Spatially Discordant Alternans in Cardiac Tissue
Role of Calcium Cycling

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Abstract—Spatially discordant alternans, where the action potential duration (APD) and intracellular calcium transient (Ca) alternate with opposite phase in different regions of tissue, is known to promote wave break and reentry. However, this phenomenon is not completely understood. It is known that alternans at the cellular level can be caused by dynamical instabilities arising from either membrane voltage (V_m) attributable to steep APD restitution or to calcium (Ca) cycling. Here, we used a mathematical model of intracellular Ca cycling, coupled with membrane ion currents, to investigate the dynamics of V_m and Ca transient alternans in an isolated cell, in two electrotonically coupled cells, and in 1D spatially homogeneous tissue. Our main finding is a novel instability mechanism in which the bidirectional coupling of V_m and Ca can drive the Ca transient of two neighboring cells to be out of phase. This instability is manifested in cardiac tissue by the dynamical formation of spatially discordant alternans. In this case, Ca transient alternans can reverse phase over a length scale of one cell, whereas APD alternans reverses phase over a much longer length scale set by the electrotonic coupling. We analyze this mechanism in detail and show that it is a robust consequence of experimentally established properties of the bidirectional coupling between Ca cycling and V_m dynamics. Finally, we address the experimental relevance of these findings and suggest physiological conditions under which these patterns can be observed. (Circ Res. 2006;99:520-527.)

Key Words: discordant alternans ■ calcium cycling

Ventricular fibrillation (VF) is initiated when a propagating electrical wave fractionates as it passes over tissue with nonuniform electrophysiological properties. This dispersion of refractoriness has traditionally been attributed to the presence of fixed structural and electrical heterogeneities.1,2 However, recent studies have shown that single-cell dynamics can play an important role in promoting VF.3,4 In particular, alternans, a beat-to-beat alternation in the action potential duration (APD) at the single-cell level, can lead to the formation of spatially discordant alternans in tissue,1,5–7 where regions of long–short APD alternation occur adjacent to regions with short–long APD alternation. This dynamical phenomenon is arrhythmogenic because it leads to the formation of steep gradients of refractoriness that can promote wave break and reentry.1,2

The mechanism underlying spatially discordant alternans is still not well understood. Pastore et al1 have suggested that spatially discordant alternans is a consequence of fixed electrophysiological heterogeneity in cardiac tissue. On the other hand, theoretical studies have also shown that this phenomenon can occur in spatially homogeneous tissue8–10 because of the interaction between APD alternans induced via a steep APD restitution curve and restitution of conduction velocity (CV). However, a further complexity in elucidating the mechanism of discordant alternans at a tissue level arises from the fact that, at the cellular level, APD alternans can be caused either by (1) A dynamical instability of V_m dynamics that is attributable to the gating kinetics of membrane ion channels that regulate V_m. Previous studies3,11 have typically attributed alternans to gating kinetics that lead to a steep APD restitution curve; (2) Unstable intracellular Ca cycling, which in turn drives APD alternans via its effects on Ca-sensitive membrane currents.12,13 Because V_m and Ca cycling are bidirectionally coupled, it is difficult to pinpoint which of these two possibilities is responsible for alternans. As a result, it is not known how the formation of discordant alternans in cardiac tissue depends on the cellular instability mechanism.

In this article, we apply mathematical modeling to test the hypothesis that discordant alternans in cardiac tissue can be initiated by a new mechanism that does not require CV restitution when alternans originate from a dynamical instability of calcium cycling. To test this hypothesis, we carry out simulations of V_m dynamics and Ca cycling in a cable of electrotonically coupled cells. The results demonstrate that
discordant alternans can be formed, independently of CV restitution, when the Cai transient and APD of an isolated myocyte are electrometrically out of phase, i.e., a large-small-large Cai transient corresponds to a short-long-short APD. Moreover, when the Cai transient and APD are in phase, sufficiently steep CV-restitution is required to initiate discordant alternans as shown in previous studies.8–10 We explain these findings based on experimentally established properties of cardiac cells, and suggest experimental conditions under which this phenomenon can be observed.

Materials and Methods

Numerical Simulations

We modeled a 1D strand of homogeneous tissue using the cable equation:

\[ \frac{\partial V}{\partial t} = -I_{ion}/C_m + D \frac{\partial^2 V}{\partial x^2} \]

where \( C_m = 1 \mu F/cm^2 \) is the transmembrane capacitance, \( D = 5 \times 10^{-4} \text{cm}^2/\text{ms} \) is the effective diffusion coefficient of membrane voltage in cardiac tissue, and where \( I_{ion} \) is the total ionic current density. The cable equation was integrated with an operator splitting method.14 The space step was \( \Delta x = 0.015 \text{ cm} \), and the time step was adaptively varied between 0.01 ms and 0.1 ms. The ionic current was modeled by integrating a model of Ca cycling developed by Shiferaw et al.,13 coupled with the canine action potential model of Fox et al.15 An illustration of the relevant ionic currents and Ca cycling machinery is shown in Figure 1A. Details of the mathematical formulation of the model are described in the online data supplement, available at http://circres.ahajournals.org. In addition, we modeled an isolated cell by integrating in time \( dV/dt = -I_{ion}/C_m \) and two electrotonically coupled cells, with voltage \( V_1 \) in cell 1 and \( V_2 \) in cell 2, by integrating the two coupled equations \( dV_1/dt = -I_{ion}/C_m + \kappa(V_2 - V_1) \) and \( dV_2/dt = -I_{ion}/C_m + \kappa(V_1 - V_2) \) with a coupling strength \( \kappa = D/\Delta x^2 \).

Data Analysis

To determine the spatial distribution of Cai transient alternans, we computed the difference in the peak Cai transient from one beat to the next defined as:

\[ \Delta C_{ai}(x,n) = \frac{(-1)^n}{2} \left( c_{n+1}(x) - c_n(x) \right) \]

where \( n \) is the beat number, and \( c_n(x) \) is the peak of the Cai transient measured at position \( x \) along the cable. The factor of \( (-1)^n \) was introduced such that the amplitude of alternans does not change sign at every beat. The spatiotemporal evolution of Cai transient alternans was visualized by plotting \( \Delta C_{ai}(x,n) \). Likewise, the spatiotemporal distribution of APD alternans is measured using the quantity:

\[ \Delta APD(x,n) = \frac{(-1)^n}{2} \left[ \text{APD}_{n+1}(x) - \text{APD}_n(x) \right] \]

With these definitions, the nodes separating spatially out-of-phase regions of Cai transient and APD alternans are located at the positions along the cable where \( \Delta C_{ai}(x,n) = 0 \) and \( \Delta APD(x,n) = 0 \), respectively. Positive and negative values of \( \Delta C_{ai} \) and \( \Delta APD \) on each side of these contours correspond to opposite phases of alternans.

Pacing Protocol

To study the dynamics of alternans, we paced an isolated cell, two coupled cells, and a 1D cable using a current stimulus of duration 1 ms and amplitude of 50 \( \mu A/cm^2 \). In the cable, we paced the left-most five cells to ensure propagation. In all cases, we applied a protocol where current was applied for 100 beats at a pacing cycle length (PCL) of 500 ms, after which the PCL was decreased by two ms every 50 beats. Following the method of Riccio et al.,16 we will refer to this protocol as the “dynamic pacing protocol.”

Realistic Cell-to-Cell Fluctuations

Spatial and temporal heterogeneities are intrinsic properties of cardiac tissue. In this article, we model cell-to-cell variations by assuring that the constituents of all the cells are identical but taking into account the stochastic fluctuations of ion currents in the cell. In particular, we simulate the total current pumping Ca from the cytosol into the sarcoplasmic reticulum (SR), by modeling the stochastic properties of a finite number of SR Ca\(^{2+}\)-ATPase (SERCA) pumps. Our approach, following the method of Fox and Lu,17 is to explicitly model the uptake current via a Langevin equation with a noise term that depends explicitly on the number of channels in the cell. Details of the current dynamics and noise formulation are given in the online data supplement.
Cellular Alternans and Bidirectional Coupling

In this work, we study the dynamics of alternans induced by unstable Ca cycling. Alternans is induced in our model by increasing the steepness, at high SR loads, of the function relating SR Ca release to SR Ca load, as described theoretically,\textsuperscript{13,15} and experimentally by Diaz et al.\textsuperscript{19} A key feature of the model is that when the cell is paced rapidly with a periodic AP clamp, Ca transient alternans develop,\textsuperscript{11} as shown experimentally in isolated rabbit myocytes\textsuperscript{12} and also in guinea pig myocytes.\textsuperscript{20} Moreover, when the Ca transient alternates, APD alternates secondarily, because Ca\textsubscript{2} affects ionic currents that regulate APD, primarily via the Na\textsubscript{+}/Ca\textsubscript{2}\textsuperscript{+} exchanger and the L-type Ca current inactivation rate. Hereafter, we will refer to alternans induced via a steep SR Ca release-load gain as Ca-driven alternans.

The relationship between APD and Ca transient alternans is determined on the bidirectional coupling between Ca and V\textsubscript{m}. First, let us consider how the Ca transient at a given beat is influenced by the membrane voltage. This coupling is determined by the well-established property of graded SR Ca release,\textsuperscript{21–23} whereby the amount of SR Ca released is graded with respect to the whole cell L-type Ca current. The availability of the L-type Ca current at a given beat depends critically on the previous diastolic interval (DI). A larger DI gives more time for recovery of L-type Ca channels at the resting membrane potential. Thus, in our physiologically based cell model, graded release requires that the peak of the Ca transient increases in response to an increase of DI at the previous beat, as illustrated in Figure 1B. We refer to this relationship as graded release coupling. Note that although this coupling is typically observed in cardiac myocytes, other factors may override its effect. For example, I\textsubscript{Na} may shorten APD while, at the same time, increasing the driving force for early Ca entry via the L-type Ca current potentiate SR Ca release.\textsuperscript{24,25} However, we did not study this case here.

Next, we consider the unidirectional coupling of Ca on V\textsubscript{m} (Ca\textsubscript{2}→V\textsubscript{m} coupling). Two distinct cases can be distinguished. The first, referred to as positive Ca\textsubscript{2}→V\textsubscript{m} coupling, illustrated in Figure 1C, corresponds to the case in which an increase in the peak Ca\textsubscript{2} transient amplitude lengthens the APD. The second, referred to as negative Ca\textsubscript{2}→V\textsubscript{m} coupling (Figure 1C), corresponds to the case in which an increase in the peak Ca\textsubscript{2} transient amplitude shortens the APD. Both the sign and the magnitude of the coupling is dictated by the relative contributions of the L-type Ca current and the Na\textsubscript{+}/Ca\textsubscript{2}\textsuperscript{+} exchange current to APD. A larger Ca\textsubscript{2} transient tends to inactivate the whole cell L-type Ca current more rapidly via Ca-induced inactivation, which tends to shorten the APD. However, a large Ca\textsubscript{2} transient concomitantly increases the net inward current from extracellular Na\textsubscript{+}/Ca\textsubscript{2}\textsuperscript{+} exchange, which tends to prolong APD. The Ca\textsubscript{2}→V\textsubscript{m} coupling was varied in our ionic model by changing the inactivation rate of cells with negative Ca\textsubscript{2} transient in cell 1 and cell 2. In Figure 1B, we show APD alternans (indicated by the vertical green line), which is earlier than the onset of alternans for the single-cell case (PCL=340 ms), and once alternans developed, ∆APD always had an opposite sign compared with ∆Ca\textsubscript{2} (electromechanically out of phase).

Electromechanically In-Phase and Out-of-Phase Alternans

The bidirectional coupling between the APD and the Ca transient determines the relative phase of APD and Ca transient alternans during steady-state pacing.\textsuperscript{26–28} For Ca-driven alternans, positive Ca\textsubscript{2}→V\textsubscript{m} coupling always leads to electromechanically in-phase alternans, as illustrated in Figure 1D, where a long→short→long APD pattern corresponds to a large-small-large Ca\textsubscript{2} transient. In contrast, negative Ca\textsubscript{2}→V\textsubscript{m} leads to electromechanically out-of-phase alternans (Figure 1D), where a long→short→long APD corresponds to a small-large-small Ca\textsubscript{2} transient. When alternans are attributable to an instability of V\textsubscript{m} dynamics, steady-state electromechanical alternans are always in phase. This is because, in this case, the Ca transient is slaved to V\textsubscript{m} via the graded release coupling (Figure 1B), so that Ca transient alternans is always in phase with APD alternans.

Results

Synchronization and Desynchronization of Two Coupled Cells

In this section, we study systematically the relationship between Ca\textsubscript{2}→V\textsubscript{m} coupling and Ca transient alternans, for the case when alternans are attributable to unstable Ca cycling. As a starting point, we first consider a simple system of two electrotonically coupled cells, with the main result that two cells alternate out of phase because of an intrinsic dynamical instability when the Ca\textsubscript{2}→V\textsubscript{m} coupling is negative. We then study the case of a cable of several hundred cells to show that the same instability mechanism leads to spatially discordant alternans that are formed independently of CV restitution.

Single-Cell Alternans

As a starting point, we first plotted the amplitude of APD alternans (ΔAPD) and Ca transient alternans (ΔCa\textsubscript{2}) as a function of PCL, for an isolated cell paced using the dynamic pacing protocol. The amplitude of alternans is measured after steady state is reached, using the 49th and 50th beats at each PCL. We considered both positive (Figure 2A) and negative (Figure 2B) Ca\textsubscript{2}→V\textsubscript{m} coupling by adjusting the inactivation kinetics of the L-type Ca current, as described in the online supplement. As shown in Figure 2A, for positive Ca\textsubscript{2}→V\textsubscript{m} coupling, alternans onset occurred at PCL=315 ms and APD alternans was in phase with Ca\textsubscript{2} transient alternans, ie, ΔAPD and ΔCa\textsubscript{2} always had the same sign after the bifurcation to alternans (electromechanically in phase). On the other hand, for the negative Ca\textsubscript{2}→V\textsubscript{m} coupling parameters, alternans onset was at PCL=340 ms, and once alternans developed, ΔAPD always had an opposite sign compared with ΔCa\textsubscript{2} (electromechanically out of phase).

Two Coupled Cells

We then paced two electrotonically coupled cells with the same model parameters used in the single-cell simulations. Here, both cells had identical parameters but differed only by small (≈0.1%) stochastic fluctuations in the intracellular Ca cycling dynamics. Fluctuations were incorporated as described in the methods section. In Figure 2C we show the amplitude of alternans for both cells as a function of pacing rate for the case of positive Ca\textsubscript{2}→V\textsubscript{m} coupling ie, same model parameters as used in Figure 2A. As shown, alternans onset occurs at the same pacing rate as the single-cell case (PCL=315 ms), and alternans phase is synchronized, ie, ΔAPD and ΔCa\textsubscript{2} had the same sign in cell 1 and cell 2. In Figure 2D, we applied the dynamic pacing protocol to a pair of cells with negative Ca\textsubscript{2}→V\textsubscript{m} coupling ie, same model parameters as in Figure 2B. As shown, Ca\textsubscript{2} transient alternans occurred simultaneously in both cells at a PCL=355 ms (indicated by the vertical green line), which is earlier than the onset of alternans for the single cell (vertical black line). Also, APD alternans were not observed in both cells. Furthermore, Ca\textsubscript{2} transient alternans in cell 1 and cell 2 are out of phase. That is, ΔCa\textsubscript{2} is positive in cell 1 and negative in cell 2, as shown. Moreover, despite the significant degree of Ca\textsubscript{2} transient alternans, APD did not alternate in either cell. This effect is attributable to electrotonic interaction between the two cells, such that the APD shortening effect of a large Ca\textsubscript{2} transient in one cell was balanced by the APD prolonging effect of the opposite phase small Ca\textsubscript{2} transient in the other cell.

Formation of Spatially Discordant Alternans in a Cable of Many Cells

The simplified system studied above illustrates the rich dynamical behavior that can arise by coupling two cardiac
cells. Here, we study the case of a cable of many cells (200 cells) that is paced at 1 end. For the case of positive $Ca_{i} ightarrow V_m$ coupling, Figure 3A through 3C shows the steady-state spatial distribution of both $\Delta$APD and $\Delta$Cai at 3 different PCLs. As shown, alternans was spatially synchronized at PCL = 310 ms. However, at a more rapid stimulation rate (PCL = 280 ms), spatially discordant alternans formed spontaneously. In this case, both Ca and APD alternans were electromechanically in phase for all cells along the cable but reversed phase spatially at a node along the cable.

As shown in Figure 3D through 3F, we repeated the above simulation with negative $Ca_{i} ightarrow V_m$ coupling parameters. Alternans amplitude was 0 across the cable at a PCL of 400 ms, but as the PCL was gradually shortened to 340 ms, spatially discordant alternans formed spontaneously. In this case, both Ca and APD alternans were electromechanically in phase for all cells along the cable but reversed phase spatially at a node along the cable.

Figure 2. Alternans dynamics during the dynamic pacing protocol. A, Steady-state normalized amplitude of APD ($\Delta$APD) and Cai transient ($\Delta$Cai) alternans as a function of PCL for an isolated cardiac cell paced using the dynamic pacing protocol. Here, the amplitude of alternans is normalized to the maximum value. The cell model is adjusted so that the $Ca_{i} ightarrow V_m$ coupling is positive. Here, alternans amplitude is measured by subtracting the APD and peak Cai at the 50th and 49th beats at the given PCL. The onset of alternans is denoted by the vertical dashed line. B, Same simulation as in A using cell model parameters with negative $Ca_{i} ightarrow V_m$ coupling. C, Amplitude of alternans within two electrotonically paced cardiac cells. Here, the cell model used is the same as in A. D, Same simulation as in C, using the same model parameters as in B. Notice that the onset of alternans (vertical green line) occurs at an earlier PCL than in the isolated cell (vertical black line).

Role of Conduction Velocity Restitution
To uncover the mechanism that leads to the formation of spatially discordant alternans, we also computed the spatial distribution of CV during the discordant alternans patterns shown in Figure 3C and 3F. Figure 4A shows the spatial distribution of CV along the cable during the steady-state pattern shown in Figure 3C. Here, the dashed/solid line corresponds to CV along the cable for the 49th/50th paced beat. As shown, the CV of the pulse changed by roughly 2 cm/ms as it traveled down the cable. Figure 4B shows the CV restitution curve computed for the model. The two vertical dashed lines mark the range of DI engaged along the cable. Figure 4C shows the spatial distribution of CV during the dynamic simulation protocol 16 times and found that the average spacing between $\Delta$Ca nodes, at PCL = 340 ms, was roughly 0.045 ± 0.004 cm, whereas the average spacing of $\Delta$PD nodes was 0.6 ± 0.1 cm.
Alternans pattern shown in Figure 3F. In this case, CV did not vary along the cable during discordant alternans. We also plotted the maximum range of DI engaged and found that indeed CV restitution was flat over the range of DIs engaged at that pacing rate (PCL = 340 ms).

Discussion

**Ca**<sub>3</sub>**V**<sub>m** Coupling Determines the Relative Synchrony of Two Coupled Cells

The main finding of this work is that the bidirectional coupling between **Ca**<sub>3</sub> and **V**<sub>m** dictates whether two coupled cells are synchronized or desynchronized. In particular, we find that if the **Ca**<sub>3**→**V**<sub>m coupling is positive, such that alternans at the single-cell level is electromechanically in phase, then **Ca** transient alternans in neighboring cells will synchronize. On the other hand, if the **Ca**<sub>3**→**V**<sub>m coupling is negative such that single-cell alternans is electromechanically out of phase, then **Ca** transient alternans desynchronize.

Mechanistic Explanation of Desynchronization Mechanism

To understand the mechanism for desynchronization, we analyze how **Ca**<sub>3**→**V**<sub>m coupling, along with electrotonic coupling, dictates the evolution of **Ca** alternans. In Figure 5A, we illustrate the **V**<sub>m** and **Ca** transients of two independent cells

![Figure 3](image-url) Distribution of **Ca** transient and APD alternans amplitude along a cable of cells with the same model parameters as in Figure 2A (positive **Ca**<sub>3**→**V**<sub>m coupling. During the dynamic pacing protocol alternans amplitude was measured for each cell by computing ∆**Ca** and ∆APD from the 49th and 50th beats at PCL = 400 ms (A), PCL = 310 ms (B), and PCL = 280 ms (C). D through F, Same simulation using the model parameters used in Figure 2B (negative **Ca**<sub>3**→**V**<sub>m coupling) for PCL = 400, 358, 340 ms. In all graphs, ∆**Ca** and ∆APD are normalized to the maximum value observed during the dynamic pacing protocol.

![Figure 4](image-url) CV during discordant alternans. A, CV computed for the 49th (dashed line) and 50th (solid line) paced beats at PCL = 280 ms, during the same simulation shown in Figure 3C. B, CV restitution curve for the ionic model parameters used in Figure 3C. The vertical dashed lines marks the maximum range of DI engaged along the cable during the 50th beat. C, CV measured along the cable for the 2 beats used to compute Figure 3F. D, CV restitution curve for the model parameters used in Figure 3F. Again, the dashed lines denote the range of DI engaged during the discordant alternans pattern shown in Figure 3F.

![Figure 5](image-url) Schematic illustration of the desynchronization/synchronization mechanism when single-cell alternans are electromechanically in phase and out of phase. A, **V**<sub>m** and **Ca** vs time for neighboring cells 1 and 2 with negative **Ca**<sub>3**→**V**<sub>m coupling. The cells are uncoupled before time **t**<sub>1**, after which they are coupled electrotonically. The red line denotes the time evolution after electrotonic coupling at time **t**<sub>2**. B, Same illustration as A with positive **Ca**<sub>3**→**V**<sub>m coupling.
(cell 1 and cell 2) that are out of phase and which are then electrotonically coupled at time $t_1$. Here, we assume negative $\text{Ca}_r \rightarrow V_m$ coupling so that alternans is electromechanically discordant in both cells. The black and red lines depict schematically the time evolution of $V_m$ and $\text{Ca}_i$ in the absence and in the presence of electrotonic coupling, respectively. Two key factors determine the subsequent evolution of alternans:

**Electrotonic Coupling of $V_m$**

This effect is illustrated with the red $V_m$ traces after time $t_1$. Here, electronic coupling simply averages the APD of the 2 cells, thereby forcing them to have an identical $V_m$ time course. Thus, the APD in cell 1 and cell 2 shorten and lengthen, respectively, in comparison with the APD that would have occurred if the 2 cells were not coupled.

**Graded Release Coupling**

The change in APD between times $t_1$ and $t_2$ will influence the amount of Ca release at time $t_2$ (the next beat) via the graded release coupling illustrated in Figure 1B. Hence, the large DI in cell 1 will lead to a larger Ca release on the next beat, whereas the smaller DI in cell 2 will yield a smaller Ca release. Note that APD shortening/lengthening will change the Ca influx into the cell and thus influence the amount of Ca released. However, we find that this effect is much smaller than that induced by graded release coupling because the change in SR load over 1 beat, caused by the change in APD, is relatively small in the present model.

The combined effects of 1 and 2 above is to cause the Ca transient of cell 1 and cell 2 to be more markedly different on the next beat, ie, the large release is larger, whereas the small release is smaller. Hence, the difference in phase of $\text{Ca}_i$ transient alternans in the two neighboring cells is amplified from one beat to the next. This mechanism explains why the two coupled cells in our simulations exhibited out-of-phase $\text{Ca}_i$ transient alternans during dynamic pacing. There, even very small differences in alternans phase, caused by stochastic fluctuations in Ca cycling properties, are amplified from beat to beat so that Ca transient alternans are forced to alternate with opposite phase. Hence, even though all cells in the cable have identical ionic properties, small ($<0.1\%$) stochastic differences are amplified dynamically by the above mechanism, to yield spatially discordant patterns after many beats.

On the other hand, in the case of positive $\text{Ca}_r \rightarrow V_m$ coupling, where alternans are electromechanically in phase, applying the same arguments given above shows that Ca transient alternans in neighboring cells (Figure 5B) tend to synchronize. Hence, in this case, small cell-to-cell differences between neighboring cells are diminished from beat to beat to yield spatially discordant alternans. In this case, steep CV restitution must be invoked to induce spatially discordant alternans via a different mechanism.

**Initiation of Spatially Discordant Alternans in Homogeneous Tissue**

When the $\text{Ca}_r \rightarrow V_m$ coupling is positive, spatially discordant alternans form only when CV alternates from beat to beat, ie, discordant alternans are associated with substantial CV variation along the cable. This mechanism for the induction of spatially discordant alternans is well known and has been extensively studied. In these studies, it was shown that discordant alternans form in homogeneous tissue because of the interaction between APD alternans, induced via a steep APD restitution curve, and CV alternans. Similarly, here, the same interaction between APD alternans and steep CV restitution suffices to initiate spatially discordant alternans in homogeneous tissue for short enough PCL. The main difference is that the APD alternans are driven by $\text{Ca}_i$ alternans, which are attributable to an instability of Ca cycling, instead of an instability of $V_m$ dynamics.

In the case of negative $\text{Ca}_r \rightarrow V_m$ coupling, we find that Ca transient alternans form into discordant patterns with many out-of-phase regions in the cable. These patterns were not dictated by CV restitution because the measured variations of CV along the cable were negligible. In this case, the spatial patterns are formed by the same mechanism that drives the $\text{Ca}_i$ transient of 2 neighboring cells out of phase. That is, small cell-to-cell differences are amplified from beat to beat by the desynchronization effect of negative bidirectional coupling of $V_m$ and $\text{Ca}_i$. Hence, arbitrarily small stochastic fluctuations grow and develop into spatially discordant alternans were the Ca transient of neighboring cells can alternate out of phase. The consequences of this desynchronization mechanism on subcellular scales, where Ca diffusion needs to be taken into account, has been the subject of a recent theoretical study.
region of a size comparable to the diffusion scale of $V_m$, this tissue scale sets the spacing between nodes of APD alternans.

**Robustness of the Simulation Findings**

To what extent do the simulation findings depend on the detailed formulation of the ionic model used in the present study? To answer this question, it is necessary to outline the essential physiological ingredients that underlie the desynchronization mechanism illustrated in Figure 5A. There are two essential conditions:

**Alternans at the Single-Cell Level Must Be Attributable to a Dynamical Instability of Ca Cycling**

This is an essential requirement because Ca, transient alternans must not be slaved to APD alternans to be able to desynchronize on a cellular scale and hence must not originate from an instability of $V_m$ dynamics. If the latter is true, graded release coupling ensures that Ca, transient alternans are always electromechanically in phase with APD alternans.

**$Ca_{trans} \rightarrow V_m$ Coupling Must Be Negative**

This is the crucial requirement that ensures that Ca cycling alternans drive electromechanically out-of-phase APD alternans, so that the arguments illustrated in Figure 5A can be applied. Here, the important feature is that a large Ca, transient shortens the APD at the same beat. In this case, if alternans are attributable to unstable Ca cycling, a large-small-large Ca, transient is always associated with a short-long-short APD.

We expect our findings to be robust in that any detailed ionic model that satisfies these conditions should exhibit qualitatively similar patterns of alternans as in the present simulations. This robustness, however, does not preclude the fact that the above conditions could in general be fulfilled by different ionic mechanisms.

**Concluding Remarks**

To test experimentally the novel predictions of this study, it is necessary to identify cardiac cells in which alternans are electromechanically out of phase. Our main prediction is that Ca alternans drive electromechanically out-of-phase APD alternans. Ca alternans must not be slaved to APD alternans to be able to desynchronize on a cellular scale and hence must not originate from an instability of $V_m$ dynamics. If the latter is true, graded release coupling ensures that Ca, transient alternans are always electromechanically in phase with APD alternans.

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$Ca_{trans} \rightarrow V_m$ Coupling Must Be Negative

This is the crucial requirement that ensures that Ca cycling alternans drive electromechanically out-of-phase APD alternans, so that the arguments illustrated in Figure 5A can be applied. Here, the important feature is that a large Ca, transient shortens the APD at the same beat. In this case, if alternans are attributable to unstable Ca cycling, a large-small-large Ca, transient is always associated with a short-long-short APD.

We expect our findings to be robust in that any detailed ionic model that satisfies these conditions should exhibit qualitatively similar patterns of alternans as in the present simulations. This robustness, however, does not preclude the fact that the above conditions could in general be fulfilled by different ionic mechanisms.

Concluding Remarks

To test experimentally the novel predictions of this study, it is necessary to identify cardiac cells in which alternans are electromechanically out of phase. Our main prediction is that if a tissue of these cells is paced into alternans, then spatially discordant alternans should organize into complex spatiotemporal patterns similar to those shown in Figure 3E and 3F. A crucial feature of these patterns is that Ca, transient alternans can form discordant patterns on the cellular scale, whereas APD alternans vary over a much larger length scale. In contrast, if Ca and APD are electromechanically in phase, then the spatial distribution of Ca alternans will be similar to that of APD alternans.

From the experimental stand point both in-phase and out-of-phase electromechanical alternans have been observed in different cell types and under a variety of experimental conditions. For example, in rabbit cardiac myocytes alternans are always electromechanically in phase with APD alternans. However, this is not the case in rat myocytes paced with a clamped AP waveform. These studies demonstrate that Ca alternans can change phase over subcellular length scales, much shorter than the diffusive length scale of $V_m$.

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Disclosures

None.

References

Spatially Discordant Alternans in Cardiac Tissue: Role of Calcium Cycling

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Fluctuations due to ion channel kinetics

Cell-to-cell differences may arise due to different expression levels of membrane ion channels or Ca cycling proteins. Moreover, even in the idealized “perfectly homogeneous” situation where all cells have the same constituents (same expression level of ion channels, same spatial distribution of T-tubules, etc), the dynamics of two nearby cells can still differ due to the inherently stochastic nature of the transitions between different individual channel states. In situations where the dynamics of membrane voltage and calcium cycling are stable, the effect of these stochastic fluctuations is negligible when averaged over a typical number of 100,000-1,000,000 channels of a given type. For this reason, stochastic effects are generally neglected in mathematical models of tissue scale behavior. In contrast, in situations where the dynamics is unstable, small fluctuations are exponentially amplified in time and play a crucial role in triggering instabilities. This is especially true in the present study where, as we shall show, the instability causing discordant Ca transient alternans is manifested on a cellular scale.

We model fluctuations of ionic processes involved in the Ca cycling dynamics by simulating the stochastic properties of $N_{up}$ SR Ca$^{2+}$-ATPase (SERCA) channels, which pump Ca from the cytosol into the SR. Following Fox and Lu $^1$ we model fluctuations by constructing a Langevin equation for the fraction $p(t)$ of SERCA channels which are open at time $t$. The total ionic flux into the SR is given by

$$J_{up}(t) = N_{up} \cdot p(t) \cdot i,$$  \hfill (1)

where $i$ is the single channel flux. We assume that each SERCA channel obeys a simple two state kinetic scheme $C \leftrightarrow O$, where the open rate is $k_+ c_i^2$, where $c_i$ is the Ca concentration in the cytosol, and the closing rate is $k_-$. The open probability then satisfies the Langevin equation

$$\frac{dp}{dt} = k_+ c_i^2 (1 - p) - k_- p + \eta(t),$$  \hfill (2)

where, following Ref. 1, the noise $\eta(t)$ satisfies the correlation function...
This Langevin equation allows us to find the total current flux into the SR given by Eq. 1, along with fluctuations due to the stochastic properties of the individual SERCA channels. Notice, that the strength of the fluctuations decrease with increasing number of SERCA channels as $\sim 1/\sqrt{N_{up}}$.

To make contact between the Langevin formulation and established models of the uptake flux, we follow Ref 2. were the uptake flux is modelled as an instantaneous function of cytosolic Ca given by

$$J_{up} = \frac{v_{up} c_i^2}{c_i^2 + c_{up}^2},$$  \hspace{1cm} (4)

where $v_{up}$ and $c_{up}$ are constants. These constants can be related to the parameters of the Langevin formulation using $v_{up} = N_{up} \cdot i$ and $c_{up}^2 = k_+ / k_-$. To fix all parameters we assume further that the channel kinetics are indeed fast and occur on a time scale of 1 ms, so that the time scale which governs the approach to equilibrium is $\tau = 1/(k_- (c_{up}^2 + c_i^2)) = 1 ms$. In this way all parameters used in Eq. 2 can be found in terms of model parameters used in well established formulations of the uptake flux. The values used for $v_{up}$ and $c_{up}$ are given in Table 3. To model noise we use a large number of channels $N_{up} = 10^6$. So that the magnitude of current fluctuations is roughly 0.1% of the average value.

It is important to emphasize that, in the context of the present study, the main role of fluctuations is to trigger dynamical instabilities. The instabilities, themselves, only depend on the deterministic dynamics of the model. Therefore, the main results of the present study turn out to be ostensibly independent of the way in which fluctuations are incorporated into the model. We chose to include current fluctuations at the cellular level, caused by the stochastic dynamics of ion channels at the molecular level, because these fluctuations are unavoidably present even when all cells have the same molecular constituents. From this

$$\langle \eta(t)\eta(t') \rangle = \frac{k_+ c_i^2 (1 - p) + k_- P}{N_{up}} \delta(t-t').$$  \hspace{1cm} (3)

\begin{align*}
\langle \eta(t)\eta(t') \rangle = \frac{k_+ c_i^2 (1 - p) + k_- P}{N_{up}} \delta(t-t').
\end{align*}
standpoint, we have incorporated the minimum magnitude of fluctuations applicable to spatially homogeneous tissue.

**Ionic Model**

The electrophysiology of an isolated cardiac myocyte was modeled using a recently developed model of Ca cycling by Shiferaw et al. \(^2\), which was incorporated into the ionic model of Fox et al. \(^3\). The ionic currents, along with the relevant aspects of the Ca cycling machinery, are illustrated in Fig. 1A. The dynamics of membrane voltage \((V_m)\) is described by the equation

\[
\frac{dV_m}{dt} = - \frac{1}{C_m} \left( I_{\text{ion}} + I_{\text{stim}} \right),
\]  

(5)

where \(I_{\text{ion}}\) is the total membrane current density, \(I_{\text{stim}}\) is the stimulus current, and where \(C_m\) is the cell membrane capacitance. Following Fox et al. \(^3\) all ion currents are computed for \(1 \mu F\) of cell membrane capacitance and have units of \(\mu A/\mu F\). The total membrane current is given by

\[
I_{\text{ion}} = I_{Na} + I_{To} + I_{Kr} + I_{Ks} + I_{Kp} + I_{K1} + I_{Ca} + I_{NaCa}.
\]  

(6)

The original Fox et al. \(^3\) model included the ion currents \(\{I_{Na}, I_{Ca}, I_{pCa}, I_{CaK}\}\), which we have omitted since these currents are small and have a negligible effect on the dynamical features of the model.

Ca cycling was simulated using a model due to Shiferaw et al. \(^2\). This model describes Ca released from the sarcoplasmic reticulum (SR) as a summation of local release fluxes distributed throughout the cell. The equations for Ca cycling are:
\[
\frac{dc_s}{dt} = \frac{\beta s v_l}{v_s} \left[ J_{rel} = \frac{c_s - c_i}{\tau_s} + J_{Ca} + J_{NaCa} \right],
\]
\[
\frac{dc_i}{dt} = \beta_i \left[ \frac{c_s - c_i}{\tau_s} - J_{up} \right],
\]
\[
\frac{dJ_{rel}}{dt} = -J_{rel} + J_{up},
\]
\[
\frac{dc_j}{dt} = \frac{c_j - c_j'}{\tau_j},
\]
\[
\frac{dJ_{rel}'}{dt} = g J_{Ca} \cdot Q(c_j') - \frac{J_{rel}}{\tau_r},
\]

where \(c_s, c_i, c_j\) and \(c_j'\) are the average concentrations of free Ca \(i\) in a thin layer just below the cell membrane (the submembrane space), in the cytosol, and the SR, with volumes \(v_s, v_i\) and \(v_r\) respectively. Here the SR volume includes both the junctional SR (JSR) and the network SR (NSR). Also \(c_j'\) is the average JSR concentration within dyadic junctions in the whole cell. The factors \(\beta_j\) and \(\beta_i\) describe instantaneous buffering to Calmodulin, the SR membrane, and Troponin C.

All Ca fluxes are divided by \(v_j\) and have units of \(\mu M / ms\), which can be converted to units of \(\mu A / \mu F\) using the conversion factor \(nFv_i / C_m\), where \(n\) is the ionic charge of the charge carrier, and where \(F\) is Faraday's constant. Thus, ionic fluxes can be converted to currents using

\[
I_{Ca} = -2\alpha J_{Ca}, \quad I_{NaCa} = \alpha J_{NaCa},
\]

where \(\alpha = Fv_i / C_m\), and where the ion currents are in units of \(\mu A / \mu F\). The detailed formulation for the current fluxes are given bellow.
Model Properties

Instability Mechanisms

Ca\textsubscript{i} transient alternans can occur due to Ca cycling dynamics alone as shown in experiments with a single cell paced with a periodic voltage clamp waveform\textsuperscript{4}. It has been argued both theoretically\textsuperscript{2,5}, and in recent experiments\textsuperscript{6}, that this period doubling instability arises due to a steep dependence of SR Ca release on SR Ca\textsubscript{i} load. The steepness of the SR release vs. SR Ca load, at high loads, is determined by the parameter

\[ u = \frac{dQ}{dc_j}. \]  

(9)

For a large enough value of \( u \) the model produces alternans that is due to a nonlinear dynamical instability of calcium cycling.

Alternans can also occur via instability in the voltage dynamics, independent of Ca\textsubscript{i} cycling. In this scenario alternans can be induced by a steep APD restitution curve, which is the functional relationship between the APD and the previous DI. This is the classic mechanism for APD alternans\textsuperscript{7}, which, due to the bi-directional coupling between V\textsubscript{m} and Ca\textsubscript{i}, will also lead to Ca\textsubscript{i} transient alternans. In the ionic model that we have used, this instability in the voltage dynamics is sensitive to the recovery from inactivation of the L-type Ca current

\[ I_{Ca} = d \cdot f \cdot f_{Ca} \cdot i_{Ca}, \]  

(10)

where \( i_{Ca} \) is the single channel current and \( d(f) \) is the fast(slow) voltage-dependent activation and inactivation gate respectively. For the range of pacing rates investigated in this study increasing the time constant \( \tau_f \) of the \( f \) gate steepens the APD restitution and promotes alternans. Ca\textsubscript{i} induced inactivation of the L-type Ca current is modeled by the \( f_{Ca} \) gate which relaxes to a steady state value.
\[ f_{Ca}^\infty = \frac{1}{1 + (c_s / \tilde{c}_s)^\gamma}, \]  

(11)

where the exponent \( \gamma \) controls the degree of \( Ca_i \) induced inactivation.

**Alternans properties**

The ionic model used in our simulation is adjusted so that at rapid rates, alternans can occur either due to purely \( V_m \) dynamics while \( Ca_i \) cycling is stable, or vice versa. The former case, referred to as the \( V_m \)-driven model, is obtained by making APD restitution slope steep by setting the time constant of recovery from inactivation of the L-type \( Ca \) channel to be large (\( \tau_f = 55ms \)), while the slope of the SR release vs SR-load is kept small (\( u = 2ms^{-1} \)). When alternans are due to unstable \( Ca \) cycling, we have two cases referred to as positive and negative \( Ca_i \rightarrow V_m \) coupling. For both cases unstable \( Ca_i \) cycling is induced by making the SR release vs. SR-load large (\( u = 8-14 \text{ ms}^{-1} \)), while keeping APD restitution slope flat by making the time constant of recovery small (\( \tau_f = 40-45 \text{ ms} \)). To change the sign of the \( Ca_i \rightarrow V_m \) coupling we adjust \( \gamma \), so that \( \gamma = 0.7 \) and \( \gamma = 1.5 \) give positive and negative coupling respectively. The rationale here is that when \( Ca_i \) induced inactivation is enhanced by increasing \( \gamma \), then negative \( Ca_i \rightarrow V_m \) is favored since the negative feedback of \( Ca_i \) on APD is increased.

**CV restitution**

The dynamics of cardiac wave propagation is governed by the CV restitution curve, which relates the speed of a planar wave to the preceding diastolic interval (DI). The CV restitution curve is primarily dependent on the kinetics of the Na current, formulated as

\[ I_{Na} = g_{Na} \cdot m^3 \cdot h \cdot j \cdot (V - E_{Na}) \]  

(12)

where \( m \) models the fast activation, while \( h \) represents the fast inactivation of the sodium current. The slow recovery from inactivation is governed by the gate \( j \), which is
where \( \tau_j \) governs the time constant of recovery. In order to generate spatially discordant alternans via the mechanism proposed by Qu et al.\(^8\) and Watanabe et al.\(^9\), we found it necessary to control the shape of the CV restitution curve of the Fox et al.\(^3\) ionic model. In order to accomplish this we have increased the time constant of the \( j \) gate by a factor of two (\( \tau_j \rightarrow 2\tau_j \)).

**Detailed formulation of ionic model**

**The fast sodium current (\( I_{Na} \)).**

\[
I_{Na} = g_{Na} m^3 h j (V - E_{Na})
\]

\[
E_{Na} = \frac{RT}{F} \ln \left( \frac{[Na^+]_o}{[Na^+]_i} \right)
\]

\[
\frac{dm}{dt} = \alpha_m (1 - m) - \beta_h m
\]

\[
\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h
\]

\[
\frac{dj}{dt} = \alpha_j (1 - j) - \beta_j j
\]

\[
\alpha_m = 0.32 \frac{V + 47.13}{1 - e^{-0.1(V+47.13)}}
\]

\[
\beta_m = 0.08 e^{-V/11}
\]
\[
\alpha_n = 0.135e^{(V-80)/-6.8}
\]
\[
\beta_n = \frac{7.5}{1 + e^{-0.1(V+11)}}
\]
\[
\alpha_f = \frac{0.175e^{(V+100)/-23}}{1 + e^{0.15(V+79)}}
\]
\[
\beta_f = \frac{0.3}{1 + e^{-0.1(V+32)}}
\]

The slow time constant of recovery of the sodium channel is given by \( \tau_j = \frac{1}{2}(\alpha_j + \beta_j) \). To model steep CV restitution we increase \( \tau_j \) by a factor of two i.e. \( \alpha_j \rightarrow \alpha_j / 2 \) and \( \beta_j \rightarrow \beta_j / 2 \).

**The transient outward K\(^+\) current (\( I_{to} \)):**

\[
I_{to} = g_{to}X_{to}Y_{to}(V - E_K)
\]
\[
E_K = \frac{RT}{F}\ln \left( \frac{[K^+]_o}{[K^+]_i} \right)
\]
\[
\frac{dX_{to}}{dt} = \alpha_{X_{to}}(1 - X_{to}) - \beta_{X_{to}}X_{to}
\]
\[
\frac{dY_{to}}{dt} = \alpha_{Y_{to}}(1 - Y_{to}) - \beta_{Y_{to}}Y_{to}
\]
\[
\alpha_{X_{to}} = 0.04516e^{0.03577V}
\]
\[
\beta_{X_{to}} = 0.0989e^{-0.063237}
\]
\[
\alpha_{Y_{to}} = \frac{0.05415e^{(V+3.5)/-5}}{1 + 0.051335e^{(V+3.5)/-5}}
\]
\[
\beta_{Y_{to}} = \frac{0.005415e^{(V+3.5)/5}}{1 + 0.051335e^{(V+3.5)/5}}
\]

**Inward rectifier K\(^+\) current (\( I_{K1} \)):**
\[ I_{K_{1}} = g_{Kr}K_{1}^{\infty} \frac{[K^{+}]_{o}}{[K^{+}]_{o} + 13}(V - E_{K}) \]
\[ K_{1}^{\infty} = \frac{1}{2 + e^{\frac{1.62F}{RT}(V - E_{K})}} \]

The Rapid component of the delayed rectifier K\(^{+}\) current \((I_{Kr})\):

\[ I_{Kr} = g_{Kr} \sqrt{\frac{[K^{+}]_{o}}{4}}x_{Kr}R(V)(V - E_{K}) \]
\[ R(V) = \frac{1}{1 + 2.5e^{0.1(V + 28)}} \]
\[ dx_{Kr} \]
\[ dt = \frac{x_{Kr}^{\infty} - x_{Kr}}{\tau_{Kr}} \]
\[ x_{Kr}^{\infty} = \frac{1}{1 + e^{-2.182 - 0.1819V}} \]
\[ \tau_{Kr} = 43 + \frac{1}{e^{-5.495 + 0.1601V}} + e^{-7.677 - 0.0128V} \]

The slow component of the delayed rectifier K\(^{+}\) current \((I_{Ks})\):

\[ I_{Ks} = g_{Ks}x_{Ks}^{2}(V - E_{Ks}) \]
\[ dx_{Ks} \]
\[ dt = \frac{x_{Ks}^{\infty} - x_{Ks}}{\tau_{Ks}} \]
\[ x_{Ks}^{\infty} = \frac{1}{1 + e^{-(V - 16)/13.6}} \]
\[ \tau_{Ks} = \frac{1}{\left( 0.0000719(V - 10) + 0.00031(V - 10) \right)} \]
\[ E_{Ks} = \frac{RT}{F} \ln \left( \frac{[K^{+}]_{o} + 0.01833[Na^{+}]_{o}}{[K^{+}]_{o} + 0.01833[Na^{+}]_{o}} \right) \]
The plateau potassium current ($I_{Kp}$):

$$I_{Kp} = g_{Kp} K_{Kp}(V)(V - E_K)$$

$$K_{Kp}(V) = \frac{1}{1 + e^{(7.488 - V)/5.98}}$$

The sodium-calcium exchanger ($J_{NaCa}$):

$$J_{NaCa} = g_{NaCa} \frac{1}{K_{m,Na}^2 + [Na^+]_o} \frac{1}{K_{m,Ca}^2 + [Ca^{2+}]_o} \frac{e^{2a}[Na^+]_o [Ca^{2+}]_o - e^{(\xi - 1)a}[Na^+]_o c_s}{1 + k_s e^{(\xi - 1)a}}.$$  

The L-type Ca$_i$ current flux ($J_{Ca}$):

$$J_{Ca} = -g_{Ca} d \cdot f \cdot f_{Ca} \cdot i_{Ca}$$

$$\frac{df}{dt} = \frac{f_{Ca} - f}{\tau_f}$$

$$\frac{df_{Ca}}{dt} = \frac{f_{Ca} - f_{Ca}}{\tau_{fca}}$$

$$f_{Ca} = \frac{1}{1 + e^{((V + 5)/6.24)}}$$

$$d_{Ca} = \frac{1}{1 + e^{-(V + 5)/6.24}}$$

$$f_{Ca}^{(\infty)} = \frac{1}{1 + (c_s/c_s)^{\gamma}}$$

$$i_{Ca} = \frac{4VF^2}{RT} \left( \frac{c_s e^{2\alpha} - 0.34 [Ca^{2+}]_o}{e^{2\alpha} - 1} \right)$$

with $\alpha = VF / RT$. 
The SR \(Ca_i\) release vs. SR \(Ca_i\) load relationship:

\[
Q(c'_j) = \begin{cases} 
0 & 0 < c'_j < 50, \\
1.5(c'_j - 50) & 50 < c'_j < 110, \\
u c'_j + s & c'_j > 110, 
\end{cases}
\]

where the flux \(Q(c'_j)\) is in units of \(10^{-6} \mu\text{M}/\text{ms}\), and where \(s = 90 - 110u\).

**Nonlinear buffering:** Instantaneous buffering of calcium to SR, calmodulin, and Troponin C sites in the submembrane and bulk myoplasm are accounted for by the functions

\[
\beta_s = \left(1 + \frac{B_{SR}K_{SR}}{(c_s + K_{SR})^2} + \frac{B_{cd}K_{cd}}{(c_s + K_{cd})^2} + \frac{B_{T}K_{T}}{(c_s + K_{T})^2}\right)^{-1},
\]

\[
\beta_i = \left(1 + \frac{B_{SR}K_{SR}}{(c_i + K_{SR})^2} + \frac{B_{cd}K_{cd}}{(c_i + K_{cd})^2} + \frac{B_{T}K_{T}}{(c_i + K_{T})^2}\right)^{-1}.
\]

**TABLES**
The parameters used in the \(Ca_i\)-driven and \(V_m\)-driven cell models are given below. Parameters in square and curly brackets correspond to the \(Ca_i\)-driven positive and negative coupling cell models respectively. Unbracketed parameters correspond to the \(V_m\)-driven cell model. Parameters where brackets are not shown are common to all models.
Table 1: cytosolic buffering parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_T$</td>
<td>Total concentration of Troponin C</td>
<td>70 µmol/l cytosol</td>
</tr>
<tr>
<td>$B_{SR}$</td>
<td>Total concentration of SR binding sites</td>
<td>47 µmol/l cytosol</td>
</tr>
<tr>
<td>$B_{Cd}$</td>
<td>Total concentration of Calmodulin binding sites</td>
<td>24 µmol/l cytosol</td>
</tr>
<tr>
<td>$K_T$</td>
<td>Dissociation constant for Troponin C</td>
<td>0.6 µM</td>
</tr>
<tr>
<td>$K_{SR}$</td>
<td>Dissociation constant for SR binding sites</td>
<td>0.6 µM</td>
</tr>
<tr>
<td>$K_{Cd}$</td>
<td>Dissociation constant for Calmodulin binding sites</td>
<td>7 µM</td>
</tr>
</tbody>
</table>

Table 2: SR release parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_r$</td>
<td>Spark lifetime</td>
<td>20 ms</td>
</tr>
<tr>
<td>$\tau_a$</td>
<td>NSR-JSR diffusion time constant</td>
<td>50 ms</td>
</tr>
<tr>
<td>$g$</td>
<td>Release current strength</td>
<td>$7.5 \times 10^4$ sparks/µM</td>
</tr>
<tr>
<td>$u$</td>
<td>Release slope</td>
<td>2 ms$^{-1}$ [8ms$^{-1}$][14ms$^{-1}$]</td>
</tr>
</tbody>
</table>

Table 3: Exchanger and uptake parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_{up}$</td>
<td>Uptake threshold</td>
<td>0.5 µM</td>
</tr>
<tr>
<td>$v_{up}$</td>
<td>Strength of exchanger</td>
<td>0.25 µM/ms</td>
</tr>
<tr>
<td>$g_{Na,Ca}$</td>
<td>Luo-Rudy II constant</td>
<td>6 µM/ms</td>
</tr>
<tr>
<td>$k_{sat}$</td>
<td>Luo-Rudy II constant</td>
<td>0.1</td>
</tr>
<tr>
<td>$\xi$</td>
<td>Luo-Rudy II constant</td>
<td>0.35</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
<td>Luo-Rudy II constant</td>
<td>87.5 mM</td>
</tr>
<tr>
<td>$K_{m,Ca}$</td>
<td>Luo-Rudy II constant</td>
<td>1.38 mM</td>
</tr>
</tbody>
</table>
Table 4: Physical constants and ionic concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$</td>
<td>Cell capacitance</td>
<td>$1.66 \times 10^{-4}$ µF</td>
</tr>
<tr>
<td>$V_i$</td>
<td>Cell volume</td>
<td>$2.58 \times 10^{-3}$ µl</td>
</tr>
<tr>
<td>$V_s$</td>
<td>Submembrane volume</td>
<td>$0.1V_i$</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
<td>96.5 C/mmol</td>
</tr>
<tr>
<td>$R$</td>
<td>Universal gas constant</td>
<td>8.315 Jmol⁻¹K⁻¹</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
<td>308 K</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>Internal sodium concentration</td>
<td>10 mM</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>External sodium concentration</td>
<td>140 mM</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>Internal potassium concentration</td>
<td>149.4 mM</td>
</tr>
<tr>
<td>$[K^+]_o$</td>
<td>External potassium concentration</td>
<td>4.0 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>External calcium concentration</td>
<td>1.8 mM</td>
</tr>
</tbody>
</table>

Table 5: Ion current conductance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Na}$</td>
<td>Peak $I_{Na}$ conductance</td>
<td>12 mS/µF</td>
</tr>
<tr>
<td>$g_{to}$</td>
<td>Peak $I_{to}$ conductance</td>
<td>0.1 mS/µF</td>
</tr>
<tr>
<td>$g_{K1}$</td>
<td>Peak $I_{K1}$ conductance</td>
<td>2.8 mS/µF</td>
</tr>
<tr>
<td>$g_{Kr}$</td>
<td>Peak $I_{Kr}$ conductance</td>
<td>0.0136 mS/µF</td>
</tr>
<tr>
<td>$g_{Ks}$</td>
<td>Peak $I_{Ks}$ conductance</td>
<td>0.0245 mS/µF</td>
</tr>
<tr>
<td>$g_{Kr}$</td>
<td>Peak $I_{Kr}$ conductance</td>
<td>0.00221 mS/µF</td>
</tr>
</tbody>
</table>
Table 6: L-type Ca channel parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{Ca}$</td>
<td>Adjustable strength of $J_{Ca}$</td>
<td>$1.46 \times 10^{-3} \mu$M/C/ms</td>
</tr>
<tr>
<td>$\tau_f$</td>
<td>Voltage dependent inactivation gate constant</td>
<td>55 ms [40 ms] {45 ms}</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Exponent for Ca-induced inactivation</td>
<td>0.7 [0.7] {1.5}</td>
</tr>
<tr>
<td>$\tilde{c}_s$</td>
<td>Calcium inactivation threshold</td>
<td>1.0 $\mu$M</td>
</tr>
<tr>
<td>$\tau_d$</td>
<td>Voltage dependent activation gate time constant</td>
<td>5 ms</td>
</tr>
<tr>
<td>$\tau_{fca}$</td>
<td>Calcium-induced inactivation gate time constant</td>
<td>20 ms</td>
</tr>
</tbody>
</table>
REFERENCES


