Transition of spiral calcium waves between multiple stable patterns can be triggered by a single calcium spark in a fire-diffuse-fire model

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Spiral patterns have been found in various nonequilibrium systems. The Ca^{2+} -induced Ca^{2+} release system in single cardiac cells is unique for highly discrete reaction elements, each giving rise to a Ca^{2+} spark upon excitation. We imaged the spiral Ca^{2+} waves in isolated cardiac cells and numerically studied the effect of system excitability on spiral patterns using a two-dimensional fire-diffuse-fire model. We found that under certain conditions, the system was able to display multiple stable patterns of spiral waves, each exhibiting different periods and distinct routines of spiral tips. Transition between these different patterns could be triggered by an internal fluctuation in the form of a single Ca^{2+} spark. © 2009 American Institute of Physics. [DOI: 10.1063/1.3207814]

Lethal arrhythmia, the leading cause of sudden cardiac death, is usually characterized by self-sustained spiral activity, as well as turbulent wavelets after spiral break up. With the motivation of understanding and preventing various cardiac arrhythmias, spiral waves in excitable media have long been the subject of numerous studies from different disciplines. Here, using the Ca^{2+} -induced Ca^{2+} release (CICR) system in single heart cells as a discrete excitable model, we imaged spiral Ca^{2+} waves with high-speed confocal microscope and simulated their nonlinear properties using a well-defined two-dimensional fire-diffuse-fire (FDF) model. The simulation showed that the spiral waves in a single heart cell displayed multiple stable patterns. These spiral patterns exhibited different periods with distinct behavior of spiral centers depending on the initial conditions. When a spiral wave was rotating, a single molecular event of Ca^{2+} release in the form of a Ca^{2+} spark, which hits the wave at a proper location and timing, was able to trigger a transition between these different patterns. The multistability of spiral waves represents a novel behavior of a discrete excitable media and may have important implications in understanding the arrhythmogenesis in heart cells and the complex behaviors in other discrete biological systems.

I. INTRODUCTION

CICR is a general mechanism that most cells use to generate cell-wide Ca^{2+} signals. This nonlinear process allows for the formation of complex spatiotemporal patterning in a variety of forms, from stochastic local Ca^{2+} release events (puffs, sparks) to waves traveling across the whole cell. In cardiac myocytes, elementary Ca^{2+} release events, in the stereotypical form of Ca^{2+} sparks,^{1–3} are generated by a few ryanodine receptor (RyR) Ca^{2+} release channels residing on the sarcoplasmic reticulum (SR).^{4,5} The RyR molecules usually cluster into discrete Ca^{2+} release units (CRUs) separated from one another by 0.4–2 μm .⁶ As the reflection of intracellular Ca^{2+} release at the molecular level, Ca^{2+} sparks can

either occur stochastically at random locations, or, when an action potential fires, be activated in a synchronized manner summing to cell-wide Ca^{2+} transients.^{1,2} In some pathological conditions, spatial coupling between CRUs is enhanced so that spontaneous Ca^{2+} sparks can activate neighboring CRUs via the CICR mechanism, leading to a propagating Ca^{2+} release activity known as a Ca^{2+} wave.^{7,8} Therefore, the CICR system in cardiac cells is a typical reaction-diffusion system, in which excitable CRUs are coupled with the diffusive Ca^{2+} .

As a general feature of the excitable media, spiral waves have been observed and studied in a variety of different physical,⁹ chemical,¹⁰ and biological^{11–15} systems. These studies are largely motivated by potential application in understanding and preventing cardiac reentrant tachycardia and lethal arrhythmias, which are believed to result from self-sustained spiral waves and turbulent jumble of small wavelets, respectively.^{12,13} In cardiac cells, Ca^{2+} waves in spiral form have also been observed.¹⁴ However, the properties of the spiral Ca^{2+} waves in single heart cells have not been fully understood.^{16–18} As a unique feature, the CICR system is a highly discrete excitable medium. Given the ~ 2 μm distance between CRUs and the 50–100 $\mu\text{m/s}$ wave propagation speed,^{6,8,19} it usually takes 20–40 ms for the wave front to propagate among neighboring CRUs. The discreteness makes the diffusion process much slower than the reaction process (the opening of CRUs takes only ~ 10 ms). As a result, unlike chemical waves, Ca^{2+} waves in cardiac cells are not continuous but saltatory.^{7,16} Since oscillators of most biological systems are compartmental more or less, it is very important to understand the properties of spirals in such systems with highly discrete oscillators.

In the present study, we observed spiral Ca^{2+} waves in freshly isolated heart cells and studied the spiral dynamics in a modified FDF model.

II. METHODS

A. Imaging of spiral Ca^{2+} waves in cardiac myocytes

Ventricular cardiac myocytes were isolated from adult Sprague–Dawley rats (2–3 months old, weight 225–300 g) using standard enzymatic techniques, as described previously.²⁰ Freshly isolated single cells were stored in Tyrode's solution containing (in millimolar) 135 NaCl, 1 CaCl_2 , 4 KCl, 1 MgCl_2 , 10 glucose, 10 HEPES, and pH 7.4. Before imaging, isolated myocytes were loaded with the Ca^{2+} indicator Fluo-4-AM.²⁰ Two-dimensional imaging used a Zeiss LSM-5Live fast confocal microscope equipped with a 40 $\times$, 1.3 NA oil immersion objective. All experiments were performed at room temperature (23–25 °C).

Digital image analysis used IDL software (Research Systems). Spatial filtering was applied to Ca^{2+} images to optimize the signal/noise ratio. The phase (θ) of each pixel in Ca^{2+} images was computed following the method by Gray *et al.*,¹² $\theta(x, y, t) = \arctan[F(x, y, t) - F_{\text{mean}}, F(x, y, t - \tau) - F_{\text{mean}}]$, where $F(x, y, t)$ is the fluorescence of Ca^{2+} , F_{mean} is the mean value of $F(x, y, t)$ during the spiral wave period T , and $\tau = 125$ ms is roughly $T/4$ unless otherwise specified. Phase singularity was detected as a point with an arbitrary phase and surrounded by a continuous phase cycle.

B. Modeling spiral Ca^{2+} waves in cardiac myocytes

The simulations were performed using a modified FDF model.^{16,17} The model consists of a regular array of 25×25 point-source CRUs with spacing, $d = 1.0$ μm , embedded in a continuum with spatially homogeneous Ca^{2+} removal and diffusion coefficient. Note that in cardiac cells, the distance between neighboring CRUs is ~ 0.4 μm in transverse direction and ~ 1.8 μm in longitudinal direction.⁶ Here, to simplify the system, 1.0 μm was used as the distance in both directions.

The partial differential equation describing the density of Ca^{2+} , denoted by $u(x, y, t)$, can be expressed as

$$\frac{\partial u}{\partial t} = D \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) + J_{\text{CRU}} - J_{\text{removal}} + J_{\text{leak}}, \quad (1)$$

where $D = 10$ $\mu\text{m}^2/\text{s}$ represents the apparent Ca^{2+} diffusion constant. Because there are multiple kinds of Ca^{2+} buffers in the cytoplasm and binding to the buffer molecule will greatly reduce the diffusion velocity of Ca^{2+} , to account for the effect of buffering, the Ca^{2+} diffusion constant D was reduced here compared to those models with Ca^{2+} buffers.^{7,8,16,21}

J_{CRU} represents Ca^{2+} release from CRUs and J_{removal} describes Ca^{2+} removal by Ca^{2+} pumps. In order to maintain a fixed basal Ca^{2+} level (0.1 μM), a constant leaking Ca^{2+} current J_{leak} is employed to compensate J_{removal} at resting Ca^{2+} level.

In cardiac cells, Ca^{2+} is removed from the cytoplasm mainly by Ca^{2+} pumps on SR membrane in a $[\text{Ca}^{2+}]_i$ -dependent manner,

$$J_{\text{removal}} = V_m u^4 / (k^4 + u^4), \quad (2)$$

where $V_m = 5$ $\mu\text{M}/\text{s}$ is the maximum rate and $k = 0.2$ μM is the pump affinity.²²

The opening of CRUs is a use-dependant process. The CRU (i, j) cannot start the k th release until (1) $u(i, j, t)$ is above a threshold $u_c = 0.2$ μM and (2) the time from the ($k-1$)th release is more than a refractory period $\tau_R = 210$ ms. Once a CRU begins releasing, it remains open for a time $\tau = 10$ ms, and then closes after having released a quantity σ of Ca^{2+} . We took the shape of a Ca^{2+} spark as the rectangular pulse, so this process is described as

$$\eta(t) = \frac{\sigma}{\tau} \Theta(t) \Theta(\tau - t), \quad (3)$$

where Θ is a Heaviside step function. Because the binding time for Ca^{2+} with immobile buffers is faster than the other time scales in the problem,²¹ we treat the action of the Ca^{2+} buffers as instantaneous. To account for the effect of buffering, we define σ as the total amount of Ca^{2+} released divided by a buffering factor of ~ 100 here. $\sigma = 0.365$ μM unless otherwise specified.

Therefore, Ca^{2+} release process from CRUs, J_{CRU} , can be expressed as

$$\begin{aligned} J_{\text{CRU}} &= \sum_{i,j,k \in \mathbb{Z}} \delta(x - x_i) \delta(y - y_j) \eta(t - T_{i,j}^k) \\ &= \sum_{i,j,k \in \mathbb{Z}} \frac{\sigma}{\tau} \delta(x - x_i) \delta(y - y_j) \Theta(t - T_{i,j}^k) \Theta(\tau + T_{i,j}^k - t), \end{aligned} \quad (4)$$

where δ is the Dirac delta function and $T_{i,j}^k$ denotes the time of the k th release at the (i, j) release site. Experimental studies reveal that the refractory period of CRUs is not constant but stochastic, and the amplitude of Ca^{2+} spark is also use-dependant.^{23,24} To embody these properties, in some simulations, we described τ_R by the following equation which comes from the sigmoid function of CRU recovery after firing,²³ $\tau_R = 210 - 14 \ln(1/\zeta - 1)$, where ζ is a random value between 0 and 1. Spark amplitude is described with an exponential recovery function, $\sigma_{i,j}^k = 0.44 \times (1 - \exp[-(T_{i,j}^k - T_{i,j}^{k-1})/\tau_A])$, where $\tau_A = 150$ ms is the recovery time constant of spark amplitude.²⁴ A spontaneous firing rate of 0.02 Hz is giving for each CRU and it is not restricted by refractory period.

The simulations were performed using an IDL routine with a spatial step $dl = 0.05$ μm and a time step $dt = 0.01$ ms. No-flux boundary conditions were imposed. Data were stored at 1 ms intervals in 512×512 16-bit TIFF format. The figures shown below were 3×3 smoothed three times, resized to 128×128 , and rescaled to 8-bit gray color.

III. RESULTS AND DISCUSSION

A. Imaging and modeling spiral Ca^{2+} waves in cardiac cells

In rat ventricular cardiomyocytes loaded with the Ca^{2+} indicator, fluo-4, stochastic opening of RyR Ca^{2+} release channels residing on the SR could be visualized as Ca^{2+} sparks using confocal microscopy. Occasionally, a Ca^{2+} spark may couple to more Ca^{2+} sparks in the neighboring area via the CICR mechanism, resulting in a concentric Ca^{2+} wave propagating outward from the center of the initial Ca^{2+}

FIG. 1. (Color) Spiral Ca^{2+} waves in single heart cell. (a) Snapshot of Ca^{2+} fluorescent image. Interval between each is 160 ms. (b) Reconstructed x - t images along the line shown in the last image of (a). (c) Phase singularity trajectory of the spiral (enhanced online).[\[URL: http://dx.doi.org/10.1063/1.3207814.1\]](http://dx.doi.org/10.1063/1.3207814.1)

spark. The occurrence rate of Ca^{2+} waves increased when the extracellular Ca^{2+} was changing from 1 to 2 mM. To track two-dimensional microdynamics of Ca^{2+} sparks and Ca^{2+} waves, we visualized the intracellular Ca^{2+} dynamics at 120 frames per second. While most Ca^{2+} waves, known as circular waves, propagated concentrically from their origin, some cells ($\sim 1\%$) exhibited spiral waves [Fig. 1(a) and supplemental movie]. The wave front rotated [Fig. 1(b)] at a period from 270 to 910 ms, in line with those for heart, but at an initial wavelength from 17 to 48 μm , much shorter than that in other biological systems.^{11,12} If we reconstructed the Ca^{2+} signal along a line through the rotating center to a stereotype line-scanning image, the spiral wave exhibited a unique shape in which wave fronts propagate out from the center alternately [Fig. 1(b)]. Phase singularity of the spiral meandered randomly in the cell [Fig. 1(c)].

We used a modified FDF model¹⁶ to simulate the Ca^{2+} dynamics in single heart cell. To initiate the first spiral wave we used the initial data corresponding to a broken wave front, the break being located at the middle of the excitable cell. As shown in Fig. 2(a), we opened ten neighboring CRUs in a single middle row and set states of the ten CRUs in the left neighboring row in refractory period. The rightward propagating wave front curved in the broken end and formed a spiral wave. In the reconstructed x - t images [Fig. 2(b)], the spiral wave exhibited a similar form as in experiments [Fig. 1(b)]. The spiral had a period of 210 ms and an initial wavelength of 13 μm . Note that the period of 210 ms is exactly the refractory period τ_R (210 ms), which represents the maximal oscillation frequency in this system. The spiral was very stable and the center was almost fixed. In simulations of 15 s, there was no movement of the rotating center.

FIG. 2. Initiation of a spiral Ca^{2+} wave in FDF model. (a) Snapshots of Ca^{2+} concentration taken every 100 ms. (b) Reconstructed x - t images along the lines shown in (a).

The oscillation of the Ca^{2+} concentration was very regular in difference to the living cell. As is shown in Fig. 1, the phase singularity of the spiral had a randomly meandering trajectory, just like spirals in heart tissue.¹²

In heart cells, the recovery of CRUs from refractoriness is not uniform but follows a sigmoid function;²³ spark amplitudes depend on the firing history;²⁴ meanwhile, there are spontaneous Ca^{2+} sparks firing even at the resting Ca^{2+} level. To embody the randomness of a living cell in the model, we described τ_R and σ with functions obtained from experimental data (see Sec. II for details) and gave CRUs a spontaneous firing rate of 0.02 Hz. These stochastic properties made the spiral wave more similar with those we detected in living cells [Fig. 3(a)]. The oscillation of Ca^{2+} concentration u was no longer precisely regular with a period of 272 ± 42 ms [mean $\pm$ SD, Fig. 3(b)]. As a result of the slowed rotation, the mean initial wavelength increased to 20 μm . The spiral meandered in the media randomly [Fig. 3(c)].

These observations and simulations proved that the CICR system in single heart cells can support spiral waves. It is significant not only to provide a new model to study the spiral dynamics but to reveal a new level of excitability that may be related with arrhythmia genesis. The occurrence of spiral Ca^{2+} waves is not an event merely confined within a particular cell. Due to the activation of the electrogenic Na/Ca exchange current,²⁵ intracellular Ca^{2+} dynamics causes depolarization of the membrane potential, which increases the chance of initiating ectopic excitation in heart tissues, a process recently conceptualized as “reversed

FIG. 3. Spiral Ca^{2+} waves in FDF model with stochastic properties. (a) Ca^{2+} dynamics in the simulation system. Snapshots in upper panel are taken every 130 ms. Lower panel is the Ca^{2+} dynamics along the line in (a). (b) Ca^{2+} dynamics of a typical point in the media. (c) Phase singularity trajectory of the spiral.

FIG. 4. Multistability of spiral Ca^{2+} waves in FDF model. (a) Initiation of a line-core spiral wave when excitability is enhanced ($\sigma = 0.42 \mu M$). Snapshots are taken every 100 ms. Thick white line denotes the rotating center of the spiral. (b) Ca^{2+} dynamics along the lines in (a). (c) Initiation of a square-core spiral wave when excitability is reduced ($\sigma = 0.361 \mu M$). Snapshots are taken every 190 ms. Dashed square denotes the rotating center of the spiral. (d) Ca^{2+} dynamics along the line in (c). (e) Ca^{2+} dynamics along a typical line when σ is reduced from 0.365 to 0.361 μM .

excitation-contraction coupling.²⁶ While circular Ca^{2+} waves only transiently disturb membrane potential, spiral Ca^{2+} waves would result in sustained instable depolarization. Under circumstances such as local ischemia during heart attack, spiral wave-induced and metabolism-induced membrane depolarization and catecholamine level increase, as well as the resulting spark frequency elevation, would form a vicious circle, rendering the waving cell an ectopic pacemaker. This could result in a series of pathological consequences at the tissue level.

B. Multistability of spiral Ca^{2+} waves in FDF model

The simplified model was again used to study the behavior of spirals in FDF model, since it has no randomness and every step is reproducible. We changed the excitability of the CICR system by changing σ , the total release amount of CRU for each Ca^{2+} spark.

When σ was increased, it is easier for the broken end of the initial wave front to curve and to form a rotating center. Since the frequency of oscillation had already reached the maximum, the CRUs which fired at the beginning of simulation were still in their refractory period when the curved wave front was rotating back [Fig. 4(a) second image, $\sigma = 0.42 \mu M$]. Therefore the free end traveled along this row of CRUs and was unable to curve back until the refractory period was over [Fig. 4(a) third image]. This led to a line-shaped spiral core [Figs. 4(a) and 4(b)]. A bigger σ generated a longer line core. When σ was reduced to a certain threshold ($\sigma = 0.364 \mu M$), however, the spiral core initiating from a broken wave front was enlarged to be a square, the CRUs in which failed to be activated [Figs. 4(c) and 4(d), $\sigma = 0.361 \mu M$]. This square-core spiral had a much longer period (760 ms) and wavelength (outranging the simulation area) than the point-core spiral (Fig. 2). If σ was further

FIG. 5. (Color) Single spark induced transition of Ca^{2+} waves between multiple stable patterns. (a) Snapshots during transition from square-core spiral to point-core spiral. The triggering spark is marked with a red arrowhead. (b) Transition from point-core spiral to square-core spiral. The triggering spark is marked with a blue arrowhead. (c) Phase singularity trajectory of the spiral. Black part shows the original square-core spiral, red shows the point-core spiral, and blue shows the modulated traveling spiral. (d) Projection of singularity trajectory on the x-y plane. (e) Ca^{2+} dynamics on the yellow point in (a).

reduced, the core was enlarged with the period and wavelength increased greatly. When σ approached a certain threshold ($\sim 0.358 \mu M$), the broken end of initial wave front was no longer able to curve to form a spiral, and thus the excitability of the system can only support a traveling wave.

If a spiral was already formed with $\sigma = 0.365 \mu M$, as shown in Fig. 2, increasing σ had no effect on the spiral except that the oscillation amplitude was increased, because the oscillation had already reached the maximal frequency. Decreasing σ within a certain range cannot change the oscillations but reduce the wave peak a little [Fig. 4(e)]. This may benefit from the high residual Ca^{2+} concentration around the rotating center as a result of former high frequency oscillation. For the square-core rotor, the Ca^{2+} concentration has almost declined to resting level when the spiral tip rotates back after 760 ms. These results indicate that at a proper excitability level ($0.358 < \sigma < 0.364 \mu M$ in the present model), different initial conditions generate spirals with different periods and organization center, i.e., the system was able to support at least two stable patterns.

C. Single spark induced transition between different stable oscillations

For the slow square-core spiral, the square region is like a functional obstruction because there the Ca^{2+} concentration is not high enough to activate those CRUs inside the square. What will happen if the regional Ca^{2+} concentration is el-

FIG. 6. Diagram of multistability of spiral pattern in FDF model. The symbols denote different rotating centers as demonstrated in the right panel.

evated to reach the spark threshold? We achieved this by introducing a Ca^{2+} spark within the square [red arrowhead in Fig. 5(a)]. It is relevant physiologically since spontaneous Ca^{2+} sparks always emerge in living cells.^{1,19} The Ca^{2+} spark elevated the background Ca^{2+} concentration in the vicinity, which enables the approaching spiral tip to activate the neighboring CRUs that were formerly unavailable to form a point-core spiral [Fig. 5(c) red part]. As a consequence, the whole system changed into high frequency oscillation [Fig. 5(e)].

As discussed before, at low excitation level ($\sigma = 0.361 \mu\text{M}$), maintenance of the point-core spiral needs the residual Ca^{2+} around the rotation center. Therefore, decrease in the Ca^{2+} level around the spiral tip is supposed to break the fast-rotating spiral. As shown in Fig. 5(b), a spontaneous spark beside the spiral tip arising at 10 s prolonged the refractory period of the CRU and made it unavailable at 10.1 s when the CRU is supposed to fire within the wave front. Absence of this spark significantly reduced the residual Ca^{2+} level around the spiral tip, so the rotating center was enlarged [Fig. 5(c) blue part] and the system fell into low frequency oscillation [Fig. 5(e)]. Interestingly, the rotating center of the new spiral was not as fixed as the one originating from a broken wave front [Figs. 5(c) and 5(d) black part] but exhibited some drift [Figs. 5(c) and 5(b) blue part].

These results demonstrate that the FDF model, with proper excitability, can support different spiral dynamics (e.g., single rotors and modulated traveling waves) with different oscillation frequencies for the same system parameters. There are at least two stable patterns within the excitability range E_1 – E_2 ($0.358 < \sigma < 0.364 \mu\text{M}$ in the present model), as shown in Fig. 6. In addition to square-core spiral, there may be other stable patterns with approximative oscillation frequency, e.g., the modulated traveling wave in our simulation. Small fluctuations in system, like changes in local residual Ca^{2+} or the oscillating state of a single CRU close to the rotating center, may drive the system transitions between different states. This also explains the complicated spiral behavior in living cells and the FDF model with stochastic properties, e.g., the complexity of periods and trajectories of phase singularities.

In summary, we have presented a typical example that multistability exists in the FDF model and transitions can be induced by a single Ca^{2+} spark. These results are significant for a few different reasons. First, the multistable patterns are important because they may be some intermediate states between simple spiral waves and spatiotemporally turbulence which causes deadly cardiac arrhythmia in heart tissue.

These wave structures have been found in several different experimental^{27–29} and model chemical reaction-diffusion systems.^{30,31} In our model, the high discreteness of the excitable elements is critical for the presence of multistable patterns, because no multistability of spirals was found in the model where Ca^{2+} is released homogeneously throughout the system (data not shown). Since the heart tissue is also a complex-oscillatory media comprised of discrete cells, the similar multistability may also exist on the tissue level, especially during the breakup of reentrant activities. Second, we have shown that localized fluctuation can influence the overall dynamics of the waves in single cardiac cell model in a quite nontrivial way. It suggests that local ectopic excitation may induce the transition from reentrant tachycardia to fibrillation. Meanwhile, it gives us a hint that spiral in heart tissue may also be slowed down or even tamed by localized stimulation or inhibition. Third, our simulations confirm that the period of spirals is decided by the spiral tip dynamics, even in our highly discrete system. The different trajectories of the spiral tip drive different oscillation of the whole system. This, in turn, raises questions on how the spiral tips are organized in this system with highly discrete oscillators and how local ectopic excitation interacts with the spiral tip, which may help us to fully understand how multistability is generated.

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