Spontaneous Calcium Sparks and Calcium Homeostasis in a Minimal Model of Permeabilized Ventricular Myocytes

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Abstract

Many issues remain unresolved concerning how local, subcellular Ca\(^{2+}\) signals interact with bulk cellular concentrations to maintain homeostasis in health and disease. To aid the interpretation of data obtained in quiescent ventricular myocytes, we present a minimal whole cell model that accounts for both localized (subcellular) and global (cellular) aspects of Ca\(^{2+}\) signaling. Using a minimal formulation of the distribution of local Ca\(^{2+}\) concentrations associated with a large number of Ca\(^{2+}\) release sites, the model simulates both random spontaneous Ca\(^{2+}\) sparks and the changes in myoplasmic and sarcoplasmic reticulum (SR) [Ca\(^{2+}\)] that result from the balance between stochastic release and reuptake into the SR. Ca\(^{2+}\) release sites are composed of clusters of two-state ryanodine receptors (RyRs) that exhibit activation by local cytosolic [Ca\(^{2+}\)], but no inactivation or regulation by luminal Ca\(^{2+}\). Decreasing RyR open probability in the model causes a decrease in aggregate release flux and an increase in SR [Ca\(^{2+}\)], regardless of whether RyRs inhibition is mediated by a decrease in RyR open dwell time or an increase in RyR closed dwell time. The same balance of stochastic release and reuptake can be achieved, however, by either high-frequency/short-duration or low-frequency/long-duration Ca\(^{2+}\) sparks. The results are well correlated with recent experimental observations using pharmacological RyR inhibitors and clarify those aspects of release-reuptake balance that are inherent to the coupling between local and global Ca\(^{2+}\) signals, and those aspects that depend on molecular-level details. The model of Ca\(^{2+}\) sparks and homeostasis presented here can be a useful tool for understanding changes in cardiac Ca\(^{2+}\) release resulting from drugs, mutations, or acquired diseases.

Introduction

Intracellular calcium (Ca\(^{2+}\)) is a ubiquitous biological signal that serves diverse functions in many cell types. In individual cells, information can be conveyed by both “global,” or cell-wide, changes in [Ca\(^{2+}\)] and by “local,” subcellular Ca\(^{2+}\) signals. Local signals are frequently caused by release of Ca\(^{2+}\) from intracellular stores, primarily the endoplasmic/sarcoplasmic reticulum (ER/SR). Local release events occur through closely-packed clusters of release channels, inositol 1,4,5-trisphosphate (IP\(_3\)) receptors or ryanodine receptors (RyRs), and are observable experimentally as Ca\(^{2+}\) sparks [Cheng et al., 1993] or puffs [Yao et al., 1995]. When one or several of the channels in a release site are open, the [Ca\(^{2+}\)] experienced by clustered channels is dramatically different from [Ca\(^{2+}\)] in the bulk cytosol.

In cardiac myocytes and many other cell types, local and global Ca\(^{2+}\) signals are closely linked to one another. Local signals frequently form the building blocks from which global signals are built [Lipp and Niggli, 1996] and, conversely, changes in bulk [Ca\(^{2+}\)] in the myoplasm or SR can influence the frequency, amplitude, and kinetics of local events. In ventricular myocytes, for instance, propagating Ca\(^{2+}\) waves emerge when spontaneous Ca\(^{2+}\) sparks trigger additional sparks in a regenerative fashion [Cheng et al., 1996]. Similarly, changes in channel gating at the level of individual RyRs can immediately affect the production of local events and, over time, influence bulk myoplasmic and SR [Ca\(^{2+}\)]. An increase in RyR opening will cause a gradual decrease in [Ca\(^{2+}\)]\(_{SR}\) [Trafford et al., 2000], whereas inhibition of RyR opening will lead over time to elevated [Ca\(^{2+}\)]\(_{SR}\) [Györke et al., 1997].
This principle has been elegantly demonstrated by Lukyanenko and coworkers [Lukyanenko et al., 2001] in quiescent rat ventricular myocytes treated with caffeine (to sensitize RyRs) or tetracaine (to inhibit RyRs).

In heart cells, changes in Ca\(^{2+}\) signaling due to altered RyR activity are currently receiving considerable attention because of close links to disease [Wehrens et al., 2005, George et al., 2007]. In particular, catecholaminergic polymorphic ventricular tachycardia (CPVT), an inherited disorder associated with a dramatic increase in arrhythmia risk, results from mutations in either the RyR or calsequestrin, an SR Ca\(^{2+}\) buffer protein that associates with and modulates the RyR [Liu and Priori, 2008, Győrke, 2009]. Experiments in vitro have shown that CPVT causing mutations usually increase the open probability of the RyR, resulting in a hyperactive or “leaky” channel [Jiang et al., 2002, George et al., 2003, Lehnart et al., 2004]. Studies also suggest that leaky RyRs are characteristic of several experimental heart failure models [Marx et al., 2000, Ai et al., 2005]. Thus, a quantitative understanding of how changes in RyR gating influence local and global Ca\(^{2+}\) responses provides insight into disease pathophysiology and can potentially suggest novel therapies.

The difference in spatial scales between local and global Ca\(^{2+}\) signals, however, creates significant challenges for the development of mechanistic mathematical models. In particular, gating of RyRs depends on both myoplasmic and SR [Ca\(^{2+}\)] [Laver, 2007], and concentrations within clusters during local events can be dramatically different from the bulk concentrations. In addition, because of the relatively small number of RyRs responsible for Ca\(^{2+}\) sparks [Cheng and Lederer, 2008], the stochastic gating of these channels must be considered when simulating local events. Prior studies have used Monte Carlo simulation methods to investigate stochastic triggering of Ca\(^{2+}\) sparks [Stern et al., 1999, Sobie et al., 2002, DeRemigio and Smith, 2005, Huertas and Smith, 2007], but these have generally treated myoplasmic and bulk SR Ca\(^{2+}\) concentrations as fixed boundary conditions. Conversely, modeling studies focused on cellular Ca\(^{2+}\) transients have usually used representations of SR Ca\(^{2+}\) release that do not account for the stochastic nature of the local events [Faber and Rudy, 2000, Shannon et al., 2004, Bondarenko et al., 2004]. Attempting to simulate Ca\(^{2+}\) signaling at both spatial scales simultaneously is a daunting prospect, because the stochastic behavior of thousands of local events must be considered to determine the effects on the bulk concentrations. As a result, only a few studies have attempted to capture both phenomena [Greenstein and Winslow, 2002, Greenstein et al., 2006, Williams et al., 2007, Williams et al., 2008].

In this paper we introduce a computationally efficient minimal model of the coupling between local and global Ca\(^{2+}\) signals in permeabilized ventricular myocytes. The model accounts for the random generation and termination of spontaneous Ca\(^{2+}\) sparks, the resulting changes in myoplasmic and SR [Ca\(^{2+}\)], and the feedback of these changes on spark frequency. We make few assumptions about the factors influencing RyR gating, which allows us to distinguish between those results that are inherent to the coupling between local and global signals and those that are specific to the RyR gating scheme. To validate the model, we consider experiments recently performed by Zima et al. [Zima et al., 2008], who showed that tetracaine, an RyR inhibitor, caused an initial suppression of Ca\(^{2+}\) sparks followed by an increase in SR [Ca\(^{2+}\)] and a partial recovery of spark frequency. Surprisingly, these authors found that prolonged exposure to tetracaine led to an increase in Ca\(^{2+}\) spark duration (see Fig. 1C in ref. [Zima et al., 2008]). The simulations presented here recapitulate this experimental result, suggesting that the observed increase in spark duration results directly from
the interplay between RyR inhibition and the resulting changes in SR [Ca\(^{2+}\)]. More broadly, this model provides a powerful yet minimal framework for understanding how mutations, post-translational modifications, or drugs can alter diastolic SR Ca\(^{2+}\) release in ventricular myocytes.

Model Formulation

The minimal whole cell model of local and global Ca\(^{2+}\) responses developed here takes into account stochastic Ca\(^{2+}\) release site dynamics as well as the balance of release and re-uptake fluxes leading to Ca\(^{2+}\) homeostasis in quiescent ventricular myocytes (Fig. 1). The model assumes that ryanodine receptor Ca\(^{2+}\) channels (RyRs) are clustered on the endoplasmic/sarcoplasmic reticulum (ER/SR) membrane in release sites composed of 10–30 channels. All channels in a given release site experience the same local Ca\(^{2+}\) concentrations (myoplasmic and SR), but these “domain” Ca\(^{2+}\) concentrations are heterogenous throughout the cell, i.e., different release sites experience different domain [Ca\(^{2+}\)]. Similar to prior work by Hinch et al. [Hinch et al., 2004, Hinch et al., 2006, Greenstein et al., 2006], we assume that when the number of open channels in a Ca\(^{2+}\) release site changes, the local Ca\(^{2+}\) concentrations rapidly equilibrate in a manner that balances the fluxes into and out of the spatially restricted domains. In our model formulation, a large number of stochastically gating Ca\(^{2+}\) release sites are coupled to the bulk myoplasmic and SR [Ca\(^{2+}\)] in a manner that allows spontaneous Ca\(^{2+}\) sparks to change the balance of Ca\(^{2+}\) release, or “leak,” and reuptake by SERCA pumps. The bulk myoplasmic and SR [Ca\(^{2+}\)] determine the relationship between the number of open channels in a Ca\(^{2+}\) release site and the resulting domain Ca\(^{2+}\) concentrations and, consequently, changes in these bulk concentrations influence the dynamics of spontaneous sparks. This minimal yet realistic representation of bi-directional coupling between local and global aspects of Ca\(^{2+}\) handling is a novel aspect of our model formulation that has not been emphasized in prior work.

Ca\(^{2+}\) release site model

In our model formulation, Ca\(^{2+}\) release sites are composed of \(N\) coupled Markov chains representing individual RyRs. For simplicity we utilize a RyR model with two states, closed (\(C\)) and open (\(O\)), but the model formulation can be generalized for channel models with more states [Groff and Smith, 2008b, Groff and Smith, 2008a]. Each RyR opens at a rate that depends on the local myoplasmic domain (i.e., the diadic subspace) [Ca\(^{2+}\)], denoted by \(c_{\text{myo}}\), and closes with a Ca\(^{2+}\)-independent transition rate,

\[
\begin{align*}
C & \xrightleftharpoons[k_{oc}]{k_{co}(c_{\text{myo}}^d)^2} O,
\end{align*}
\]

where we assume cooperative Ca\(^{2+}\) binding, \(k_{co}(c_{\text{myo}}^d)^2\) and \(k_{oc}\) are transition rates with units of reciprocal time, and \(k_{co}\) is an association rate constant with units of \(\text{conc}^{-2}\ \text{time}^{-1}\).

We assume the local [Ca\(^{2+}\)] experienced by the Ca\(^{2+}\) regulatory site of each channel depends only on the number of open channels at the Ca\(^{2+}\) release site, \(N_O(t)\), and the bulk
Ca\(^{2+}\) concentrations, \(c_{\text{myo}}\) and \(c_{\text{sr}}\), as described below. Because the channels are identical and indistinguishable, the state space for the \(N\)-channel Ca\(^{2+}\) release site includes \(N + 1\) states (0 \(\leq N_O \leq N\),
\[
\begin{array}{cccccc}
0 & \equiv & 1 & \equiv & \ldots & \equiv & N - 1 & \equiv & N \\
Nk_{co}(c_{\text{myo}}^{d,0})^2 & & (N - 1)k_{co}(c_{\text{myo}}^{d,1})^2 & & 2k_{co}(c_{\text{myo}}^{d,N-2})^2 & & k_{co}(c_{\text{myo}}^{d,N-1})^2 \\
k_{oc} & & 2k_{oc} & & (N - 1)k_{oc} & & Nk_{oc}
\end{array}
\]  
(2)
where the state labels (0, 1, \(\ldots\), \(N - 1\), \(N\)) indicate the possible numbers of open channels and \(c_{\text{myo}}^{d,n}\) is the local myoplasmic domain [Ca\(^{2+}\)] that applies when there are \(n\) open channels. The infinitesimal generator matrix, denoted by \(Q = (q_{ij})\), that corresponds to Eq. 2 is tridiagonal,
\[
Q = \begin{pmatrix}
\diamond & Nk_{co}(c_{\text{myo}}^{d,0})^2 & 0 & \ldots & 0 & 0 & 0 \\
k_{oc} & \diamond & (N - 1)k_{co}(c_{\text{myo}}^{d,1})^2 & \ldots & 0 & 0 & 0 \\
\vdots & \vdots & \ddots & \ddots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & \ldots & (N - 1)k_{oc} & \diamond & k_{co}(c_{\text{myo}}^{d,N-1})^2 \\
0 & 0 & 0 & \ldots & 0 & Nk_{oc} & \diamond \\
\end{pmatrix}
\]  
(3)
where \(q_{ij}\) is the transition rate from state \(i\) to state \(j\) and the diamonds (\(\diamond\)) indicate a diagonal entry leading to a row sum of zero.

Once the local [Ca\(^{2+}\)] concentrations \(c_{\text{myo}}^{d,n}\) that apply for any given number of open channels are specified, all of the statistical properties of the Ca\(^{2+}\) release site model can be determined using Eq. 3. In particular, the time-evolution of the probability distribution of the number of open channels in the release site can be found by solving the ODE initial value problem
\[
\frac{d\boldsymbol{\pi}}{dt} = \boldsymbol{\pi}Q
\]  
(4)
where the row vector \(\boldsymbol{\pi} = (\pi_0, \pi_1, \ldots, \pi_N)\), \(\pi_i(t)\) is the probability of finding a release site in state \(i\), and \(\boldsymbol{\pi}(0)\) is the initial condition. The numerical solution of Eq. 4 can also be interpreted as providing the probability distribution for the state of a release site randomly sampled at time \(t\) from a large population that was initially prepared with a distribution of states given by \(\boldsymbol{\pi}(0)\).

Myoplasmic and SR domain Ca\(^{2+}\)

As shown schematically in Fig. 1, the domain Ca\(^{2+}\) concentrations for each release site \(c_{\text{myo}}^{d}\) and \(c_{\text{sr}}^{d}\) are coupled to the bulk compartments via the fluxes \(J_{\text{myo}}\) and \(J_{\text{sr}}\), and coupled to one another through the release flux \(J_{\text{rel}}\). We assume these fluxes take the form
\[
J_{\text{rel}}^n = v_{\text{rel}} \gamma_n \left(c_{\text{sr}}^{d,n} - c_{\text{myo}}^{d,n}\right)
\]  
(5)
\[
J_{\text{myo}}^n = v_{\text{myo}} \left(c_{\text{myo}}^{d,n} - c_{\text{myo}}^{d}\right)
\]  
(6)
\[
J_{\text{sr}}^n = v_{\text{sr}} \left(c_{\text{sr}}^{d,n} - c_{\text{sr}}^{d}\right)
\]  
(7)
where \(c_{\text{myo}}\) and \(c_{\text{sr}}\) are the bulk myoplasmic and SR concentrations, \(\gamma_n = n/N\) indicates the fraction of channels at any given Ca\(^{2+}\) release site that are open, \(v_{\text{rel}}\) is the maximum
release rate. Because the parameter \( v_{myo} \) is related to the exponential time constant for the decay of elevated myoplasmic domain \([Ca^{2+}]\) to the myoplasmic bulk \([Ca^{2+}]\) when an open release site closes, we will refer to \( v_{myo} \) as the rate of myoplasmic domain collapse [Mazzag et al., 2005]. Similarly, the parameter \( v_{sr} \) is the rate of luminal domain recovery that determines the exponential time constant for relaxation of depleted junctional SR \([Ca^{2+}]\) as this compartment is refilled via \(Ca^{2+}\) translocation from the bulk SR [Huertas and Smith, 2007].

Recall that Eqs. 2 and 3 require the specification of the local \([Ca^{2+}]\) concentration \(c_{d,n}^{\text{myo}}\) that applies for any given number \(n\) of open channels. Assuming the dynamics of domain \(Ca^{2+}\) are fast compared to the gating of RyRs, these domain \(Ca^{2+}\) concentrations are found by balancing the fluxes \(J_{rel}^n = J_{myo}^n\) and \(J_{sr}^n = J_{rel}^n\). Solving these equations simultaneously for \(c_{d,n}^{\text{myo}}\) and \(c_{d,n}^{\text{sr}}\) yields

\[
c_{d,n}^{\text{myo}} = \frac{v_{myo}}{v_{myo} + \tilde{v}_{sr}} c_{myo} + \frac{\tilde{v}_{sr}}{v_{myo} + \tilde{v}_{sr}} c_{sr} \quad (8)
\]

\[
c_{d,n}^{\text{sr}} = \frac{v_{myo}}{v_{myo} + \tilde{v}_{sr}} c_{myo} + \frac{v_{sr}}{v_{myo} + v_{sr}} c_{sr} \quad (9)
\]

where

\[
\tilde{v}_{myo} = \frac{\gamma_n v_{rel} v_{myo}}{\gamma_n v_{rel} + v_{myo}}, \quad \tilde{v}_{sr} = \frac{\gamma_n v_{rel} v_{sr}}{\gamma_n v_{rel} + v_{sr}} \quad \text{and} \quad \gamma_n = \frac{n}{N}. \quad (10)
\]

The filled circles in Fig. 2 show the myoplasmic and SR domain concentrations given by Eqs. 8 and 9 as functions of the number of open channels \((N_O = n)\). Note that as the number of open channels increases, \(c_{d,n}^{\text{myo}}\) increases and \(c_{d,n}^{\text{sr}}\) decreases, but always \(c_{d,n}^{\text{myo}} < c_{d,n}^{\text{sr}}\). The open circles in Fig. 2 show how an increase in the bulk SR \([Ca^{2+}]\) \((c_{sr})\) influences the relationship between the number of open channels in a \(Ca^{2+}\) release site and the resulting cytosolic \((c_{d,n}^{\text{myo}})\) and luminal \((c_{d,n}^{\text{sr}})\) domain \(Ca^{2+}\) concentrations. In particular, note that an increase in the bulk SR \([Ca^{2+}]\) \((c_{sr})\) leads to an increase in cytosolic domain \([Ca^{2+}]\) \((c_{d,n}^{\text{myo}})\) provided one or more RyRs are open \((n \geq 1\) in Eq. 8).

**Conventional Monte Carlo simulation**

As diagrammed in Fig. 1, the bulk myoplasmic and SR \(Ca^{2+}\) concentrations are affected by the SERCA pumps \((J_{pump})\), a passive leak from the SR to the myoplasm that is independent of release site activity \((J_{leak})\), as well as the fluxes from the myoplasmic domains to the bulk myoplasm \((J_{myo})\) and from the bulk SR to the SR domains \((J_{sr})\). In a conventional Monte Carlo simulation involving a large number of \(Ca^{2+}\) release sites, concentration balance equations for the myoplasmic and SR \(Ca^{2+}\) concentrations consistent with Fig. 1 are written,

\[
\frac{dc_{myo}}{dt} = J_{myo}^T + J_{leak} - J_{pump} + J_{pm}
\]

\[
\frac{dc_{sr}}{dt} = \frac{1}{\lambda_{sr}} \left( J_{sr}^T - J_{leak} + J_{pump} \right),
\]

where \(\lambda_{sr} = V_{sr}/V_{myo}\), \(V_{myo}\) and \(V_{sr}\) are the effective myoplasmic and SR volumes (i.e., accounting for \(Ca^{2+}\) buffering capacity), and \(J_{myo}^T\) and \(J_{sr}^T\) are total fluxes obtained summing
over all release sites. Under the assumption of fast domain Ca$^{2+}$, these fluxes can be expressed as a sum over all possible release site states,

$$J_{\text{myo}}^T = \sum_{n=0}^{N} f_n v_{\text{myo}}^T (c_{\text{myo}}^{d,n} - c_{\text{myo}})$$

and

$$J_{\text{sr}}^T = \sum_{n=0}^{N} f_n v_{\text{sr}}^T (c_{\text{sr}} - c_{\text{sr}}^{d,n})$$

where $f_n(t)$ are random variables denoting the fraction of release sites with $n$ open channels, $0 \leq f_n \leq 1$, $\sum_{n=0}^{N} f_n = 1$, $c_{\text{myo}}^{d,n}$ and $c_{\text{sr}}^{d,n}$ are given by Eqs. 8–10, and the rate constants $v_{\text{myo}}^T$ and $v_{\text{sr}}^T$ are proportional to $v_{\text{myo}}$ and $v_{\text{sr}}$ but scaled by the total number of release sites. In a conventional Monte Carlo approach to simulation of a whole cell model of local and global Ca$^{2+}$ dynamics, the ODEs for bulk myoplasmic and SR Ca$^{2+}$ are integrated while bi-directionally coupled (through the $f_n$ in Eqs. 11–14) to a stochastic simulation of a large finite number of Markov chains (Eq. 2), each one of which represents a Ca$^{2+}$ release site.

**Accelerated simulation assuming large population of release sites**

In our model formulation, we avoid Monte Carlo simulation of a large number of Markov chains by assuming that the number of release sites (and associated domains) is large enough that the probability distribution of release site states is well-approximated by $\pi(t)$ solving Eq. 4. In a naive application of this approach, Eqs. 11–12 would be integrated simultaneously with Eq. 4 with the substitution of $\pi_n$ for $f_n$ in Eqs. 13–14. Unfortunately, this approach is invalid because the assumption of rapid Ca$^{2+}$ domain formation and collapse is a singular limit of the corresponding whole cell model formulation in which ODEs are used to solve for the dynamics of domain Ca$^{2+}$ (see Supporting Material). Instead, our model formulation is based on numerical solution of concentration balance equations for the total myoplasmic ($\hat{c}_{\text{myo}}$) and SR ($\hat{c}_{\text{sr}}$) Ca$^{2+}$ concentrations,

$$\frac{d\hat{c}_{\text{myo}}}{dt} = J_{\text{rel}}^T + J_{\text{leak}} - J_{\text{pump}} + J_{\text{pm}}$$

and

$$\frac{d\hat{c}_{\text{sr}}}{dt} = \frac{1}{\lambda_{\text{sr}}} (-J_{\text{rel}}^T - J_{\text{leak}} + J_{\text{pump}})$$

where the total release flux $J_{\text{rel}}^T$ is given by

$$J_{\text{rel}}^T = \sum_{n=0}^{N} \pi_n \gamma_n v_{\text{rel}}^T (c_{\text{sr}}^{d,n} - c_{\text{myo}}^{d,n})$$

where $\gamma_n = n/N$, $c_{\text{myo}}^{d,n}$ and $c_{\text{sr}}^{d,n}$ are given by Eqs. 8–10, $\pi_n$ is the probability that a randomly sampled release site has $n$ open channels, and as mentioned above $\pi = (\pi_0, \pi_1, \cdots, \pi_N)$ is found by integrating Eq. 4. The total myoplasmic ($\hat{c}_{\text{myo}}$) and SR ($\hat{c}_{\text{sr}}$) Ca$^{2+}$ concentrations that solve Eqs. 15 and 16 are sums of the bulk and domain concentrations weighted by
effective volume ratios,
\[
\hat{c}_{\text{myo}} = c_{\text{myo}} + \Lambda_{\text{myo}}^d \bar{c}_{\text{myo}}, \\
\hat{c}_{\text{sr}} = c_{\text{sr}} + \Lambda_{\text{sr}}^d \bar{c}_{\text{sr}},
\]
(18) (19)

In these definitions, \(\bar{c}_{\text{myo}}^d\) and \(\bar{c}_{\text{sr}}^d\) are the average myoplasmic and SR domain \(Ca^{2+}\) concentrations that would be obtained upon randomly sampling release sites from within the cell, that is,
\[
\bar{c}_{\text{myo}}^d = \sum_{n=0}^{N} \pi_n c_{\text{myo}}^d; n, \\
\bar{c}_{\text{sr}}^d = \sum_{n=0}^{N} \pi_n c_{\text{sr}}^d.
\]
(20) (21)

The effective volume ratios that appear in Eqs. 18 and 19 are given by \(\Lambda_{\text{myo}}^d = V_{\text{myo}}^{d,T}/V_{\text{myo}}, \Lambda_{\text{sr}}^d = V_{\text{sr}}^{d,T}/V_{\text{myo}}, \) and \(\lambda_{\text{sr}} = V_{\text{sr}}/V_{\text{myo}}\) where \(V_{\text{myo}}\) and \(V_{\text{sr}}\) are the effective myoplasmic and SR volumes, and \(V_{\text{myo}}^{d,T}\) and \(V_{\text{sr}}^{d,T}\) are the effective volumes of the aggregated myoplasmic and SR domains, respectively.

Because the experimental observations that are of primary relevance to this paper involve permeabilized cells [Zima et al., 2008], the plasma membrane flux is chosen to be
\[
J_{\text{pm}} = k_{\text{pm}} (c_{\text{ext}} - c_{\text{myo}}),
\]
(22)

where \(k_{\text{pm}}\) is chosen large enough to “clamp” the bulk myoplasmic \([Ca^{2+}]\) \((c_{\text{myo}})\) at the level of the extracellular bath \((c_{\text{ext}} = 0.1 \mu M)\) in the standard parameter set. Even so, the total myoplasmic \([Ca^{2+}]\) \((\hat{c}_{\text{myo}}, \text{Eq. 18})\) that solves Eq. 15 is not fixed, because this concentration includes \(Ca^{2+}\) in the myoplasmic domains. The fluxes between the bulk myoplasm and bulk SR that occur in Eqs. 15 and 16 include \(Ca^{2+}\) reuptake by SERCA pumps,
\[
J_{\text{pump}} = \frac{v_{\text{pump}} c_{\text{myo}}^2}{k_{\text{pump}}^2 c_{\text{myo}}^2 + c_{\text{myo}}^2},
\]
(23)

and a passive leakage flux,
\[
J_{\text{leak}} = v_{\text{leak}} (c_{\text{sr}} - c_{\text{myo}}).
\]
(24)

This minimal model of the relationship between \(Ca^{2+}\) sparks and \(Ca^{2+}\) homeostasis was implemented in Matlab (The Mathworks, Inc.) running on a 1.67 GHz Power PC with 1GB memory. The model ODEs are stiff and were integrated using Matlab’s built in function \texttt{ode15s} using an adaptive time step and relative and absolute tolerances of \(10^{-3}\) and \(10^{-6}\).

Summary and significance of the model

Although minimal in nature, the whole cell model of local and global \(Ca^{2+}\) signaling that is the focus of this paper accounts for the changes in myoplasmic and SR \([Ca^{2+}]\) mediated by the balance of stochastic release and reuptake by the SR, and the feedback of myoplasmic and
SR \( [Ca^{2+}] \) on spark frequency. As discussed in the introduction, previously published models of \( Ca^{2+} \) signaling in cardiac myocytes that include stochastic release of SR \( Ca^{2+} \) have either not included bi-directional coupling between local \( Ca^{2+} \) release and global \( Ca^{2+} \) homeostasis or, because of the computational challenge of the required Monte Carlo simulations, have not emphasized the phenomenon. To our knowledge this is the first systematic modeling study of the relationship between RyR kinetics, spontaneous and stochastic release of SR \( Ca^{2+} \), and the resulting balance of bulk \( Ca^{2+} \) concentrations in permeabilized ventricular myocytes. It is also the first model of \( Ca^{2+} \) sparks and homeostasis that bypasses Monte Carlo simulation by assuming both a large number of \( Ca^{2+} \) release sites and rapid \( Ca^{2+} \) domain dynamics, resulting in a minimal formulation that facilitates parameter studies.

The minimal whole cell model of local and global \( Ca^{2+} \) signaling that is the focus of this paper includes \( N + 3 \) ODEs and several algebraic relations. Two ODEs are concentration balance equations for the total myoplasmic \( (\hat{c}_{\text{myo}}) \) and SR \( (\hat{c}_{\text{sr}}) \) \( Ca^{2+} \) concentrations (Eqs. 15 and 16). The additional \( N + 1 \) ODEs (Eq. 4) account for the dynamics of a large number of \( Ca^{2+} \) release sites, each composed of \( N \) two-state RyRs (Eqs. 1 and 2). Algebraic relations include the fluxes (Eq. 17 and Eqs. 22–24) that appear in the concentration balance equations as well as the assumed relationship between myoplasmic \( (c_{\text{myo}}^{d,n}) \) and SR \( (c_{\text{sr}}^{d,n}) \) domain \( Ca^{2+} \) concentrations and the number of open channels (Eqs. 8–10). Note that the fluxes \( J_{\text{rel}}^T \), \( J_{\text{pump}} \), and \( J_{\text{leak}} \) are functions of \( c_{\text{myo}} \) and \( c_{\text{sr}} \), which are functions of \( \hat{c}_{\text{myo}}, \hat{c}_{\text{sr}}, \) and \( \pi \). The algebraic relationship between these quantities is found by inverting Eqs. 18 and 19 after substitution of Eqs. 8, 9, 20, and 21 (see Supporting Material).

Although our model formulation assumes a large population of \( Ca^{2+} \) release sites, we do not have to specify a precise number. To see this, note that the domain concentrations \( c_{\text{myo}}^{d,n} \) and \( c_{\text{sr}}^{d,n} \) do not depend on the number of release sites in the cell \( (M) \) when the rate constants are defined by \( v_{\text{rel}} = v_{\text{rel}}^{T}/M, \) \( v_{\text{myo}} = v_{\text{myo}}^{T}/M, \) and \( v_{\text{sr}} = v_{\text{sr}}^{T}/M \) (Eqs. 8–9). Because the rate \( v_{\text{rel}}^{T} \) that appears in Eq. 17 does not correspond to release through one release site, but rather the entire population, it is convenient to specify \( c_{\text{myo}}^{d,n} \) and \( c_{\text{sr}}^{d,n} \) using Eqs. 8–10 with the replacement of \( v_{\text{rel}}^{T}, v_{\text{myo}}^{T}, \) and \( v_{\text{sr}}^{T} \) for \( v_{\text{rel}}, v_{\text{myo}}, \) and \( v_{\text{sr}} \).

The minimal model of local and global \( Ca^{2+} \) signaling includes 14 parameters, far fewer than most mathematical models of \( Ca^{2+} \) handling in cardiac myocytes (see Table ?? in Supporting Material). Some parameters—such as the effective volume ratios \( \lambda_{\text{sr}}, \Lambda_{\text{sr}}^{d}, \Lambda_{\text{myo}}^{d} \) and the SERCA pump maximum rate \( (v_{\text{pump}}) \) and dissociation constant \( (k_{\text{pump}}) \)—are either chosen consistent with prior work [Williams et al., 2007, Williams et al., 2008] or do not require extensive consideration because model responses to changes in these parameters are obvious and intuitive. Because the ventricular myocyte is assumed to be permeabilized, the precise value of the parameter \( k_{\text{pm}} \) is unimportant so long as there is rapid equilibration of bulk myoplasmic \( Ca^{2+} \) with the extracellular \( [Ca^{2+}] \) \( (c_{\text{ext}}) \). The assumed number of RyRs in each release site \( (N = 10) \) is chosen to be consistent with estimates of the number of channels activated during a \( Ca^{2+} \) spark [Cheng et al., 1993, Gonzalez et al., 2000, Sobie et al., 2002]. This is a smaller number of RyRs than reported in electron microscopic (EM) studies performed a decade ago [Loesser et al., 1992, Franzini-Armstrong et al., 1999], but consistent with more recent estimates based on super-resolution optical techniques and 3D electron tomography [Hayashi et al., 2009, Baddeley et al., 2009]. The most important of the model parameters—the kinetic parameters for the stochastic gating of the two-state RyR \( (k_{\text{co}} \) and \( k_{\text{occ}} \)
and \(k_{oc}\) and the rates constants for Ca\(^{2+}\) release (\(v_{rel}^{T}\)), myoplasmic domain collapse (\(v_{myo}^{T}\)), and luminal domain recovery (\(v_{sr}^{T}\))—are more difficult to constrain and, consequently, these parameters are the focus of numerous sensitivity studies (see below).

The following aspects of the model behavior suggest that our standard parameter set is physiologically realistic. At 100 nM cytosolic [Ca\(^{2+}\)] (\(c_{myo}\)) the average duration of a spontaneous Ca\(^{2+}\) release event is on the order of 20 ms, similar to the observed rise time of Ca\(^{2+}\) sparks [Cheng et al., 1993, Cheng and Lederer, 2008]. The Ca\(^{2+}\) spark rate with \(c_{myo} = 100\) nM is 0.043 sparks per second per release site (approximately one spark every 23 seconds). Assuming 20,000 release sites in a ventricular myocyte, this corresponds to 860 sparks per second per cell, that is, 86 sparks per second in a fast 2D confocal frame scan that samples 10% of the cell volume. This value is consistent with experimental studies performed in intact cells that report spontaneous spark rates of \(1-4\times10^{-5}\) \(\mu m^{-2}ms^{-1}\), which corresponds to 30–120 sparks s\(^{-1}\) assuming a cross-sectional area of 100 \(\mu m \times 30\) \(\mu m\) [Bányaš et al., 2007]. Consistent with experiment, an increase in myoplasmic [Ca\(^{2+}\)] in the model leads to an increase in the spontaneous Ca\(^{2+}\) spark rate.

## Results

### RyR open probability and spontaneous Ca\(^{2+}\) sparks

The minimal model of local and global Ca\(^{2+}\) signaling that is the focus of this paper simulates stochastic Ca\(^{2+}\) release by clusters of RyRs and the resulting whole cell Ca\(^{2+}\) homeostasis in quiescent ventricular myocytes. The modeling formalism—described in the previous section—is chosen to be as simple as possible while still provide mechanistic insight into the perturbation of SR Ca\(^{2+}\) leak that results from pharmacological agents, mutations, or post-translational modifications of the RyR as may occur in disease states. For example, tetracaine—a potent local anesthetic that allosterically blocks Ca\(^{2+}\) release channels—reduces the open probability of RyRs in planar lipid bilayer experiments [Zima et al., 2008] by increasing the mean closed dwell time of channels [Györke et al., 1997]. Because the the mean closed time of the two-state RyR model is given by \(\tau_C = 1/k_{co}(c_{myo}^{d})^2\) (Eq. 1), we simulate the application of tetracaine to permeabilized ventricular myocytes by decreasing the rate constant \(k_{co}\), which influences the stochastic dynamics of the Ca\(^{2+}\) release sites (Eq. 2) in the minimal whole cell model. We wish to understand how the simulated application of tetracaine influences the dynamics of Ca\(^{2+}\) sparks and homeostasis in the permeabilized ventricular myocyte model.

Fig. 3 summarizes 60 numerical calculations of the stationary dynamics of the minimal whole cell model performed using different values of the RyR Ca\(^{2+}\)-activation rate constant \(k_{co}\). The circles highlight the result of two particular simulations: one corresponding to the standard parameter values (\(k_{co} = 4.5\) \(\mu M^{-2}s^{-1}\), filled circles) and the other corresponding to the simulated application of tetracaine (\(k_{co} = 0.5\) \(\mu M^{-2}s^{-1}\), open circles). In the latter case, the value of \(k_{co}\) is chosen so that the single channel \(P_{open}\) given by

\[
P_{open} = \frac{(c_{myo}^{d})^2}{(c_{myo}^{d})^2 + K^2}
\]

where \(K^2 = \frac{k_{oc}}{k_{co}}\) (Eq. 25)
is decreased by 88% upon action of tetracaine, consistent with experiments in which 0.7 mM tetracaine is applied [Zima et al., 2008] (see Table ??).

Fig. 3A shows that the simulated application of tetracaine leads to increased bulk SR [Ca$^{2+}$] in the whole cell model (c$_{sr}$ = 342 to 1112 µM; compare filled and open circles). As expected, the steady-state bulk SR [Ca$^{2+}$] increases as RyR open probability decreases, due to a decrease in the total release flux (Eq. 17). Because the SERCA pump flux (Eq. 23) is independent of the bulk SR [Ca$^{2+}$], the maximum bulk SR [Ca$^{2+}$] (c$_{sr}$) asymptotically approaches 2.5 mM when the association rate constant k$_{co}$ is very small. When a non-specific passive leak is not included in the model, c$_{sr}$ increases further (not shown). Results similar to Fig. 3A can be obtained without a passive leak by extending the SERCA pump model to include both forward and reverse modes [Shannon et al., 2000].

Fig. 3B shows that during the simulated application of tetracaine the fraction of open channels in a randomly sampled release site (f$_O$) is reduced by 79%, less than the reduction in the single channel open probability given by Eq. 25 (88%). This result indicates that the elevated bulk SR [Ca$^{2+}$] and the interaction between the RyRs combine to attenuate the decrease in channel activity occurring during the simulated application of tetracaine. That is, increased SR [Ca$^{2+}$] increases the driving force during stochastic Ca$^{2+}$ release events and elevates the myoplasmic domain [Ca$^{2+}$] (compare filled and open circles of Fig. 2B). The fraction of open channels is calculated as

$$f_O = \frac{E[N_O]}{N}$$

(26)

where

$$E[N_O] = \sum_{n=0}^{N} n \pi_n.$$

Fig. 3C and D show the frequency and duration of spontaneous Ca$^{2+}$ sparks occurring in the whole cell model as a function of the parameter k$_{co}$. The presence or absence of Ca$^{2+}$ sparks is assessed by calculating the Ca$^{2+}$ spark Score,

$$Score = \frac{1}{N} \frac{\text{Var}[N_O]}{E[N_O]}$$

(27)

where

$$\text{Var}[N_O] = \sum_{n=0}^{N} (n - E[N_O])^2 \pi_n.$$  

(28)

The Ca$^{2+}$ spark Score takes values from 0 to 1, and a Score of 0.2 or higher indicates robust sparks [Nguyen et al., 2005]. The solid lines in Fig. 3C and D show that Ca$^{2+}$ sparks were observed when the RyR Ca$^{2+}$-activation rate constant (k$_{co}$) was between 0.04 and 322 µM$^{-2}$s$^{-1}$, a range spanning four orders of magnitude.

With spark initiation defined as a release site reaching a threshold number of open channels (a $N_O = 4$ to 5 transition), and spark termination defined as all channels closing (a $N_O = 1$ to 0 transition), spark frequency and mean duration were calculated using the matrix analytic method described in ref. [Groff and Smith, 2008a]. The filled and open circles of Fig. 3C and D show that the simulated application of tetracaine decreases Ca$^{2+}$ spark frequency but increases mean Ca$^{2+}$ spark duration, consistent with experimental observations [Zima et al., 2008]. While bulk SR [Ca$^{2+}$] (c$_{sr}$) and spark frequency are monotone
functions of RyR open probability (decreasing and increasing, respectively), mean spark duration is a biphasic function of RyR open probability (first increasing, then decreasing).

Spark frequency and duration upon application of tetracaine

The left panel of Fig. 4A shows representative Ca\(^{2+}\) release events exhibited by a Ca\(^{2+}\) release site in the standard whole cell simulation. Spontaneous Ca\(^{2+}\) sparks were simulated using Gillespie’s method [Gillespie, 1976]. The parameters used correspond to the filled circles in Fig. 3 \((k_{co} = 4.5 \, \mu\text{M}^{-2}\text{s}^{-1})\) and result in robust Ca\(^{2+}\) sparks \((Score = 0.51)\) for the bulk concentrations \((c_{myo} \text{ and } c_{sr})\) of the equilibrated whole cell model. Note the high frequency of spontaneous release events, including five sparks \((N_O \geq 5)\) and a large number of smaller release events. These small release events, termed “Ca\(^{2+}\) quarks” [Lipp and Niggli, 1996], would not be detectable with standard confocal microscopy and would therefore contribute to “invisible” SR Ca\(^{2+}\) leak [Sobie et al., 2006]. The right panel of Fig. 4A shows an expanded version of the first spark (asterisk in left panel), which has a duration (17.5 ms) close to the mean spark duration with these standard parameters (18.3 ms; filled circle, Fig. 3D).

Fig. 4B shows representative Ca\(^{2+}\) release events during the simulated addition of tetracaine. The parameters used correspond to the open circles in Fig. 3 \((k_{co} = 0.5 \, \mu\text{M}^{-2}\text{s}^{-1})\). While robust Ca\(^{2+}\) sparks are observed \((Score = 0.51)\), the simulated application of tetracaine significantly reduces the spark frequency; one spark and three quarks are observed. While the mean spark duration is 26.8 ms (open circle, Fig. 3D), the right panel of Fig. 4B shows an expanded version of the observed spark, which is over 90 ms in duration. Consistent with experimental observations [Zima et al., 2008], such long duration sparks are not infrequent during the simulated addition of tetracaine, in spite of the fact that they almost never occur with the standard parameter set (see below).

In order to confirm that this decreased spark frequency and increased mean spark duration is due to overloading of bulk SR \([\text{Ca}^{2+}]\), Fig. 4C shows a control simulation using the single channel RyR parameters of Fig. 4B \((k_{co} = 0.5 \, \mu\text{M}^{-2}\text{s}^{-1})\) with bulk SR \([\text{Ca}^{2+}]\) “clamped” at the value observed in Fig. 4A \((c_{sr} = 342 \, \mu\text{M})\). The resulting simulation shows only a few release events, none with more than three channels open \((Score = 0.11)\). We conclude that the overloading of SR \([\text{Ca}^{2+}]\) that occurs when RyR open probability is decreased is required for the presence of prolonged sparks in the whole cell model.

As mentioned above, the simulated addition of tetracaine results in Ca\(^{2+}\) sparks whose duration tends to be longer than that observed with the standard parameters (compare right panels of Fig. 4A and B). To further quantify this effect, Fig. 5A shows the numerically calculated distribution of spark durations for the standard (solid line) and tetracaine (dashed line) parameter sets. While the mode of these distributions is nearly identical (standard: 4.8 ms, tetracaine: 4.6 ms), in the case of tetracaine the distribution extends further to the right, consistent with the higher probability of long sparks (Fig. 4B, right panel). Integrating Fig. 5A leads to cumulative probability distributions (Fig. 5B) that show 21.5\% of the sparks in the tetracaine simulations, but only 9.4\% of the sparks in the standard simulations, are longer than 40 ms (compare filled and open circles).
Magnitude of Ca$^{2+}$ release due to spontaneous sparks

As discussed above, the SR [Ca$^{2+}$] overload induced by tetracaine leads to higher myoplasmic domain [Ca$^{2+}$] (compare open and filled circles of Fig. 2A), higher SR domain [Ca$^{2+}$] (Fig. 2B), and higher release flux ($J_{rel}$, Eq. 17) for any given number of open channels. The resulting changes in the dynamics of Ca$^{2+}$-mediated coupling of RyRs leads to a decrease in spark frequency and an increase in spark duration (Fig. 3C and D). Nevertheless, filled and open circles of Fig. 5C show that the application of tetracaine decreases the aggregate release flux in the whole cell model. In fact, the solid line of Fig. 5C shows that the aggregate release flux is a monotone increasing function of RyR open probability, in spite of the fact that the SR [Ca$^{2+}$] is monotone decreasing (Fig. 3A).

During experimental observations of spontaneous Ca$^{2+}$ release, small amplitude events may not be detectable. Thus, it is of interest to dissect the aggregate release flux of the whole cell model to determine the fraction of spontaneous release that occurs via release sites that have few open channels. The dotted and dashed lines of Fig. 5C show that the release flux mediated by release sites with one or two open channels is a monotone increasing function of the RyR Ca$^{2+}$-activation rate constant $k_{co}$. Fig. 5D shows the aggregate flux $J_{rel}$ jointly distributed with the number of open channels for both the tetracaine and standard parameter sets. Both distributions are bimodal; a peak is observed at $N_O = 1$ as well as $N_O = 6$ or 7. Tetracaine suppressed the proportion of Ca$^{2+}$ released released through sites with seven or fewer open channels ($N_O \leq 7$), while release mediated by sites with eight or more open channels ($N_O \geq 8$) increases slightly in the presence of tetracaine. These observations are consistent with the increased probability of long duration Ca$^{2+}$ sparks observed upon application of tetracaine (Fig. 5A).

Because detectability of sparks recorded with fluorescent dyes is primarily determined by spark amplitude (i.e., integrated Ca$^{2+}$ release) [Smith et al., 1998, Cheng et al., 1999], Fig. 6A summarizes Monte Carlo simulations analyzing how spark amplitude and duration are jointly influenced by the simulated application of tetracaine (compare filled and open circles). Spark events are here defined as beginning with a $N_O = 0 \rightarrow 1$ transition, while spark amplitude is the integrated stochastic release flux (Eq. 5) prior to spark termination via a $N_O = 1 \rightarrow 0$ transition. Fig. 6B and C show the cumulative probability distributions of spark amplitude and duration, respectively. Sparks have both larger amplitude and extended duration when tetracaine is applied (consistent with Fig. 5A). Nevertheless, the decrease in spark frequency in the presence of tetracaine leads to the overall decrease in the aggregate release flux discussed above (Fig. 5C).

Fig. 6D shows the percentage of undetected spark events—and the “hidden” Ca$^{2+}$ release flux mediated by undetected sparks—as a function of a detection threshold on spark amplitude. The vertical dashed lines in Fig. 6B and D indicate a sensitive detection threshold equivalent to the amount Ca$^{2+}$ released by an average quark; in the standard simulation this is a single channel release event with duration 1.1 ms. With this sensitive detection threshold 36% of release events are not observed; most of these release events are quarks, brief single channel openings through which less than 1% of the stochastic Ca$^{2+}$ release occurs. Because the simulated application of tetracaine leads to increased SR load and greater release flux for any given number of open RyRs (Fig. 2), the tetracaine condition leads to fewer hidden events (15%) and decreased hidden release (< 0.1%). The solid and dashed lines of Fig. 6D
show that the percentage of hidden release events and hidden release flux are both increasing functions of detection threshold. For the range of possible detection thresholds shown, the percentage of hidden events decreases by 2–3 fold upon application of tetracaine.

**Transient effects upon application of tetracaine**

While the above simulations focused on steady-state dynamics of the whole cell model, Fig. 7 shows transient effects upon bulk SR $[\text{Ca}^{2+}] (c_{sr})$, mean spark duration, and spark frequency that occur during simulated application and washout of tetracaine. Consistent with experimental observations [Zima et al., 2008, Györke et al., 1997], the initial application of tetracaine causes spark frequency to decrease; the mean spark duration during this phase (5.0 ms) is much shorter than the baseline value (18.3 ms). However, this reduced spontaneous Ca$^{2+}$ release causes a slow increase in SR load that ultimately increases the mean spark duration to 26.8 ms, consistent with the steady-state results (Fig. 3D).

Fig. 7 also shows that upon simulated washout of tetracaine (right arrow) there is a transient increase in spark frequency (maximum of approximately 4 times the baseline value) and a rapid depletion of SR $[\text{Ca}^{2+}]$ from elevated to baseline values. For a short period of time the mean spark duration is quite large (see asterisk); however, the value attained is not relevant because it is greater than duration of the phase itself (400 ms). Shortly after this burst of spark activity, the mean spark duration returns to baseline.

**Model parameters and Ca$^{2+}$ homeostasis**

Fig. 8 summarizes 2500 calculations of the stationary dynamics of the whole cell model as a function of $v_{sr}^T$ and $v_{myo}^T$. These parameters control the rate of 'diffusion' or translocation of Ca$^{2+}$ between the different cellular subspaces represented in the minimal model, for example, from the individual myoplasmic domains (diadic subspaces) to the cytoplasm and from the bulk SR to the luminal domains (network to junctional SR). These domain rate constants also influence the strength of the Ca$^{2+}$-mediated coupling between RyRs and the extent of SR domain depletion during sparks (recall Eqs. 8–10). Because $v_{sr}^T$ and $v_{myo}^T$ are difficult to constrain via experiment, they are good choice for a parameter study designed to determine their effect on experimentally observable quantities such as the steady state bulk SR $[\text{Ca}^{2+}]$ and mean spark frequency and duration.

Fig. 8A shows that bulk SR $[\text{Ca}^{2+}]$ is an increasing function of rate of myoplasmic domain collapse ($v_{myo}^T$) and a decreasing function of the rate of SR domain recovery ($v_{sr}^T$). When $v_{myo}^T$ is very large or $v_{sr}^T$ is very small, RyRs become decoupled such that most openings fail to trigger neighboring channels, and the reduced leak causes an increase in bulk SR $[\text{Ca}^{2+}]$. Fig. 8B and D show, respectively, that the fraction of open channels and the mean spark duration are much more sensitive to $v_{myo}^T$ than $v_{sr}^T$. When $v_{myo}^T$ is small, sparks are extremely long because subspace $[\text{Ca}^{2+}]$ remains elevated after RyRs close (cf. [Mazzag et al., 2005, Huertas and Smith, 2007]). Fast SR refilling alone is not sufficient to induce long duration Ca$^{2+}$ sparks of the sort observed upon simulated application of tetracaine (cf. Fig. 4B).

Robust Ca$^{2+}$ sparks are observed in the whole cell model even when the domain rate constants are ranged over several orders of magnitude (Fig. 9A). This robust Ca$^{2+}$ spark behavior is a consequence of the homeostatic changes in bulk SR $[\text{Ca}^{2+}]$ accounted for in our
model formulation; when these simulations are repeated with bulk myoplasmic and SR Ca$^{2+}$ concentrations fixed at baseline values ($c_{myo} = 0.1 \mu M$, $c_{sr} = 342 \mu M$), the range of domain rate constants leading to Ca$^{2+}$ sparks is considerably smaller (Fig. 9B).

Fig. 10 shows the stationary dynamics of the whole cell model as a function of the bulk myoplasmic [Ca$^{2+}$] ($c_{myo}$) and the maximum rate of the SERCA pump ($v_{pump}$), two parameters that can be easily manipulated in experiment. The bulk SR [Ca$^{2+}$] is (as expected) an increasing function of $v_{pump}$; however, the SR Ca$^{2+}$ load is a biphasic function of $c_{myo}$ (Fig. 10A). The fraction of open channels and mean spark duration are both increasing functions of the SERCA pump activity (Fig. 10B and D). Spark frequency is not sensitive to SERCA activity, but is a rapidly increasing function of the bulk myoplasmic [Ca$^{2+}$] (Fig. 10C). Spark duration is a biphasic function of $c_{myo}$ (Fig. 10D), consistent with the biphasic effect of this parameter on SR load (Fig. 10A). The bulk myoplasmic [Ca$^{2+}$] ($c_{myo}$) significantly changes the functional dependence of the stationary dynamics of the whole cell model on the RyR Ca$^{2+}$-activation rate constant ($k_{co}$). On the other hand, when the application of tetracaine is simulated by comparing $k_{co}$ values that correspond to a decrease in RyR activity from $f_O = 9.6 \times 10^{-4}$ to $2.0 \times 10^{-4}$ (Table 1), we observe increased SR load, decreased spark frequency, and increased spark duration for $c_{myo}$ in the range 0.1–0.5 \mu M (see Fig. ??).

**RyR inhibition mechanism and spark duration**

We have modeled the action of tetracaine as a decrease in the Ca$^{2+}$-activation rate constant $k_{co}$, which reduces the open probability (Eq. 25) of the RyR model (Eq. 1) by increasing the mean closed dwell time, $\tau_C = 1/k_{co}(c_{myo}^4)^2$. However, the open probability of the RyR can also be reduced by increasing the rate constant $k_{oc}$, thereby decreasing the mean open dwell time ($\tau_O = 1/k_{oc}$). Such a change would be analogous to the pharmacological action of the antiarrhythmic agent flecainide, which has been shown to reduce the dwell time of RyR open states [Watanabe et al., 2009].

Fig. 11C and D show Ca$^{2+}$ spark frequency and mean spark duration as a function of the RyR kinetic constants $k_{co}$ and $k_{oc}$ when these spark statistics are well-defined ($Score \geq 0.2$). The contours dividing the plane into the areas where sparks are present (gray) and absent (white) follow constant $K = \sqrt{k_{oc}/k_{co}}$, indicating that sparks occur provided the single channel RyR open probability (Eq. 25) is neither too low or too high. If RyR parameters are changed from the standard values (asterisk), the resulting change in SR [Ca$^{2+}$] also depends only on RyR open probability (Fig. 11A). The SR [Ca$^{2+}$] is too high for sparks in the upper left white region and too low for sparks in the lower right (cf. Fig. 3A). In contrast, spark frequency and mean spark duration depend strongly on whether an pharmacological perturbation of RyR kinetics is assumed to affect $k_{co}$, $k_{oc}$, or both (diamonds). A reduction in $k_{co}$ (increase in $\tau_C$), analogous to effect of tetracaine, leads to fewer sparks but an increase in spark durations. Conversely, an increase in $k_{oc}$ (decrease in $\tau_O$), analogous to the effect of flecainide, causes more sparks with decreased mean duration. Examples of Monte-Carlo simulations under these different conditions are shown in Fig. 12. Similarly, we can assume that low dose caffeine leads to an increase $k_{co}$ accompanied by a smaller decrease in $k_{oc}$ (+ symbol) [Kong et al., 2008]. The model predicts that this will cause a decrease in SR [Ca$^{2+}$], an increase in spark frequency, and little change in spark duration, roughly consistent with
the results observed by Lukyanenko et al. [Lukyanenko et al., 2001].

These results illustrate a fundamental point about the interplay between local and global Ca$^{2+}$ signals during pharmacological interventions designed to manipulate spontaneous cellular responses. Local (microscopic) aspects of cell response—such as spark frequency and duration—are highly dependent on the molecular details, that is, precisely how the kinetics of RyR stochastic gating kinetics have been modified. Global (macroscopic) aspects of cell response—such as the steady state bulk SR [Ca$^{2+}$]—are less dependent on the kinetic details of the perturbation, but remain determined by equilibrium quantities such as the dissociation constant for Ca$^{2+}$ binding to the RyR.

Discussion

We have presented a minimal whole cell model of local and global Ca$^{2+}$ signals in quiescent ventricular myocytes. The modeling formalism accounts for the effect of random spontaneous Ca$^{2+}$ sparks, changes in bulk myoplasmic and SR [Ca$^{2+}$] mediated by the balance of stochastic release and reuptake by the SR, and feedback of myoplasmic and SR [Ca$^{2+}$] on spark frequency. The functional organization of the model (Fig. 1) is similar to previously published Monte Carlo models of local control [Greenstein and Winslow, 2002]. The assumptions made here regarding rapid equilibration of domain Ca$^{2+}$ are similar to the assumptions made in previously published local control models that represent the stochastic dynamics of a large number of Ca$^{2+}$ release sites [Hinch et al., 2004, Greenstein et al., 2006], but these prior studies do not make a distinction between domain and bulk SR Ca$^{2+}$ as done in our minimal whole cell model.

To our knowledge this is the first theoretical study of the relationship between RyR kinetics, spontaneous and stochastic Ca$^{2+}$ release, and the resulting balance of bulk myoplasmic and SR [Ca$^{2+}$] in quiescent ventricular myocytes. Because of the computational challenge of large-scale simulations, a traditional Monte Carlo approach is not well-suited to investigate these phenomena. The whole cell modeling approach introduced here bypasses Monte Carlo simulation by assuming a large number of Ca$^{2+}$ release sites and rapid Ca$^{2+}$ domain dynamics, resulting in a minimal formulation that facilitates parameter studies.

The minimal model of local and global Ca$^{2+}$ responses in quiescent ventricular myocytes presented here is able to recapitulate recent experiments by Zima et al. [Zima et al., 2008] showing that tetracaine, an inhibitor of RyRs, causes a transient suppression of Ca$^{2+}$ sparks that is followed by an increase in bulk SR [Ca$^{2+}$], partial recovery of spark frequency, and an increase in Ca$^{2+}$ spark duration (Fig. 11). Conversely, if flecainide is assumed to decrease RyR mean open time while not affecting closed times, the model predicts that this drug will cause an increase in spark frequency and decrease in spark duration, similar to recent experimental observations [Hilliard et al., 2010]. However, it should be noted that this study found no change in steady-state SR [Ca$^{2+}$], suggesting that the effects of flecainide on RyR gating are somewhat more complex.

More broadly, these examples illustrate how the model provides insight into the relationships between RyR gating, Ca$^{2+}$ spark characteristics, and the balance of bulk myoplasmic and SR [Ca$^{2+}$] (Figs. 3–5). For example, simulations showing that mean spark duration is a biphasic function of the RyR Ca$^{2+}$-activation rate constant $k_{co}$ suggest that the increase
in spark duration observed after application of tetracaine may be concentration dependent (Fig. 3D). In spite of the fact that spark duration is biphasic, dissection of model responses suggests that the release flux is a monotone function of RyR open probability. The model also predicts that tetracaine suppresses the hidden flux mediated by Ca\(^{2+}\) release events below detection threshold more strongly than observable release events (Fig. 6D). Parameter studies exploring the effect of different mechanisms of RyR inhibition (Table 1 and Fig. 12) indicate that whole cell Ca\(^{2+}\) balance is largely determined by RyR open probability (a function of \(K = \sqrt{k_{oc}/k_{co}}\)). Conversely, the frequency and duration of Ca\(^{2+}\) sparks are sensitive to the RyR’s open and closed dwell times that are determined by \(k_{co}\) and \(k_{oc}\) independently (see Fig. 11).

The Ca\(^{2+}\) release site model used here is quite simple and assumes ‘instantaneous mean field coupling’ [Nguyen et al., 2005] of \(N = 10\) two-state RyRs. When model simulations are performed with release sites composed of larger numbers of channels, qualitatively similar results are observed (Fig. 11). Our whole cell modeling approach can be applied with single channel models of arbitrary complexity, so long as the state space of the resulting Ca\(^{2+}\) release site model is not so large that integrating Eq. 4 is impractical. Our use of a two-state RyR in this first study of the relationship between Ca\(^{2+}\) sparks and homeostasis allows us to focus on aspects of the cellular response to perturbations that are likely to be fundamental and general. A more complicated RyR model would call into question the specific details of how we (arbitrarily) suppose pharmacological perturbations influence the kinetic constants that determine RyR stochastic gating, assumptions that could easily influence the whole cell response and our conclusions. We find it intriguing that this model of local and global Ca\(^{2+}\) responses in quiescent ventricular myocytes is able to reproduce changes in spark frequency and duration caused by application of both tetracaine and flecainide, in spite of fact that the RyR model used does not include regulatory processes such as Ca\(^{2+}\)-dependent inactivation and/or sensitization by SR [Ca\(^{2+}\)]. The effect of these well-established aspects of RyR Ca\(^{2+}\) regulation is beyond the scope of this work.

The Ca\(^{2+}\) activation process in the RyR model is mediated by myoplasmic domain Ca\(^{2+}\) (\(c_{\text{myo}}^{d}\), Eq. 9) and it would be possible to augment the model to include sensitization of RyRs by SR domain Ca\(^{2+}\) (\(c_{\text{sr}}^{d}\), Eq. 9). However, the assumption of instantaneous coupling of RyRs (i.e., rapid equilibration of myoplasmic and SR domain Ca\(^{2+}\)) may not work well when luminal Ca\(^{2+}\) plays a role in spark termination [Sobie et al., 2002, Huertas and Smith, 2007]. A computational study of the contribution of luminal RyR regulation to the bi-directional coupling of spontaneous Ca\(^{2+}\) release and Ca\(^{2+}\) homeostasis will likely require more subtle mathematical formulations similar to probability density and moment closure techniques that accelerate ‘local control’ simulations of high-gain graded Ca\(^{2+}\) release in voltage-clamped cardiac myocytes [Williams et al., 2007, Williams et al., 2008]. These representations of heterogenous domain Ca\(^{2+}\) concentrations associated with a large number of Ca\(^{2+}\) release sites remain valid even when the dynamics of SR domain Ca\(^{2+}\) are not fast compared to channel gating. Indeed, the model presented here can be viewed as a reduction of the moment closure formulation [Williams et al., 2008] that is valid when SR Ca\(^{2+}\) domains rapidly equilibrate with myoplasmic domain and bulk SR Ca\(^{2+}\), in which case there is negligible variance of SR domain [Ca\(^{2+}\)] for any given Ca\(^{2+}\) release site state.

In preliminary work we have studied whole cell responses using RyR models that in-
clude both Ca\(^{2+}\) activation and inactivation by myoplasmic domain Ca\(^{2+}\), for example, the following three state model,

\[
\begin{align*}
C & \xrightleftharpoons[k_{oc}]{k_{co}(c_{myo}^{d})^{2}} O & \xrightleftharpoons[k_{ro}]{k_{or}(c_{myo}^{d})^{2}} R,
\end{align*}
\] (29)

that includes a long-lived closed state \(R\). As expected, release sites composed of such channels exhibit Ca\(^{2+}\) sparks and homeostasis similar to that observed for the two-state RyR when the dissociation constant \(K_{\text{inact}} = \sqrt{k_{ro}/k_{or}}\) is large (see Fig. ??). While fast Ca\(^{2+}\) inactivation (large \(k_{ro}\) and \(k_{or}\) with fixed \(K_{\text{inact}}\)) is associated with decreased spark duration and increased spark frequency, extremely fast Ca\(^{2+}\) inactivation can preclude sparks (Fig. ??C and D). Provided Ca\(^{2+}\) inactivation is sufficiently fast, decreasing \(K_{\text{inact}}\) leads to increased SR Ca\(^{2+}\) load, decreased RyR activity, increased spark frequency, and decreased spark duration (Fig. ??A–D). Interestingly, decreasing the Ca\(^{2+}\)-activation rate constant \((k_{co})\) in the three-state model with Ca\(^{2+}\) inactivation (Eq. 29) to simulate application of tetracaine may lead to longer or shorter duration sparks depending on whether the rate of Ca\(^{2+}\) inactivation is slow or fast, respectively, in spite of the fact that neither RyR activity or SR load are strongly affected by the rate of Ca\(^{2+}\) inactivation in either condition (see Fig. ??). This sensitivity of the stochastic dynamics of Ca\(^{2+}\) release to the rate of Ca\(^{2+}\) inactivation is consistent with results obtained using Ca\(^{2+}\) release site models that do not account for Ca\(^{2+}\) homeostasis [Groff and Smith, 2008a]. However, the significance of these observations is unclear given recent experimental results showing that, even at 50 \(\mu M\) myoplasmic [Ca\(^{2+}\)], inactivation is unable to suppress SR Ca\(^{2+}\) release in permeabilized myocytes [Stevens et al., 2009].

While the simulated spark duration histograms of Fig. 5A are unimodal, the experimentally observed spark duration histogram shows two peaks in the presence of tetracaine, suggesting two distinct populations of sparks [Zima et al., 2008]. Zima and coworkers suggested that the prolonged sparks occurred at release sites with highly interconnected junctional SR and high refilling rates. Our simulations presented in Fig. 8 address this idea by investigating the effects of a larger domain refilling rate \((v_{sr}^{T})\). Consistent with the hypothesis of Zima et al. [Zima et al., 2008], an increase in \(v_{sr}^{T}\) from 10 to 50 \(s^{-1}\) causes a slight increase in baseline Ca\(^{2+}\) spark duration, as well as a greater percent increase upon application of tetracaine (57\% versus 47\%, not shown). This suggests that the long sparks observed upon application of tetracaine [Zima et al., 2008] may indeed be associated with fast rather than slow SR refilling.

Finally, it is intriguing that robust Ca\(^{2+}\) sparks are observed in the whole cell model even when the domain rate constants are ranged over several orders of magnitude (Fig. 8). Because the range of domain rate constants leading to Ca\(^{2+}\) sparks is considerably smaller when bulk myoplasmic and SR Ca\(^{2+}\) concentrations are fixed at baseline values (compare Fig. 9A and B in Supporting Materials), we conclude that this robust Ca\(^{2+}\) spark behavior is a consequence of homeostatic changes in bulk SR [Ca\(^{2+}\)] accounted for in our model formulation. The fact that the feedback of spontaneous SR leak on spark frequency extends the regime where spontaneous sparks occur is potentially physiologically relevant. Speaking teleologically, the homeostatic mechanisms appear to encourage SR leak mediated by Ca\(^{2+}\) sparks and discourage the alternatives: SR leak mediated by quarks or tonically active release.
sites. These observations underscore the importance of accounting for global Ca\(^{2+}\) balance in models of localized Ca\(^{2+}\) release events.

**Acknowledgements**

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**References**


Table 1: Three mechanisms of RyR inhibition have different consequences on mean spark duration and spark frequency. Results for whole cell model with standard parameters and modified Ca\(^{2+}\)-activation rate constant \(k_{co}\) that represents application of tetracaine and leads to an increase in RyR open time (cf. filled and open circles in Fig. 3, respectively). When the same level of RyR inhibition is modeled as a decrease in open dwell time (\(\tau_O\downarrow\)) as may occur upon application of flecainide, or a dual mechanism (\(\tau_C\uparrow \& \tau_O\downarrow\)), the mean spark duration changes. Hyphens (-) indicate results identical to the tetracaine case.

<table>
<thead>
<tr>
<th></th>
<th>standard</th>
<th>tetracaine (\tau_C\uparrow)</th>
<th>flecainide (\tau_O\downarrow)</th>
<th>dual mechanism (\tau_C\uparrow &amp; \tau_O\downarrow)</th>
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<td>(k_{co}) ((\mu\text{M}^{-2}\text{s}^{-1}))</td>
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<td>4.5</td>
<td>1.5</td>
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<td>(k_{oc}) (\text{s}^{-1})</td>
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<td>450</td>
<td>1500</td>
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<tr>
<td>(f_O)</td>
<td>9.6 \times 10^{-4}</td>
<td>2.0 \times 10^{-4}</td>
<td>-</td>
<td>-</td>
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<tr>
<td>(c_{sr}) ((\mu\text{M}))</td>
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<td>0.59</td>
<td>-</td>
<td>-</td>
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<tr>
<td>duration (ms)</td>
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<td>26.7</td>
<td>2.97</td>
<td>8.90</td>
</tr>
<tr>
<td>frequency (sparks/s)</td>
<td>0.043</td>
<td>6.5 \times 10^{-4}</td>
<td>0.053</td>
<td>0.0059</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1

The minimal whole cell model represents two bulk compartments: the SR ($c_{sr}$) and the myoplasm ($c_{myo}$). The membrane of the SR includes multiple release sites, each of which includes a local Ca$^{2+}$ domain on both sides of the membrane. The domain Ca$^{2+}$ concentrations ($c^{d}_{myo}$ and $c^{d}_{sr}$) are influenced by fluxes that involve the bulk Ca$^{2+}$ concentrations (myoplasmic and SR) and the state of the release site. Fluxes include diffusion from myoplasmic domains to the bulk myoplasm ($J_{myo}$), diffusion from the bulk SR to the luminal domains ($J_{sr}$), the SERCA pump flux that re-sequesters Ca$^{2+}$ into the SR ($J_{pump}$), a passive leak from the SR to the myoplasm ($J_{leak}$), and the fluxes across the plasma membrane ($J_{pm}$).

Figure 2

A: Myoplasmic domain [Ca$^{2+}$] ($c^{d,n}_{myo}$ in Eq. 8) as a function of the number of open channels ($N_{O} = n$). B: SR domain [Ca$^{2+}$] ($c^{d,n}_{sr}$ in Eq. 9). Filled and open circles correspond to different bulk SR Ca$^{2+}$ concentrations ($c_{sr} = 342$ and 1112 µM, respectively). The myoplasmic [Ca$^{2+}$] is $c_{myo} = 0.1$ µM in both cases.

Figure 3

Steady-state responses of the minimal whole cell model of local and global Ca$^{2+}$ signaling as a function of the Ca$^{2+}$-activation rate constant ($k_{co}$) in the single channel RyR model (Eq. 1). A: The bulk SR [Ca$^{2+}$] ($c_{sr}$, Eq. 18). B: The fraction of open channels in whole cell model ($f_{O}$, solid line) as well as single channel RyR open probability ($P_{O}$, Eq. 25, dashed line). C. Spark frequency. D. Mean spark duration. The filled circles (●) indicate the standard parameter value; the open circles (○) designate the reduction in $k_{co}$ corresponding to the simulated addition of tetracaine. Other parameters as in Table ??.

Figure 4

Representative Ca$^{2+}$ sparks in the minimal whole cell model under three different steady-state conditions. Left panels show time and amplitude of Ca$^{2+}$ quarks and sparks exhibited by a representative release site. Right panels show the Ca$^{2+}$ release events indicated by an asterisk in more detail. A: Standard parameters corresponding to filled circles in Fig. 3 ($k_{co} = 4.5$ µM$^{-2}$s$^{-1}$ resulting in spark $Score = 0.51$). B: Simulated application of tetracaine corresponding to open circles in Fig. 3 ($k_{co} = 0.5$ µM$^{-2}$s$^{-1}$, $Score = 0.59$). C: Control simulation with RyR parameters as in B ($k_{co} = 0.5$ µM$^{-2}$s$^{-1}$) and bulk SR [Ca$^{2+}$] as in A ($c_{sr} = 342$ µM) resulting in $Score = 0.11$. The durations of the Ca$^{2+}$ release events shown in the right panels are 17.54 (A), 91.02 (B), and 3.76 ms (C). Fluctuations in the number of open channels prior to termination of the Ca$^{2+}$ release event are always present (A–D), but are most obvious in long duration sparks (B).
Figure 5
A, B: Probability density (A) and cumulative probability (B) of spark duration for the standard (solid line) and tetracaine-based (dashed line) parameter sets (see Table ??). Circles indicate probability of observing a spark 40 ms or longer in the standard (filled) and tetracaine (open) cases. C: Aggregate flux ($J_{rel}^T$, solid line) through release sites in the whole cell model as a function of the Ca$^{2+}$-activation rate constant ($k_{co}$) in the single channel RyR model (Eq. 1). The flux due to release sites with a small number of open channels is also shown ($N_O = 1; \text{and } N_O = 1 \text{ or } 2$). The open (o) and filled (●) circles indicate the tetracaine and standard parameter sets, respectively. D: Release site flux ($J_{rel}^T$) jointly distributed with the number of open channels ($N_O$) for the tetracaine (white) and standard (black) parameter sets.

Figure 6
A: Monte Carlo sampling of blip/spark duration and amplitude (integrated Ca$^{2+}$ release) for the standard (filled circles) and tetracaine-based (open circles) parameter sets (see Table ??). Each blip or spark is initiated by a $N_O = 0 \rightarrow 1$ transition (cf. Fig. 5 where $N_O = 4 \rightarrow 5$ is interpreted as a spark initiation event). B, C: Cumulative probability distribution of spark amplitude (B) and duration (C) for the standard (solid line) and tetracaine-based (dashed line) parameter sets. Amplitude of release is expressed in units of femtocoulomb (fC) under the assumption of a total myoplasmic volume of $2 \times 10^{-5}$ µL and $2 \times 10^4$ release sites per cell (see Supporting Material). D: The percentage of “hidden” (undetected) spark events and the percentage of Ca$^{2+}$ release flux mediated by hidden sparks as a function of spark amplitude detection threshold.

Figure 7
Transient effects upon bulk SR [Ca$^{2+}$] ($c_{sr}$), mean spark duration, and spark frequency during simulated application and washout of tetracaine (arrows). The Ca$^{2+}$-activation rate constant is decreased from $k_{co} = 4.5 \mu M^{-2}s^{-1}$ (left arrow) and later restored to its original value (right arrow). The mean spark duration is not applicable at the time indicated by the asterisk (see text).

Figure 8
Effect of $v_{myo}^T$ and $v_{sr}^T$ on bulk SR [Ca$^{2+}$] (A), fraction of open channels (B), spark frequency (C), and mean spark duration (D) in the minimal whole cell model. Asterisks indicate standard parameters. White indicates mean spark duration was not calculated because the spark Score is less than 0.2.

Figure 9
Spark Score as a function of the rate of myoplasmic domain collapse ($v_{myo}^T$) and SR domain recovery ($v_{sr}^T$). Robust sparks correspond to a Score greater than 0.2. Asterisks indicate
standard parameters. A: In the minimal whole cell model of quiescent ventricular myocytes, the bulk SR $[Ca^{2+}]$ is an increasing function of $v_{myo}^T$ and a decreasing function of $v_{sr}^T$ (Fig. 8A) and the sparks are observed for a wide range of values of the rate constants. B: When these calculations are repeated with bulk myoplasmic and SR $Ca^{2+}$ concentrations fixed at baseline values ($c_{myo} = 0.1 \mu M$, $c_{sr} = 342 \mu M$), the range of domain rate constants leading robust sparks is much smaller.

**Figure 10**

Effect of $c_{myo}$ and $v_{pump}$ on bulk SR $[Ca^{2+}]$ (A), fraction of open channels (B), spark frequency (C), and mean spark duration (D) in the minimal whole cell model. Asterisks indicate standard parameters. White indicates mean spark duration was not calculated because the spark *Score* is less than 0.2.

**Figure 11**

Effect of rate constants $k_{co}$ and $k_{oc}$ in the RyR single channel model (Eq. 1) on bulk SR $[Ca^{2+}]$ (A), fraction of open channels (B), spark frequency (C), and mean spark duration (D). Asterisks indicate standard parameters. Diamonds indicate various changes in the open and closed dwell times of the RyR (cf. tetracaine, flecainide, and dual mechanism of Table 1 and Fig. 12). The square indicates an increase in $k_{co}$ accompanied by a smaller decrease in $k_{oc}$ corresponding to a low dose of caffeine. The steady-state SR load ($c_{sr}$) and fraction of open channels ($f_O$) are functions of the RyR $Ca^{2+}$ binding constant $K = \sqrt{k_{oc}/k_{co}}$, which is constant along lines parallel to the diagonal band for which spark frequency and duration are well-defined (dashed lines, *Score* > 0.2).

**Figure 12**

Representative $Ca^{2+}$ sparks in the minimal whole cell model under the four different steady-state conditions presented in Table 1. A, B: Stochastic $Ca^{2+}$ release events in whole cell model with standard parameters (A) and modified $Ca^{2+}$-activation rate constant $k_{co}$ that represents application of tetracaine and leads to an increase in RyR open time (B). When the same level of RyR inhibition is modeled as a decrease in open dwell time as may occur upon application of flecainide ($\tau_O \downarrow$, C), or a dual mechanism ($\tau_C \uparrow \& \tau_O \downarrow$, D), the mean spark duration changes.
Figures

Figure 1:
Figure 2:
Figure 3:
Figure 4:
Figure 6:
Figure 7:
Figure 8:
Figure 10:
A

$C^d_{\text{myo}}$

$N_O$

B

$C^d_{\text{sr}}$

$N_O$