Delta x = 0.97 \pm 0.119$ mm, n=4) was used to generate the spatial gradient of the maximal conductance of G_{Kr} ($x_0 = 17.6$ mm and slope $\Delta x = 0.98676$ mm) used in simulations. Right: Introduction of this spatial gradient into the 2D mathematical model. $5xG_{Kr}$ was used for the upregulated region. B. APD_{75} map implementing the spatial gradient in G_{Kr} with representative regional AP traces paced at 1 Hz from star. C. G_{Kr} heterogeneity induces both unequal APD and MDP distribution profiles across the model. APD_{50} profile taken perpendicular to G_{Kr} gradient, APD_{50} is 103.3 ms within the $1xG_{Kr}$ region and 48.1 ms within the $5xG_{Kr}$ region. D. Resting membrane potential (RMP) profile perpendicular to G_{Kr} gradient. RMP is -72.0 mV within the $1xG_{Kr}$ region and -75.2 mV within the $5xG_{Kr}$ region.

Supplemental Figure S5. MDP gradients are not a major mechanism of wavebreak. A. Snapshots of rotors in whole $5xG_{Kr}$ (top), regional $5xG_{Kr}$ (middle) and whole $5xG_{Kr}$ with regional MDP (bottom) simulations. Rotors are localized on the left hand side which corresponds to the $5xG_{Kr}$ and/or lower MDP regions. Wavebreak is only observed in the regional $5xG_{Kr}$ model. B. MDP as a function of distance across the gradient in whole $5xG_{Kr}$ (blue squares), Regional 1-$5xG_{Kr}$ (red triangles) and whole $5xG_{Kr}$ simulation with the application of a MDP gradient (black circles) similar to the MDP gradient generated in the Regional 1-$5xG_{Kr}$ model. C. APD_{80} as function of distance across the gradient. D. DF Profiles across the gradients indicate that only the Regional 1-$5xG_{Kr}$ and not the Regional MDP model generated a decrease in frequency.

Supplemental Figure S6. APD differences are maintained at higher pacing frequencies. A. Experimental APD restitution curve. At all points W-hERG (n=13-4) has a significantly shorter APD than Uninfected monolayers (n=14-2). Right panel: expansion of the graph between 5-15 Hz. B. APD restitution curve of whole $1xG_{Kr}$ and Whole $5xG_{Kr}$, generating similar model dependent APD differences. Right panel: expansion of the graph between 5-15 Hz.

Supplemental Movie S1. Phase Movie of rotor activity in a regionally hERG infected monolayer. hERG infection is present in the lower half of the monolayer.