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Excito-oscillatory Dynamics as a Mechanism of Ventricular Fibrillation

Richard A. Gray, Ph.D., FHRS and Delilah J. Huelsing, Ph.D.

University of Alabama at Birmingham

Abstract

Background—The instabilities associated with reentrant spiral waves are of paramount importance to the initiation and maintenance of tachyarrhythmias, especially ventricular fibrillation (VF). In addition to tissue heterogeneities there are only a few basic purported mechanisms of spiral wave breakup, most notably restitution.

Objective—We test the hypothesis that oscillatory membrane properties act to destabilize spiral waves.

Methods—We recorded transmembrane potential (V_m) from isolated rabbit myocytes using a constant current stimulation protocol. We developed a mathematical model that included both the stable excitable equilibrium point at resting V_m (−80 mV) and the unstable oscillatory equilibrium point at elevated V_m (−10 mV). Spiral wave dynamics were studied in two-dimensional grids using variants of the model.

Results—All models exhibited restitution and reproduced the experimental values of transmembrane resistance at rest and during the plateau. Stable spiral waves were observed when the model exhibited only one equilibrium point. However, spatio-temporal complexity was observed if the model exhibited both excitable and oscillatory equilibrium points (*i.e.*, “excito-oscillatory” models). The initial wave breaks resulted from oscillatory waves expanding in all directions; after a few beats, the patterns were characterized by a combination of unstable spiral waves and target patterns consistent with the patterns observed on the heart surface during VF. In our model, this VF-like activity only occurred when the single cell period of V_m oscillations was within a specific range.

Conclusion—The VF-like patterns observed in our excito-oscillatory models could not be explained by the existing proposed instability mechanisms. Our results introduce the important suggestion that membrane dynamics responsible for V_m oscillations at elevated V_m levels can destabilize spiral waves and thus may be a novel therapeutic target for preventing VF.

INTRODUCTION

Monomorphic ventricular tachycardia (VT) is typically attributed to stable reentry, while polymorphic VT and ventricular fibrillation (VF) are associated with unstable reentry. Rotating two-dimensional (2-D) “spiral” and three-dimensional (3-D) “scroll” waves (a stack of 2-D spiral waves whose tips are “connected” via a virtual filament) of electrical impulse propagation occur during monomorphic VT, polymorphic VT, and VF.[1–3] The transition

Corresponding author: Richard Gray, PhD, University of Alabama at Birmingham, Department of Biomedical Engineering, Volker Hall B140, 1670 University Blvd. Birmingham, AL 35294-0019, Ph: 205-975-4715, FAX: 205-975-4720, email: rag@crml.uab.edu.
Current address for DJH: DJH Biomedical Consulting, LLC, 3425 Halliday Avenue, St. Louis, MO 63118

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from stable to unstable reentry (*i.e.*, “spiral wave breakup”) results in life-threatening VF and has been discussed extensively in the literature.[4–9]

The “excitable” nature of individual cardiac cells and its role in action potential (AP) generation, impulse propagation, and reentry are well-recognized.[4,5,10] With regard to mechanisms of arrhythmogenesis, the “restitution hypothesis” states that the rate dependences of action potential duration (APD) and conduction velocity (CV), *i.e.*, restitution, yield wave front-tail interactions that lead to wave breaks and the formation, and subsequent breakup, of spiral waves.[5–7] In addition to 1) restitution,[5,11,12] other mechanisms thought to be responsible for spiral wave breakup include: 2) wave front-tail interactions caused by rapidly moving spiral wave tips;[2,3]; 3) intracellular calcium overload disrupting spiral waves;[13, 14] 4) 3-D filament tension;[15] and 5) 3-D filament twist.[16]

In contrast to the heart’s “excitable” nature, other generic membrane properties (*e.g.*, oscillations) and their role in the maintenance of VF in the heart remain unknown. The delicate balance of inward and outward ionic currents can give rise to transmembrane potential (V_m) oscillations during the AP plateau termed abnormal automaticity or afterdepolarizations.[17] These oscillations occur primarily at slow heart rates and can result in the initiation of waves that propagate throughout the heart, either as a single premature excitation or as a salvo of “extra” beats.[18] This abnormal sequence of activity results in a complex pattern of activation and recovery that is thought to initiate restitution-induced wave front-tail interaction, unidirectional block and the formation of rapidly rotating spiral waves in the heart.[19] The behavior of reentrant waves in the heart is thought to be largely independent of the initiation event. However, here we introduce the idea that the mechanisms of reentrant waves and automaticity in the heart are intimately related. In this manuscript, we present a sixth basic fundamental mechanism of spiral wave breakup that involves an interdependence of oscillatory and excitable membrane properties which we call “excito-oscillatory” behavior.

METHODS

Model Description

Many detailed models describing the ionic mechanisms of cardiac APs exist. However, the complexity of these models, including the interdependency of variable equations, precludes the identification of relationships between cellular properties and multi-cellular phenomena such as spiral wave stability. We used the classic approach of Fitzhugh[20] here to present a novel, simplified model of the cardiac AP using a two-variable model comprised of two ordinary differential equations:

$$\tau_v \frac{dV}{dt} = f(V) - W + R_m I_{stim}(t) \quad [1]$$

$$\tau_w \frac{dW}{dt} = g(V) - W \quad [2]$$

where $V = V_m + 80$ (in mV); V represents a fast, excitable variable (*i.e.*, transmembrane potential); W represents a slow recovery variable; I_{stim} is the stimulus current density applied to the isolated myocyte; and τ ’s represent variable time constants with $\tau_v = C_m * R_m$, where C_m is the specific membrane capacitance in $\mu\text{F}/\text{cm}^2$ and R_m is the specific membrane resistance in $\text{k}\Omega * \text{cm}^2$. All model data in the paper is presented as V_m (*i.e.*, $V - 80$). A complete model description is presented in the supplementary material online.

Following the classic approach of Fitzhugh,[20] we used “nullclines” to describe the trajectory of the change of V_m . Mathematically, nullclines are defined from Eqn. [1] and [2] as $dV/dt = 0$ (fast variable nullcline) and $dW/dt = 0$ (slow variable nullcline). The intersections of the two

nullclines define equilibrium points, and the “properties” of these equilibrium points (represented mathematically as “eigenvalues”: see online material) are determined by the slope of the nullclines at these intersections. While variations of the “excitable” Fitzhugh model utilize a cubic, or “N”, shape for the fast variable nullcline and a linear slow variable nullcline, our model differs in the representation of the slow variable nullcline. Specifically, the slow variable nullcline was represented by a sigmoid-shape rather than a line.[21]

Figure 1 shows how the structure of the nullclines and their equilibrium points shape the AP. The left-hand panels of Fig. 1 show the basic nullcline shapes for our model are shown; the right-hand side shows the corresponding V_m traces. The N-shaped fast variable nullcline (solid line) is comprised of a lower (l), middle (m), and high branch (h). The sigmoid-shaped slow variable nullcline (dashed line) results in *three* equilibrium points in contrast to the typical Fitzhugh linear slow variable nullcline (not shown) that has only *one* equilibrium point. In addition to the stable equilibrium point at rest (solid circle), our model has an unstable equilibrium point (open circle) at elevated V_m , and a third marginally stable equilibrium point (saddle point: half-filled circle) at an intermediate V_m that defines the threshold V_m . As shown in panel A, a brief suprathreshold stimulus results in a typical AP configuration in our model.

Bias Current Stimulation Protocol

To “unmask” the presence and characteristics of oscillatory phenomena in cardiac cells associated with our purported unstable equilibrium point, we used the simple approach of injecting a constant current (I_{bias}) after the AP upstroke to prolong the action potential and generate early afterdepolarizations.[22]

Isolated myocyte recordings

Single myocytes were isolated from the right ventricular epicardial surface of adult rabbit hearts as described previously.[23] V_m was recorded with an Axoclamp 2B amplifier system (Axon Instruments, Inc., Foster City, CA) using suction pipettes. V_m and stimulus current were digitized at a sampling rate of 10 kHz with a 12-bit A/D converter (Digidata 1200A, Axon Instruments) and recorded with a computer using pClamp 6 software (Axon Instruments). APs were initiated with intracellular current injection at a basic cycle length of 5000 ms (S1). The S1 stimulus duration was 3 ms, and the stimulus magnitude was approximately 1.1 times the current threshold. The second stimuli (I_{bias}) were applied either 40 ms after the S1 during the plateau or at resting V_m (no S1). The value of the delay (40 ms) was chosen arbitrarily to ensure that I_{bias} was initiated early during the plateau. The duration of I_{bias} was 4000 ms, and its magnitude and polarity were varied.

This stimulation protocol was used to construct the parameters of our model, including the equations for the nullclines. Figures 1B and 1C show the results of applying I_{bias} to our model. Depolarizing I_{bias} shifts the fast variable nullcline upward (see Eqn. [1]), resulting in “bistability”. Bistability is characterized by a stable equilibrium point on both the l and h branches of the fast variable nullcline (solid circles in panel B and C). Therefore, if I_{bias} is applied during the action potential plateau, V_m equilibrates at an elevated level of V_m (on the h branch); however, for the same magnitude I_{bias} applied during diastole, V_m equilibrates near resting levels (on the l branch). Figure 1C shows the result of smaller values of I_{bias} in our model. In this case, bistability does not occur because the presence of the unstable equilibrium point in the model results in oscillatory activity when I_{bias} is applied during the AP replicating current-induced early afterdepolarizations recorded in isolated cardiac myocytes;[22] This model still exhibits a stable single equilibrium point near rest. We call this model with three equilibrium points at $I_{bias} = 0$ that exhibits both bistability (for large I_{bias}) and current-induced early afterdepolarizations (for small I_{bias}) an “excito-oscillatory” model. It is important to note that in an excito-oscillatory model only a very small depolarizing I_{bias} can fundamentally alter

the normal action potential shape resulting in sustained oscillations at elevated V_m levels. In addition, the V_m oscillation period near the upper unstable equilibrium point which we call T_{eigen} is determined by the eigenvalues for this equilibrium point (see online material).

We used results from our single cell experiments to determine specific model parameters. A number of simulations were ultimately run in which the model variables were altered to better understand the dynamics of excito-oscillatory models.

Results

Development of the excitable model

We recorded V_m from isolated cardiac myocytes ($n=15$) using this I_{bias} stimulation protocol (Fig. 2A). The magnitude of the S1 depolarizing pulses were 1.1 times threshold (2.5 ± 0.6 nA). For hyperpolarizing and small depolarizing values of I_{bias} applied at rest, the cells responded with small changes in V_m consistent with the typical linear circuit model of a membrane, *i.e.*, a resistor and capacitor in parallel (data not shown). [24] For small positive values of I_{bias} ($0 < I_{bias} < 0.1$ nA) applied during the AP plateau, APD was prolonged and V_m stabilized ($V_{m,end}$) at values slightly depolarized from resting values (see Fig. 2B; $I_{bias} = 0.06$ nA). Bistability was observed for larger I_{bias} values ($0.1 \text{ nA} < I_{bias} < 0.35$ nA); bistability was characterized by equilibration of V_m at levels greater than -10 mV when I_{bias} was applied during the plateau (see Fig. 2B; $I_{bias} = 0.20$ nA), but equilibration of V_m near resting levels (~ -80 mV) when the same I_{bias} was applied at rest (data not shown). The experimental results from all cells are presented in the graph of $V_{m,end}$ versus I_{bias} shown in Fig. 2C; there was more variability corresponding to h compared to l. The range of bistability (*i.e.*, overlap of the l and h branches) is shown as a thick solid horizontal bar. The membrane slope resistance of both l ($30 \text{ M}\Omega$) and h ($186 \text{ M}\Omega$) branches were computed from this graph via linear regression (Fig. 2C).

Membrane resistance in our two-state variable model is governed by the slope of both the fast and slow variable nullclines as well as the fast variable time constant (τ_v). Therefore, we set the slope of the slow variable nullcline equal to zero both at rest and during the AP plateau, set the fast variable time constant to reproduce the shape of the normal action potential including the fast upstroke and long plateau (see inset in Fig. 2D), and then adjusted the slopes of the fast variable nullcline for the l and h branches to match the experimentally derived membrane resistance values. Furthermore, the model parameters were selected to reproduce the action potential amplitude (115 mV) and threshold S1 current observed experimentally (see Fig. 2D). We call such a two-state variable model with just one stable equilibrium point (filled circle) at the resting potential for $I_{bias} = 0$ an “excitable” model.

Development of the excito-oscillatory model

When intermediate values of I_{bias} (overall range: $0.056 < I_{bias} < 0.164$ nA) were applied during the AP plateau, V_m oscillations occurred at elevated V_m levels (~ -10 mV) as shown in Fig. 3A. These oscillations occurred for relatively small values of I_{bias} (less than 10% of S1 threshold current) indicating the presence of an unstable oscillatory equilibrium point (open circle) in our model at a high value of V_m (see Fig. 3B) *in addition* to the stable equilibrium point (filled circle) defining the resting V_m . [25] The slopes of both the fast (thick solid line) and slow (thick dashed line) variable nullclines at the upper equilibrium point were determined by analyzing the experimental behavior of V_m in the immediate vicinity of the unstable equilibrium point. That is, the nullcline slopes at the upper intersection of the nullclines (open circle) were adjusted such that the linear stability characteristics (*i.e.*, eigenvalues) of this equilibrium point matched the growth and frequency of oscillations in the experimental recordings (see Fig. 3C). We name such a two-state variable model with three equilibrium

points (stable; saddle; unstable) for $I_{bias} = 0$ an “excito-oscillatory” model. The complete model for the excito-oscillatory model is provided in the supplementary material online.

The response to current-induced oscillations in our experiments varied with I_{bias} . As I_{bias} increased the following sequence of events occurred: APD prolongation (see Fig. 2B; $I_{bias} = 0.06$ nA); a single afterdepolarization (data not shown); multiple afterdepolarizations (see Fig. 3C); sustained oscillations (see Fig. 3A; $I_{bias} = 0.16$ nA); and prevention of repolarization with no discernable V_m oscillations (see Fig. 2B; $I_{bias} = 0.2$ nA). The range of I_{bias} for which oscillations occurred in the experiments is shown as a thick solid horizontal bar in Fig. 2C. The open data points with error bars in Fig. 2C indicate the average minimum (-11 ± 1 mV) and maximum ($+13 \pm 3$ mV) values of V_m and associated I_{bias} (0.073 ± 0.01 nA) at the onset of oscillations recorded experimentally when I_{bias} was applied in the AP plateau; the shortest period of V_m oscillations was 160 ms and occurred during sustained oscillations (see Fig. 3A). Our excito-oscillatory model reproduced these experimental trends. For example, compare the “bifurcation diagram” of the excito-oscillatory model showing $V_{m,end}$ as a function of I_{bias} (Fig. 3D) with the corresponding one from the experimental data in Fig. 2C.

APD Restitution

As with all cardiac models, the relationship between model parameters and cellular behavior (*e.g.* APD restitution) is very complex. We kept upstroke parameters constant as to not change CV restitution, and we varied model parameters c_5 , c_6 , and f_I to cover a range of APD restitution relationships and keep the position of the upper equilibrium point relatively constant (see supplementary material online). All models exhibited APD restitution as assessed by curve fits to single exponential curves ($R^2 > 0.99$ for all models). All models could be paced at twice diastolic threshold to diastolic intervals (DIs) < 5 ms. The maximum slopes computed at DI = 0 (Slope₀) from the curve fits for each model are shown in Table 1 and range from 0.47 to 5.2 for the excitable models and from 0.78 to 8.6 for the excito-oscillatory models. In addition, all models for which Slope₀ > 1 exhibited alternans during pacing, as predicted by the restitution hypothesis, and the pacing cycle length for which alternans occurred was within 10 ms of the value predicted from the model curve fits (*i.e.*, Slope = 1). All restitution curves are available online.

2-D simulations

To test the hypothesis that the transitions between the excitable and oscillatory states destabilize spiral waves we initiated rotating spiral waves in 2-D numerical grids using variants of our mathematical model. Details of the model as well as the parameter sets corresponding to all Figures are provided in the supplementary material online. Rotating spiral waves were easily induced for all excitable models as shown in Figure 4. The tips of these spiral waves were not strictly stationary and meandered to various amounts, but the waves did not “break-up” and degenerate into multiple unstable spiral waves typical of VF. The APD was sensitive to the shape of the slow variable nullcline, and the spiral wave dynamics of these models are consistent with previous findings.[26, 27] In particular, both the APD and the dependence of CV on wave front curvature determine tip trajectories such that for long APDs the tip followed linear trajectories for each beat (see Fig. 4A), while for shorter APDs the wave front curvature dominated, resulting in circular trajectories[28] (see Fig. 4B). Therefore, the period of spiral wave rotation (T_{2D}) varied with APD as the shape of the slow variable nullcline was altered for the excitable models (see Table 1 and online movies).

To study the role of the unstable equilibrium point at elevated V_m on spiral wave dynamics, we constructed “oscillatory” only models (a two-state variable model with just only one *unstable* equilibrium point at $V_m = \sim -10$ mV for $I_{bias} = 0$). The spiral waves for oscillatory models were extremely stable (see Fig. 4C&D) and rotated at periods similar to the period

corresponding to the eigenvalue of the unstable equilibrium point, T_{eigen} , (also see Figure 5 and Table A1). In addition, the V_m excursions at each site remained elevated (minimum $V_m > -50$ mV) for the oscillatory models while V_m returned to near resting levels for the excitable models.

The spatio-temporal patterns observed in the excito-oscillatory model with three equilibrium points were fundamentally different from those models with only one equilibrium point. For many of the model parameters sets (see Table 1), rotating spiral waves were not stable and spatio-temporal complexity was observed in the form of transient spiral waves along with the coexistence of target patterns (see Figure 5 and movies online). The overall dynamics were independent of the method of spiral wave initiation. Similar to the oscillatory models, T_{2D} increased as the T_{eigen} increased. T_{eigen} appears to play a major role in the spatio-temporal behavior. For $T_{\text{eigen}} < 200$ ms oscillatory activity occurred only at the center of rotation and had minimal effect on spiral wave stability (see eo1, eo2, and eo3 in Fig. 5 and corresponding movies). When T_{eigen} was slightly larger ($0.2 \text{ sec} < T_{\text{eigen}} < 0.3 \text{ sec}$), spatio-temporal complexity characterized by unstable rotating spiral waves and *target patterns* (see below) were observed. For larger values of T_{eigen} ($0.3 \text{ sec} < T_{\text{eigen}} < 0.45 \text{ sec}$), spatio-temporal complexity was not sustained (see eo8 and eo9 in Fig. 5) while for still larger values, meandering spirals were observed with transient wavebreaks but no target patterns (see eo10 in Fig. 5).

The emergence of target patterns in 2-D tissue can not be explained by restitution or spiral tip motion, and the evolution of one such target pattern is shown in Figure 6. Time-space plots are shown for both the fast (V_m) and slow (W) variables corresponding to the horizontal (bottom) and vertical (right) cross sections at the origin of the target pattern. The “wedge” shaped patterns in all four time-space plots indicate that this target pattern originated from this site de-novo and was not the result of continued propagation of a preexisting wave front. The time series of V_m and W for this site of origin are shown at the bottom.

Discussion

Overall, this study provides the following new information. First, we present a two-state model of the cardiac myocyte that reproduces transmembrane resistance values near resting levels and during the action potential plateau as well as current-induced oscillations observed experimentally. Second, we provide strong evidence (but not proof) that three equilibrium points in this model are a necessary, but not sufficient condition, for spatio-temporal complexity. The proof regarding the requirement of the three equilibrium points for spatio-temporal complexity in this model is a formidable mathematical problem, although it should be noted that a few papers have suggested that three equilibrium points are required for spatio-temporal complexity in Fitzhugh-type two variable models.[21,29] Third, we introduce a novel transition of VT to VF, although the exact mechanism remains elusive (see below). Fourth, we demonstrate spatio-temporal complexity in a 2-D array that involves *both* unstable rotating spiral waves and target patterns in a membrane model. Fifth, we propose that the characteristics responsible for membrane oscillations (*e.g.*, the L-type calcium current, I_{CaL}) are intimately related to the instability of reentrant waves and thus VF initiation and maintenance. Finally, we introduce the heretical idea that the reentrant spiral waves and “break through” patterns observed on the heart surface during VF are the result of oscillatory membrane phenomena.

The relationship between cellular dynamics and spatio-temporal behavior remains an important and formidable theoretical problem in understanding many phenomena including VF. Restitution is widely regarded to play a primary role in VF, as rate dependence of APD and, to a lesser extent CV, lead to APD alternans, increased wave front-tail interactions, and eventually spiral wave breakup. Our cursory sampling of parameter space suggests that APD

restitution was not associated with spiral wave breakup in our model. Wave breaks did not occur via wave front-tail interactions (see online movies), and T_{2D} was less than the cycle length threshold for which alternans occurred in 5/9 VT episodes and in 9/10 VF episodes (for which $Slope_0 > 1$).

In this manuscript we hypothesize that oscillatory membrane properties are responsible for target patterns in 2-D or 3-D that destabilize excitable spiral waves, providing a novel explanation for VF instability. In our model, this occurred as a result of transitions between the excitable and oscillatory equilibrium points of individual cells. The equations for total transmembrane current density (I_m) are similar for a single cell (Eqn. [3]) and for a multi-cellular preparation with no external input (Eqn. [4]), assuming a monodomain representation, are:

$$I_m = C_m \frac{dV_m}{dt} + I_{ion} = I_{stim} \quad [3]$$

$$I_m = C_m \frac{\partial V_m}{\partial t} + I_{ion} = C_m \nabla \cdot (\tilde{D} \nabla V_m) \quad [4]$$

where I_{ion} is the transmembrane ionic current density and $\tilde{D}$ is the diffusion tensor. Eqns. [3] and [4] illustrate the analogy between I_m resulting from the spatial distribution of V_m in multi-cellular preparations and the constant bias current in our single cell experiments used to induce sustained oscillations. Typically I_m in multi-cellular preparations is not constant; however, sustained non-zero I_m resulting from V_m gradients can occur (*e.g.*, at the tip of a spiral wave). We suggest these prolonged V_m gradients can act to induce transitions between the excitable and oscillatory states similar to I_{bias} in single cells. In support of this assertion, when we included a hyperpolarizing I_{bias} to every node in a subset of VF simulations, “defibrillation” or conversion to VT resulted.

There are several features of VF that are consistent with our multi-cellular excito-oscillatory model. VF in mammalian hearts is characterized by non-stationary rotating waves and target patterns on the heart surface (Fig. 7: right, top).[3,30,31] As seen in Fig. 7 (right, middle), membrane potential during fibrillation in large hearts remains elevated with the minimum potential remaining significantly above resting membrane potential.[32–34] Electric shocks can result in a rapid (1–2 beats) transition of V_m take-off potential from resting to elevated levels[3,35] as seen in Fig. 7, and vice versa.[36,37]

The excitable aspect of our model represents the rapid upstroke of cardiac myocytes from resting levels carried by the rapid sodium current (I_{Na}), while the slower upstroke dynamics at elevated levels of V_m reflects the reactivation of I_{CaL} . The threshold for activation of I_{Na} is approximately –65 mV [38–40] while the threshold for I_{CaL} is approximately –30 mV.[41] Zhou et al. demonstrated the existence of both fast and slow channel activity during the early stages of fibrillation.[33] This fact is consistent with our supposition of the dual role of excitable (I_{Na}) and oscillatory (I_{CaL}) membrane kinetics in the complex spatio-temporal patterns of fibrillation. The VF cycle length in our studies was longer than in most species,[42] although the activation period in human VF can be as long as 220 ms.[31] We only sampled a small subset of parameter space, and it is possible that other parameter sets would produce shorter VF cycle lengths.

Despite the similarities, there are formidable difficulties in establishing proof that excito-oscillatory cellular kinetics give rise to the coexistence of spiral waves and target patterns during VF in the heart. First, both excitable and oscillatory media support propagating spiral waves with no discernable differences. Second, there is no 2-D experimental model of fibrillation. The ideal experimental model to test our hypothesis is a large (able to sustain spatio-

temporal complexity) monolayer of homogeneous adult cardiac cells. Unfortunately, the existing cardiac monolayers are comprised of neonatal cells that exhibit pacemaker activity, [43] and large spatial heterogeneities are required to establish a coexistence of target patterns and spiral waves.[44,45] In addition, discerning target patterns resulting from pacemaker activity and those arising from the unstable equilibrium point at elevated V_m levels from fluorescence recordings (which are not calibrated to absolute V_m levels) remains a formidable challenge. Third, although excitable media do not exhibit target patterns in 2-D, target patterns on the heart surface can result from intramural excitable scroll waves in 3-D. The target patterns in fibrillation are thought to result from reentrant waves whose filaments do not intersect the surface, hence the waves emanating from the reentrant core “break through” onto the surface. [46] Part of the justification for this rationale is that early afterdepolarizations occur predominately at slow rates and hence should not be observed during the fast rates of fibrillation. Our results suggest that the mechanism of these breakthrough patterns and the associated role of membrane oscillations need to be reexamined.

We hypothesize that the existence of both a stable and unstable equilibrium point are necessary to reproduce current-induced oscillations seen in isolated cardiac cells and is responsible for a fundamentally new mechanism of spatio-temporal complexity in 2-D: the coexistence of spiral waves and target patterns. The coexistence of excitable and oscillatory waves was evident from the very different take-off levels of V_m , upstroke velocities, and conduction speeds. Neither restitution,[3,5,11,12] nor the Doppler effect,[2] nor the other previously proposed mechanisms of spiral wave instabilities in the heart [7] are consistent with target patterns in 2-D.

The membrane oscillations in our simplified two-state variable are the result of *sarcolemma membrane* dynamics (presumably I_{CaL}); the interdependence of intra-cellular calcium and V_m membrane dynamics adds another level of complexity. It has been shown that altering the calcium current kinetics in the Beeler-Reuter model[47]eliminated the VT-VF transition although the purported mechanism of this instability was slow recovery waves.[48] In addition, altering I_{CaL} affected spiral wave stability in the Fox model,[49] although this effect was attributed to restitution.[50] The I_{CaL} blocker verapamil has been shown to eliminate current-induced early afterdepolarizations.[51]and also convert VF to VT.[52,53]

If oscillatory membrane processes play an essential role in the stability of reentrant waves as we suggest, then we have to consider the mechanisms of VF in a fundamentally different way as compared to “excitable” activation, recovery, and propagation. For example, the concept of wavelength (APD times CV) is thought to be quantitatively related to the “critical mass” of tissue able to sustain VF.[54] However, both APD and CV result from excitable dynamics. Oscillatory waves are not similarly “spatially constrained” thus the “critical mass” for “excito-oscillatory VF” may be considerable less than its “excitable” wavelength. *Finally, our results suggest that interventions (e.g., pharmaceuticals) that reduce the amplitude or alter the frequency of V_m oscillations in isolated myocytes may act to prevent VF.*

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic representation of excito-oscillatory dynamics. Schematic of nullclines corresponding to Eqns. [1] and [2]; $W = f(V)$ is the fast variable nullcline (solid line), and $W = g(V)$ is the slow variable nullcline (dashed line). Filled circles represent stable equilibrium points; half-filled circles represents marginally stable equilibrium points; and open circles indicate unstable equilibrium points. Asterisks indicate initial conditions, and the thin arrows with lines represent the corresponding “trajectories” in state space. **A:** When no bias current (I_{bias}) is applied, a brief S1 stimulus initiates an all-or-none action potential characteristic of excitable cells. The cardiac action potential time series is characterized by four phases (1–4) as shown on the right, while its “state-space” representation is shown by the arrows in the left

panel. **B:** The model cell demonstrates “bistability” when a large value of I_{bias} is applied, resulting in a stable equilibrium either in the plateau or near rest. **C:** The model cell exhibits both a stable equilibrium point near rest and an oscillatory equilibrium at elevated V_m when a small value of I_{bias} is applied resulting in either V_m oscillations in the plateau if I_{bias} was turned on during the action potential or equilibration near resting V_m if I_{bias} was turned on during diastole. See text for more details.

Figure 2.

Isolated cardiac myocyte dynamics. **A:** Stimulus current protocol consisted of a brief 3 ms pulse to stimulate the cell (S1) followed by a 4 sec bias current. **B:** The V_m response for various values of bias current, I_{bias} (B). **C:** The steady state V_m at the end of the 4 sec ($V_{m,end}$) as a function of I_{bias} ; two steady states can be seen, a low (l) and high (h) branch. The range of bistability and oscillations are shown as thick solid horizontal bars. Open circles with error bars represent the range of V_m at the onset of oscillations. **D:** Two-state variable excitable model; the fast (solid line) and slow (dashed line) variable nullclines are shown. The filled circle at the nullclines intersection represents the stable excitable equilibrium point. The resting and threshold values of V_m are shown graphically as well as action potential amplitude (APA).

Figure 3.

Excito-oscillatory dynamics. **A:** Sustained oscillations in an isolated myocyte at elevated V_m for intermediate I_{bias} . **B:** Fast variable nullcline (solid line) and slow variable nullcline (dashed line) and trajectory evolution away from upper equilibrium point. **C:** Experimental data were used to identify the characteristics (i.e., growth and frequency of oscillations) of the unstable equilibrium point. **D:** Bifurcation diagram of excito-oscillatory model eo5 illustrating the relationship between $V_{m,end}$ and I_{bias} . The range of bistability and oscillations are shown as thick solid horizontal bars. The two branches in the oscillation region indicate the maximum and minimum values of V_m during oscillations.

Figure 4.

Stable spiral waves (excitable models, left; oscillatory models, right). Snapshots of V_m along with trajectory of spiral wave tip with time series of V_m plotted beneath each image (horizontal scale bars indicate 500 ms). All square images (including subsequent figures) represent snapshots of a 10 cm $\times$ 10 cm area of V_m represented as greyscale with black representing -80 mV and white representing +50 mV. Model parameter sets are **A:** $e7$; **B:** $e1$; **C:** $o6$; **D:** $o2$.

Figure 5. Spiral wave stability. Period of 2-D spiral wave activity (T_{2D}) versus the corresponding eigenvalue period (T_{eigen}) for oscillatory (open circles) and excito-oscillatory (filled squares) models. The corresponding model parameters are shown in Table 1. The images represent snapshots of V_m five seconds after spiral wave initiation via cross-field gradients.

Figure 6.

Evolution of target pattern. Snapshot of V_m indicating the emergence of a target pattern; white arrows indicate the direction of wave front movement. Time space plots of both V_m and W along the vertical (horizontal) cross sections indicated by dashed lines are shown to the right (bottom). The time series of both variables at the site indicated by intersection of dashed lines is shown at the bottom. The 500 ms scale bar indicates the beat illustrated in the time-space plots and the asterisk indicates the time of V_m the snapshot.

Figure 7.

Fibrillation (model, left; experiment, right) evolution from initiation. Phase map (top). Single cell dynamics during fibrillation (middle) and its representation in state space (bottom). Phase maps for the model were computed from both state variables (true state space) with the location of the unstable equilibrium point as the state space origin. Phase maps for the experiment (snapshot area = 4 cm × 4 cm) were computed from reconstructed state space; $\tau = 10$ ms and the state space origin was set equal to 63% of the maximum fluorescence (F) value.[3,35] Asterisks indicate the origin of target patterns. V_m signal from the model (left) and fluorescence, F , from experiment (V_m surrogate) (right) both exhibit elevated take-off potential and rapid oscillations (horizontal scale bar = 1 sec). True state space for the model (bottom left) and reconstructed state space from the experimental data (bottom right) both reflect oscillations around an upper unstable equilibrium point. The experimental data was taken from an isolated pig heart used in experiments described in Ref #[55].

Table 1

Model Parameters and Results

Label	c5	c6	f1	upper equilibrium point (V,W)	T _{clon} (ms)	T ₂₀	Fig	VT/VF	Slope ₀
eo1	0	50	70	(72.95)	113	121	6	VT	0.78
eo2	0	25	70	(74.96)	159	162	6	VT	1.7
eo3	0	18	70	(75.97)	186	192	6	VT	2.4
eo4	0	12	70	(78.99)	226	266	6	VT	3.9
eo5	0	10	70	(80.100)	246	280	3,6,7,8	Y	4.8
eo6	0	8.5	70	(82.101)	265	240	4,6,9	Y	3.6
eo7	0	7	65	(85.103)	289	430	6	Y	5.8
eo8	0	6	60	(76.97)	334	379	6	NSVF	5.6
eo9	0	4	50	(74.96)	429	418	6	NSVF	6.7
eo10	0	3	45	(78.98)	501	523	6	VF	8.6
eo11	0	6	63	(80.100)	327	383		VF	6.5
eo12	0	6	65	(82.101)	321	359		VT	6.7
eo13	0	15	75	(82.101)	196	196		VF	4.3
eo14	0	5	60	(80.100)	362	345		VF	4.7
o1	50	50	70	(72.95)	113	74	6	VT	X
o2	25	25	70	(74.96)	159	111	5D, 6	VT	X
o3	18	18	70	(75.97)	186	135	6	VT	X
o4	12	12	70	(78.99)	226	180	6	VT	X
o5	10	10	70	(80.100)	246	207		VT	X
o6	8.5	8.5	70	(82.101)	265	238	5C, 6	VT	X
o7	7	7	65	(85.103)	289	265	6	VT	X
o8	6	6	60	(76.97)	334	261		VT	X
o9	4	4	50	(74.96)	429	314	6	VT	X
o10	3	3	45	(78.98)	501	405	6	VT	X
e1	0	25	20	X	x	109	5B	VT	0.47
e2	0	10	20	X	X	132		VT	1.3
e3	0	8.5	20	X	X	314	2, 4A	VT	1.4
e4	0	7	20	X	X	174		VT	1.9
e5	0	5	20	X	X	242		VT	2.7
e6	0	4	20	X	X	235		VT	3.5
e7	0	3	20	X	X	310	5A	VT	5.2