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 (Vm) attributable to steep APD restitution nor to calcium (Ca) cycling. Here, we used a mathematical model of intracellular Ca cycling, coupled with membrane ion currents, to investigate the dynamics of Vm and Ca transient alternans in an isolated cell, in 2 electrotonically coupled cells, and in 1D spatially homogeneous tissue. Our main finding is a novel instability mechanism in which the bidirectional coupling of Vm and Ca can drive the Ca transient of 2 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 1 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 Vm dynamics. Finally, we address the experimental relevance of these findings and suggest physiological conditions under which these patterns can be observed. (Circ Res. 2006;99:0-0.)

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 spatial 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 Vm dynamics that is attributable to the gating kinetics of membrane ion channels that regulate Vm. 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 Vm and Ca cycling are bidirectionally coupled, it is difficult to pinpoint which of these 2 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 Ca transient and APD of an isolated myocyte are electromechanically out of phase, i.e., a large-small large Ca transient corresponds to a short-long short APD. Moreover, when the Ca transient and APD are in phase, sufficiently steep CV restitution is required to initiate discordant alternans as shown in previous studies.\textsuperscript{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:

$$\frac{dV}{dt} = -I_{ion}/C_m + \frac{\partial^2 V}{\partial x^2}$$

where $C_m = F/cm^2$ is the transmembrane capacitance, $D = 5 \times 10^{-4} cm^2/ms$ 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.\textsuperscript{14} The space step was $\Delta x = 0.015 cm$, 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.,\textsuperscript{13} coupled with the canine action potential model of Fox et al..\textsuperscript{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 $V = -I_{ion}/C_m$ and 2 electrotonically coupled cells, with voltage $V_i$ in cell 1 and $V_j$ in cell 2, by integrating the 2 coupled equations $dV_i/dt = -I_{ion}/C_m + \kappa(V_j - V_i)$ and $dV_j/dt = -I_{ion}/C_m + \kappa(V_i - V_j)$ with a coupling strength $\kappa = D/\Delta x^2$.

**Data Analysis**

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

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

where $n$ is the beat number, and $c_{ai}(x)$ is the peak of the Ca 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 Ca transient alternans was visualized by plotting $\Delta C_{ai}(x,n)$. Likewise, the spatiotemporal distribution of APD alternans is measured using the quantity:

$$\Delta APD(x,n) = \frac{-1}{2} [APD_{ei+1}(x) - APD_{ei}(x)]$$

With these definitions, the nodes separating spatially out-of-phase regions of Ca 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, 2 coupled cells, and a 1D cable using a current stimulus of duration 1 ms and amplitude of 50 $\mu$A/$\mu$F. 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 2 ms every 50 beats. Following the method of Riccio et al.,\textsuperscript{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 assuming 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) pump. Our approach, following the method of Fox and Lu,\(^{11}\) 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,\(^{13,18}\) and experimentally by Diaz et al.\(^{19}\) A key feature of the model is that when the cell is paced rapidly with a periodic AP clamp, Ca\(_t\) transient alternans develop,\(^{13}\) as shown experimentally in isolated rabbit myocytes\(^{12}\) and also in guinea pig myocytes.\(^{20}\) Moreover, when the Ca\(_t\) transient alternates, APD alternates secondarily, because Ca\(_t\) affects ionic currents that regulate APD, primarily the Na/Ca exchanger and the L-type Ca current.

In this section, we study systematically the relationship between APD and Ca\(_t\) transient alternans in the single cell is dependent on the bidirectional coupling between Ca and V\(_m\). 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.\(^{21-23}\) where 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\(_t\) 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\(_{Na}\) may shorten APD while, at the same time, increasing the driving force for early Ca entry via the L-type Ca current to potentiate SR Ca release.\(^{24,25}\) However, we did not study this case here.

Next, we consider the unidirectional coupling of Ca on V\(_m\) (Ca\(_t\)-V\(_m\) coupling). Two distinct cases can be distinguished. The first, referred to as positive Ca\(_t\)-V\(_m\) coupling, illustrated in Figure 1C, corresponds to the case in which an increase in the peak Ca\(_t\) transient amplitude lengthens the APD. The second, referred to as negative Ca\(_t\)-V\(_m\) coupling (Figure 1C), corresponds to the case in which an increase in the peak Ca\(_t\) 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/Ca exchange current to APD. A larger Ca\(_t\) transient tends to inactivate the whole cell L-type Ca current more rapidly via Ca\(_t\)-induced inactivation, which tends to shorten the APD. However, a large Ca\(_t\) transient concomitantly increases the net inward current from electrogenic Na/Ca exchange, which tends to prolong APD. The Ca\(_t\)-V\(_m\) coupling was varied in our ionic model by changing the degree of Ca\(_t\)-induced inactivation of the L-type Ca current, as described in the online data supplement.

**Electromechanically In-Phase and Out-of-Phase Alternans**

The bidirectional coupling between the APD and the Ca\(_t\) transient determines the relative phase of APD and Ca\(_t\) transient alternans during steady-state pacing.\(^{26-28}\) For Ca-driven alternans, positive Ca\(_t\)-V\(_m\) 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–small Ca\(_t\) transient. In contrast, negative Ca\(_t\)-V\(_m\) coupling leads to electromechanically out-of-phase alternans (Figure 1D), where a long–short–long APD corresponds to a small–large–small Ca\(_t\) transient. When alternans are attributable to an instability of V\(_m\) dynamics, steady-state electromechanical alternans are always in phase. This is because, in this case, the Ca\(_t\) transient is slaved to V\(_m\) via the graded release coupling (Figure 1B), so that Ca\(_t\) 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\(_t\)-V\(_m\) coupling and Ca\(_t\) transient alternans, for the case when alternans are attributable to unstable Ca cycling. As a starting point, we first consider a simple system of 2 electrotonically coupled cells, with the main result that 2 cells alternate out of phase because of an intrinsic dynamical instability when the Ca\(_t\)-V\(_m\) 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, first plotted the amplitude of APD alternans (ΔAPD) and Ca\(_t\) transient alternans (ΔCa\(_t\)) 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\(_t\)-V\(_m\) coupling by adjusting the inactivation kinetics of the L-type Ca current, as described in Materials and Methods. As shown in Figure 2A, for positive Ca\(_t\)-V\(_m\) coupling, alternans onset occurred at PCL=315 ms and APD alternans was in phase with Ca\(_t\) transient alternans, ie, ΔAPD and ΔCa\(_t\) always had the same sign after the bifurcation to alternans. On the other hand, for the negative Ca\(_t\)-V\(_m\) coupling parameters, alternans onset was at PCL=340 ms, and once alternans developed, ΔAPD always had an opposite sign compared with ΔCa\(_t\) (electromechanically out of phase).

**Two Coupled Cells**

We then paced 2 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\(_t\)-V\(_m\) 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\(_t\) 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\(_t\)-V\(_m\) coupling ie, same model parameters as in Figure 2B. As shown, Ca\(_t\) 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\(_t\) transient alternans in cell 1 and cell 2 are out of phase. That is, ΔCa\(_t\) is positive in cell 1 and negative in cell 2, as shown. Moreover, despite the significant degree of Ca\(_t\) transient alternans, APD did not alternate in either cell. This effect is attributable to electro-
tonic interaction between the 2 cells, such that the APD shortening effect of a large Ca transient in 1 cell was balanced by the APD prolonging effect of the opposite phase small Ca 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 2 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\(_{i}\), fig. 3A through 3C shows the steady-state spatial distribution of both \(\triangle APD\) and \(\triangle Ca_i\) 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 of the Ca transient and APD gradually grew from the spatially homogeneous state. The spatial pattern of alternans is characterized by the presence of many (>30) \(\triangle Ca_i\) nodes and a few APD nodes. We repeated the dynamic simulation protocol 16 times and found that the average spacing between \(\triangle Ca_i\) nodes, at PCL=340 ms, was roughly 0.045±0.004 cm, whereas the average spacing of \(\triangle APD\) nodes was 0.6±0.1 cm.

**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

As shown in Figure 3D through 3F, we repeated the above simulation with negative Ca\(_{i}\) 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 of the Ca transient and APD gradually grew from the spatially homogeneous state. The spatial pattern of alternans is characterized by the presence of many (>30) \(\triangle Ca_i\) nodes and a few APD nodes. We repeated the dynamic simulation protocol 16 times and found that the average spacing between \(\triangle Ca_i\) nodes, at PCL=340 ms, was roughly 0.045±0.004 cm, whereas the average spacing of \(\triangle APD\) nodes was 0.6±0.1 cm.
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 2 vertical dashed lines mark the range of DI engaged along the cable. Figure 4C shows the spatial distribution of CV during the 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_3V_m Coupling Determines the Relative Synchrony of Two Coupled Cells**

The main finding of this work is that the bidirectional coupling between Ca and V_m dictates whether 2 coupled cells are synchronized or desynchronized. In particular, we find that if the Ca_3V_m coupling is positive, such that alternans at the single-cell level is electromechanically in phase, then Ca transient alternans in neighboring will synchronize. On the other hand, if the Ca_3V_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_3V_m coupling, along with electrotonic coupling, dictates the evolution of Ca alternans. In Figure 5A, we illustrate the V_m and Ca transients of 2 independent cells (cell 1 and cell 2) that are out of phase and which are then electrotonically coupled at time $t_1$. Here, we assume negative Ca_3V_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 Ca in the absence and in the presence of electrotonic coupling, respectively. Two key factors determine the subsequent evolution of alternans.

**Electrotetric 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 Ca transient alternans in the 2 neighboring cells is amplified from one beat to the next. This mechanism explains why the 2 coupled cells in our simulations exhibited out-of-phase Ca 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 Ca→$V_m$ 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 concordant 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 Ca→$V_m$ 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 restitution. 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 Ca alternans, which are attributable to an instability of Ca cycling, instead of an instability of $V_m$ dynamics.

In the case of negative Ca→$V_m$ 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 Ca 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 Ca. Hence, arbitrarily small stochastic fluctuations grow and develop into spatially discordant alternans when 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.

**Spatial Scale of Discordant Alternans in Homogeneous Tissue**
A major difference between positive and negative Ca→$V_m$ coupling is the spacing between nodes of Ca alternans. For positive Ca→$V_m$ coupling, we find that the spacing between nodes is on a tissue scale (≈1 cm). In contrast, for negative Ca→$V_m$ coupling, the spacing between nodes can range from 1 to a few cell lengths (100 to 400 μm). This short scale originates from the fact that the instability mechanism that drives discordant alternans in a cable of coupled cells is similar to the aforementioned instability mechanism that drives out-of-phase alternans in 2 electrotonically coupled identical cells. The tissue case is more complex, however, because several instability modes with different length scales can be manifested, with the spacing between nodes varying from 1 to several cells.

The difference between positive and negative Ca→$V_m$ coupling is also reflected in the relationship between Ca and APD alternans patterns. For positive coupling, the nodes of $V_m$ and Ca alternans essentially coincide. In contrast, for negative coupling, the spacing between nodes of APD alter-
nans is substantially larger than the cellular-scale spacing between nodes of Ca alternans. The reason is that the diffusion constant of $V_m$ in homogeneous tissue ($\approx 1 \text{ cm}^2/\text{sec}$) is at least 5 orders of magnitude larger than the molecular diffusion constant of Ca$^{2+}$ ions (within the myoplasm and across gap junctions). Therefore, on the time scale of 1 APD, $V_m$ diffuses on a spatial scale $[\text{rad}]D\times\text{APD}/[\text{rad}]$ of a few millimeters, whereas Ca diffuses at most by a fraction of a cell length. Because APD alternans must be in phase within a 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 2 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.

$Ca_{\text{Ca}} \rightarrow V_m$ 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–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 standpoint 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 consistently electromechanically in phase, whereas in cat atrial myocytes alternans have been observed to be electromechanically out of phase. Also, electromechanically out-of-phase alternans have been observed under ischemic conditions. Furthermore, an interesting experimental study by Rubenstein et al. has shown that in isolated cat ventricular myocytes that at 36°C, APD alternans are in phase with contraction, which mirrors the amplitude of the Ca transient, whereas at 32°C, they become out of phase. These observations suggest that the bidirectional coupling between $V_m$ and Ca can vary with cell type and can be modulated by changing experimental conditions.

The possibility of observing Ca nodes with a spacing comparable to a cell length is consistent with experimental studies that demonstrate that subcellular Ca alternans can be spatially discordant within a single cell. For example, Kockskamper et al. have imaged subcellular Ca in cat atrial cells and found that half of the cell could alternate out of phase with the other half. Also, Diaz et al. have shown subcellular discordant alternans 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**


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 100,000-1000,000 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\textsubscript{i} 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

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

(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

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

(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 $\sim \frac{1}{\sqrt{N_{\text{up}}}}$.

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

$$J_{\text{up}} = \frac{v_{\text{up}} c_i^2}{c_i^2 + c_{\text{up}}^2},$$

(4)

where $v_{\text{up}}$ and $c_{\text{up}}$ are constants. These constants can be related to the parameters of the Langevin formulation using $v_{\text{up}} = N_{\text{up}} \cdot i$ and $c_{\text{up}}^2 = k_\eta / k_\delta$. 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 $\tau = 1/(k_\eta (c_{\text{up}}^2 + c_i^2)) = 1 \text{ ms}$. 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 $v_{\text{up}}$ and $c_{\text{up}}$ are given in Table 3. To model noise we use a large number of channels $N_{\text{up}} = 10^6$. 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
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

\[
\frac{dV_m}{dt} = -\frac{1}{C_m} (I_{ion} + I_{stim}), \quad (5)
\]

where \(I_{ion}\) is the total membrane current density, \(I_{stim}\) 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

\[
I_{ion} = I_Na + I_to + I_Kr + I_Ks + I_Kp + I_Kl + I_Ca + I_{NaCa}. \quad (6)
\]

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:
\[
\frac{dc_s}{dt} = \frac{\beta_s v_s}{v_s} \left[ J_{rel} = \frac{c_s - c_i}{\tau_s} + J_{Ca} + J_{NaCa} \right],
\]
\[
\frac{dc_i}{dt} = \beta_i \left[ \frac{c_s - c_i}{\tau_s} - J_{up} \right],
\]
\[
\frac{dc_j}{dt} = -J_{rel} + J_{up},
\]
\[
\frac{dc_j'}{dt} = \frac{c_j - c_j'}{\tau_a},
\]
\[
\frac{dJ_{rel}}{dt} = gJ_{Ca} \cdot Q(c_j') - \frac{J_{rel}}{\tau_r}
\]

where \(c_s, c_i\) and \(c_j\) are the average concentrations of free \(Ca\) 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 \(v_a\) 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 \(\beta_s\) 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 below.
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

\[ u = \frac{dQ}{d\epsilon_{ij}}. \]  

(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 $V_m$ 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.
Spatially discordant alternans

\[ f_{Ca}^\infty = \frac{1}{1 + \left( \frac{c_s}{\bar{c}_s} \right)^\gamma}, \]  

(11)

where the exponent \( \gamma \) controls the degree of Ca\textsubscript{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\textsubscript{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 (\( \tau_f = 55 \text{ms} \)), while the slope of the SR release vs SR-load is kept small (\( u = 2 \text{ms}^{-1} \)). When alternans are due to unstable Ca cycling, we have two cases referred to as positive and negative Ca\textsubscript{i} \( \rightarrow V_m \) coupling. For both cases unstable Ca\textsubscript{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\textsubscript{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\textsubscript{i} induced inactivation is enhanced by increasing \( \gamma \), then negative Ca\textsubscript{i} \( \rightarrow V_m \) is favored since the negative feedback of Ca\textsubscript{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 ($I_{Na}$).**

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

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

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

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

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

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

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

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

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

\[I_{to} = g_{to} X_{to} Y_{to} (V - E_K)\]
\[E_K = \frac{RT}{F} \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)\]
\[\frac{dX_{to}}{dt} = \alpha_{Xto} (1 - X_{to}) - \beta_{Xto} X_{to}\]
\[\frac{dY_{to}}{dt} = \alpha_{Yto} (1 - Y_{to}) - \beta_{Yto} Y_{to}\]
\[\alpha_{Xto} = 0.04516e^{0.03577V}\]
\[\beta_{Xto} = 0.0989e^{-0.063237}\]
\[\alpha_{Yto} = \frac{0.005415e^{(V+33.5)/-5}}{1 + 0.051335e^{(V+33.5)/-5}}\]
\[\beta_{Yto} = \frac{0.005415e^{(V+33.5)/5}}{1 + 0.051335e^{(V+33.5)/5}}\]

Inward rectifier \(K^+\) current \((I_{K1})\):
\[ I_{K_1} = g_{Kr}K_1^\infty \frac{[K^+]_o}{[K^+]_o + 13}(V - E_K) \]

\[ K_1^\infty = \frac{1}{2 + e^{1.62F/(RT)(V - E_K)}} \]

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

\[ I_{Kr} = g_{Kr} \sqrt{\frac{[K^+]_o}{4}} x_{Kr} R(V)(V - E_K) \]

\[ R(V) = \frac{1}{1 + 2.5e^{0.1(V - 28)}} \]

\[ \frac{dx_{Kr}}{dt} = \frac{x_{Kr}^\infty - x_{Kr}}{\tau_{Kr}} \]

\[ x_{Kr}^\infty = \frac{1}{1 + e^{-2.182 - 0.1819V}} \]

\[ \tau_{Kr} = 43 + \frac{1}{e^{-5.495 + 0.1601V} + e^{-7.677 - 0.0128V}} \]

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

\[ I_{Ks} = g_{Ks}x_{Ks}^2 (V - E_{Ks}) \]

\[ \frac{dx_{Ks}}{dt} = \frac{x_{Ks}^\infty - x_{Ks}}{\tau_{Ks}} \]

\[ x_{Ks}^\infty = \frac{1}{1 + e^{-(V - 16)/13.6}} \]

\[ \tau_{Ks} = \frac{1}{\left(\frac{0.0000719(V - 10)}{1 - e^{-0.148(V - 10)}} + \frac{0.00031(V - 10)}{1 - e^{0.0687(V - 10)}}\right)} \]

\[ E_{Ks} = \frac{RT}{F} \ln \left( \frac{[K^+]_o + 0.01833[Na^+]_o}{[K^+]_o + 0.01833[Na^+]_o} \right) \]
The plateau potassium current \((I_{Kp})\):

\[
I_{Kp} = g_{Kp} K_{Kp}(V)(V - E_K)
\]

\[
K_{Kp}(V) = \frac{1}{1 + e^{(3.488 - V)/5.98}}
\]

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

\[
J_{NaCa} = g_{NaCa} \frac{1}{K_{m,Na}^2 + [Na^+]_o} \frac{1}{K_{m,Ca} + [Ca^{2+}]_o} \frac{e^{\alpha t}[Na^+]_o^3[Ca^{2+}]_o - e^{(\xi - 1)a}[Na^+]_o^3c_x}{1 + k_{sat} e^{(\xi - 1)a}}
\]

The L-type \(Ca_i\) current flux \((J_{Ca})\):

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

\[
\frac{df}{dt} = \frac{f_a - f}{\tau_f}
\]

\[
\frac{d(d)}{dt} = \frac{d_a - d}{\tau_d}
\]

\[
\frac{df_{Ca}}{dt} = \frac{f_{Ca}^\infty - f_{Ca}}{\tau_{fca}}
\]

\[
f_a = \frac{1}{1 + e^{(V+35)/8.6}}
\]

\[
d_a = \frac{1}{1 + e^{(V+5)/6.24}}
\]

\[
f_{Ca}^\infty = \frac{1}{1 + (c_a/c_x)^\gamma}
\]

\[
i_{Ca} = \frac{4VF^2}{RT} \left( c_a e^{2a} - 0.34 [Ca^{2+}]_o \right)
\]

with \(a = VF / RT\).
The SR $\text{Ca}_i$ release vs. SR $\text{Ca}_i$ load relationship:

$$Q(c_j') = \begin{cases} 
0 & 0 < c_j' < 50, \\
1.5(c_j' - 50) & 50 < c_j' < 110, \\
uc_j' + s & c_j' > 110, 
\end{cases}$$

where the flux $Q(c_j')$ is in units of $10^{-6}$ µM/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

$$\beta_s = \left(1 + \frac{B_{SR}K_{SR}}{(c_s + K_{SR})^2} + \frac{B_{cd}K_{cd}}{(c_s + K_{cd})^2} + \frac{B_TK_T}{(c_s + K_T)^2}\right)^{-1},$$

$$\beta_i = \left(1 + \frac{B_{SR}K_{SR}}{(c_i + K_{SR})^2} + \frac{B_{cd}K_{cd}}{(c_i + K_{cd})^2} + \frac{B_TK_T}{(c_i + K_T)^2}\right)^{-1}.$$

TABLES

The parameters used in the $\text{Ca}_i$-driven and $V_m$-driven cell models are given below. Parameters in square and curly brackets correspond to the $\text{Ca}_i$-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>$g_{NaCu}$</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>$K_{m,Cu}$</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>$2.58 \times 10^{-5}$ 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$^{-1}$K$^{-1}$</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>$1.46 \times 10^{-3}$ μMl/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 μM</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


