Stochastic Modeling of Calcium Release Units Reveals a Strong Dependence of Calcium Release Variability on Cluster Size

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Abstract

Intracellular calcium (Ca^{2+}) dynamics drive contractile function in cardiac myocytes. In particular, L-type Calcium Channels (LCCs) and Ryanodine Receptors (RyRs) are organized in microdomains, where LCCs trigger substantial Ca^{2+} release from the Sarcoplasmic Reticulum (SR) via RyRs. We present a minimal Calcium Release Unit (CRU) model for human ventricular myocytes that integrates a recently developed Markov Chain (MC)-based description of LCCs, replacing classical Hodgkin-Huxley gates. Our approach builds on previously published MC-based frameworks for the human heart, enabling stochastic gating and faithfully reproducing both spontaneous and evoked local Ca^{2+} release events. Despite its simplicity, our single-CRU system captures key features of microscale and macroscale Ca^{2+} cycling, revealing how the variability in Ca^{2+} release depends on microdomain size. In particular, simulations show a sharp transition in Ca^{2+} release dynamics around cluster sizes of 100 LCC channels, marking a critical threshold where stochastic behavior gives way to deterministic-like dynamics.

1. Introduction

Cardiac contraction relies on highly regulated intracellular Ca^{2+} cycling. At the onset of each heartbeat, a moderate Ca^{2+} influx through L-type Calcium Channels (LCCs) initiates a dramatic release of Ca^{2+} from the sarcoplasmic reticulum (SR) by activating clusters of Ryanodine Receptors (RyRs) in a process known as Calcium-Induced Calcium Release (CICR). While large-scale deterministic cell models [1, 2] effectively replicate global action potentials and calcium transients, they often depend on Hodgkin-Huxley-type gating variables, which average the behavior of thousands of channels. This approach fails to capture the inherent subcellular stochasticity crucial for understanding localized phenomena such as spontaneous Ca^{2+} sparks or subcellular waves [3].

Recently, we developed a robust Markov Chain (MC) parameterization specifically designed for L-type Calcium Channels in the context of human cardiac electrophysiology [4]. Recognizing the significant role of stochastic behavior in the CICR process, our research focuses on a single cluster of LCCs and RyRs within the ten Tusscher-Panfilov MC framework. We devised a stochastic adaptation of the MC-based model to systematically vary the number of LCCs and RyRs from a minimal count to several hundred or thousands. This approach effectively illustrates how the level of local randomness diminishes as the number of channels increases.

By simulating the dynamics of a single cluster, we systematically analyze the variability in Ca^{2+} release as a function of cluster size. Our results reveal a sharp transition in behavior around 100 LCCs, marking a critical threshold where release shifts from a stochastic to a more deterministic regime. Below this threshold, release events exhibit high variability, driven by fluctuations in the opening and closing of individual channels. In contrast, larger clusters produce more predictable Ca^{2+} release patterns, as the influence of single-channel stochasticity diminishes. Quantifying variability across different cluster sizes provides novel insights into how microdomain organization governs excitation-contraction coupling.

Despite its simplicity, this single-cluster release unit (CRU) system replicates essential behaviors at lower channel counts and deterministic waveforms at higher channel counts. This model not only enhances our understanding of the fundamental mechanisms governing Ca^{2+} dynamics but also provides valuable insights into the interplay between stochasticity and determinism in cardiac excitation-contraction coupling.

2. Methods

We start with a baseline deterministic cell model and introduce stochastic Markov Chain representations for L-type Calcium Channels (LCCs) and Ryanodine Receptors (RyRs). This approach allows us to adapt subcellular calcium compartments and fluxes to exhibit both deterministic and probabilistic behaviors depending on channel densities.

2.1. Defining the Single CRU Setup

Our focus is on a single Calcium Release Unit (CRU), comprised of a cluster of LCCs and RyRs within a dyadic microdomain. Let N_{LCC} represent the number of L-type channels in the cluster and N_{RyR} the number of RyRs. A typical configuration might use $(N_{LCC}, N_{RyR}) = (4, 20)$ [5, 6], although our model offers flexibility for exploring various channel densities.

Local calcium concentrations, such as $[Ca]_{ss}$, $[Ca]_{SR}$, and $[Ca]_i$, are determined by fluxes resulting from I_{CaL}, I_{rel}, and I_{up}, similar to standard deterministic modeling approaches. The fluxes I_{CaL} and I_{rel} are adjusted based on the proportion of channels that are open in the Markov Chain (MC). For example, the calcium current through L-type channels can be computed as follows:

$$I_{CaL} = o_L G_{CaL} \frac{\Delta V F^2}{RT} \left(\frac{[Ca]_{ss} e^{\frac{2\Delta VF}{RT}} - 4[Ca^{2+}]_o}{e^{\frac{2\Delta VF}{RT}} - 1} \right), \quad (1)$$

where $\Delta V = V - 15$. Similarly, the calcium release from RyRs can be expressed as:

$$I_{rel} = o_R V_{rel}([Ca]_{SR} - [Ca]_{ss}). \quad (2)$$

In these equations, o_L and o_R denote the fraction of open L-type channels and open Ryanodine Receptors, respectively. The other parameters are derived from the original model [2].

Figure 1 illustrates the Markov Chain-based model, emphasizing the components that constitute a single CRU. We use a four-state model of RyR2 and the 7 state model of the LCC. The four-state model of the RyR has cytosolic calcium regulation corresponding to the Calcium-Induced Calcium-Release (CICR) mechanism and inactivation are directly mediated by cytosolic calcium as well, mimicking Ca-Calmodulin complex regulation. Additionally, in this RyR model, both the transitions from the resting to the open (O) state or to the resting-inactivated (RI) state are also regulated by the sarcoplasmic reticulum (SR) calcium concentration, capturing luminal control of channel gating.

Meanwhile, the LCC, represented by a seven-state Markov Chain, captures voltage-dependent opening, calcium-dependent inactivation, and closed or inactivated states, thus providing a more biophysically faithful portrayal of how a modest trigger current can initiate a large-scale release of calcium from the SR.

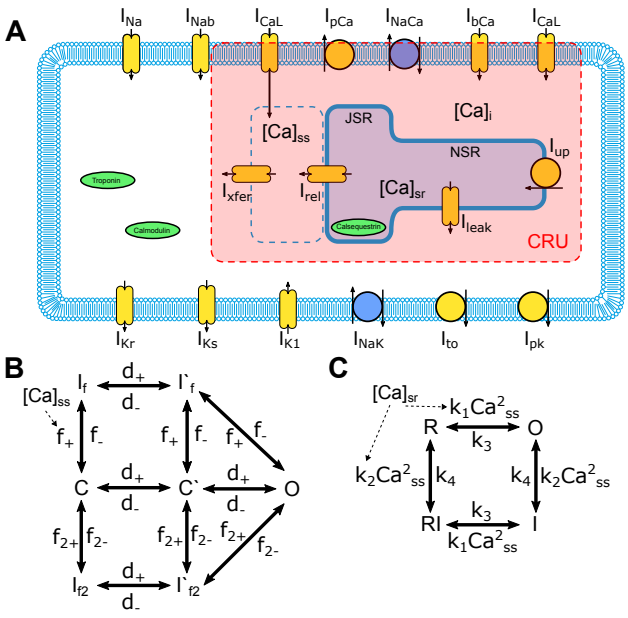


Figure 1. Panel (A) shows a schematic representation of the novel MC-based Model highlighting, in red, the structures that compose a single CRU. Panel (B) shows the LCC 7 states MC, with one open, two closed, and four inactivated states. Panel (C) shows the RyR2 model with four states: one open, two inactivated and one closed.

Since o_L and o_R can vary stochastically, the single-CRU model might exhibit beat-to-beat fluctuations in local Ca^{2+} release.

To track these fluctuations, we use tau leaping to efficiently model systems with discrete events. More specifically, to simulate the number of RyR2 in the open state, we do not track each individual change in configuration, but we compute the number of multiple events that occur within a specified time interval (dt). This is achieved by exploiting the binomial distribution to represent the number of successful transitions of states [7]. Using this approach, the model captures the stochastic dynamics of the system while significantly reducing the computation time compared to simulating each event individually.

2.2. Computational Experiments

This study introduces a stochastic Markov Chain (MC) model, where a cell consists of a single calcium release unit (CRU). Our model recovers the deterministic MC model in the limit of a large number of LCC and RyR. However, the stochastic model incorporates randomness in calcium currents, specifically the I_{CaL} (L-type calcium current) and I_{rel} (calcium release current). Such variability of the calcium dynamics within the unit allows one to address the relevance of stochasticity in the core model and explore the role of stochasticity in the underlying cellular process.

To carry out our numerical experiments, we varied the

number of L-type calcium channels (LCCs) within the CRU, exploring values ranging from 1 to 32768. This progression was made using the rule of doubling the number of LCCs with each experiment. According to established literature [5,6], for each chosen value of N_{LCC}, the number of ryanodine receptors (N_{RyR}) was determined using the relationship: $N_{RyR} = 5 \times N_{LCC}$ For each combination of N_{LCC} and the subsequently determined N_{RyR}, we simulated a sequence of 110 pulses at a pacing rate of 1 Hz. Importantly, the first ten pulses were excluded from analysis to allow the system to reach a stable state. This setup resulted in a total of 100 pulse outputs for each specific value of both N_{LCC} and N_{RyR}.

To quantify the level of stochasticity in our simulations, we focused on the influx of calcium ions from the sarcoplasmic reticulum into the intracellular medium (I_{rel}), as represented by the I_{rel} current over a 1-second interval following stimulation. The results obtained from the summations $S(I_{rel})$ will be presented using descriptive statistics to capture the variability observed across the different simulation realizations.

3. Results

Figure 2 shows the descriptive measures of the values $S(I_{rel})$ grouped by N_{LCC} and N_{RyR}. These measures are compared with the same value obtained from the deterministic version of the model.

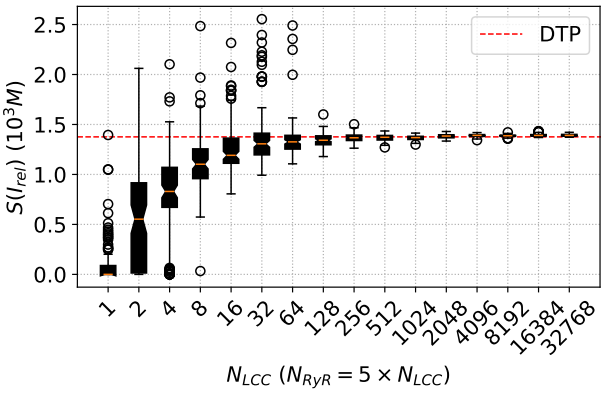


Figure 2. Descriptive Measures of the stochastic metric S asscoaited with the total release of Calcium from the SR . For each N_{LCC} and, consequently, N_{RyR}, there were generated $n = 100$ outputs. In contrast, there are presented also the S values for the deterministic model (DTP).

The results illustrate that the stochastic model effectively captures multiscale characteristics, as evidenced by the changes in the average, and standard deviation values, obtained as a function of N_{LCC} and N_{RyR}. For very low values of $N_{LCC}(N_{LCC} \leq 10)$ associated with realistic values of the number of channels in a single microdomain, we reproduce the strongly stochastic nature and variability. We also observe a sharp increase in the average release of Ca^2 as the number of channels increases. We notice that the release values at the microdomain level are much lower than those obtained with a deterministic equation.

Our stochastic simulation perfectly reproduced the deterministic limit with larger N_{LCC} and N_{RyR}. More specifically, for $N_{LCC} \geq 250$, the system exhibits a very reduced stochasticity. Surprisingly, this transition from a strong stochastic variability to an almost deterministic is very sharp. We can observe how this standard deviation drops very strongly from 64 to 128 channels. Around 100 channels there is a strong reduction in variability.

The same behavior can be seen in the traces of the output. Figure 3 presents the 100 output curves of the main variables of the STP output (color lines) compared to the same traces obtained by the DTP model (black line). Panels A, C and E show the AP; panels B, D and F show the $[Ca]_{i_{free}}$ traces. Panels A-B show the results obtained from STP using $N_{LCC} = 1$; panels C-D show the same traces obtained from STP using $N_{LCC} = 32$; and panels E-F show the same traces obtained from STP using $N_{LCC} = 32768$. For all simulations, N_{RyR} was defined as $N_{RyR} = 5 \times N_{LCC}$.

Following the same behavior shown in Figure 2, the curves presented in Figure 3 also converged to deterministic values as soon as N_{LCC} and N_{RyR} reach values of 100 LCC channels. It indicates that the STP model can properly reproduce both extreme scales, micro and macro scales.

4. Discussions and Conclusions

We have integrated a MC-based L-type current model with a four-state stochastic RyR model into a single CRU framework for human ventricular myocytes [4]. This model maintains deterministic whole-cell output at high channel counts while effectively capturing stochastic microdomain subcellular release behavior at lower channel densities. Notably, the transition from random to deterministic-like behavior occurs sharply when the number of L-type calcium channels (LCCs) in the unit surpasses the threshold of 100 LCCs.

This has significant implications for our understanding of subcellular calcium dynamics, as it clarifies the importance of effective diffusion coupling between microdomains. The coupling between the LCC and RyR microdomains is influenced by several factors, including the characteristic diffusion length, the distance between microdomains, and the amount of buffering present. Higher diffusion rates, closer microdomain proximity, and minimal buffering pathways lead to greater effective coupling. Since a single microdomain contains only a few LCCs, effective diffusion coupling that can synchronize fewer than

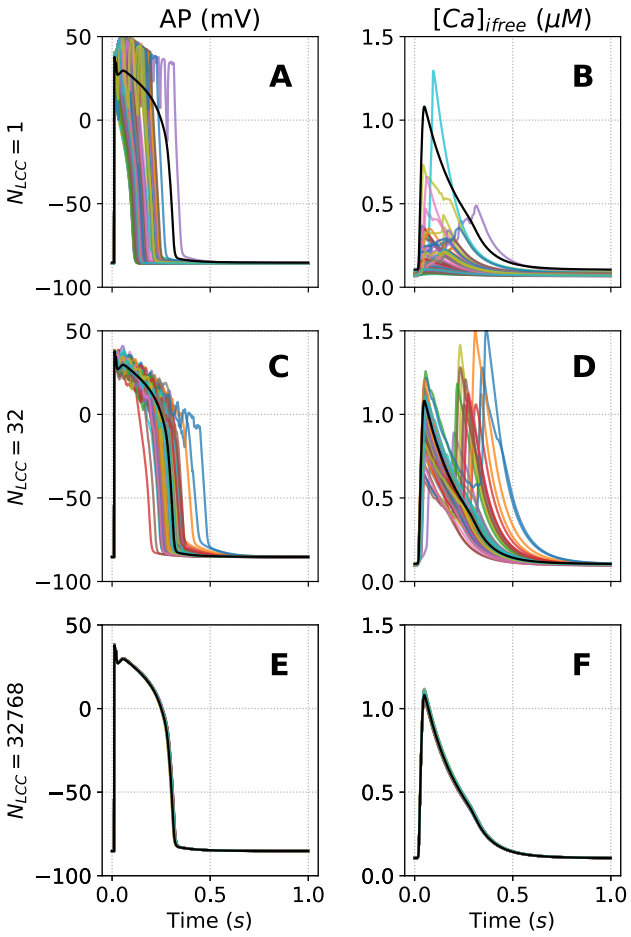


Figure 3. Output curves of the AP and $[Ca]_{i_{free}}$. The results present the outputs of the STP model varying the N_{LCC} as $N_{LCC} = \{1, 32, 32768\}$ and using $N_{RyR} = 5N_{LCC}$. For each N_{LCC} and, consequently, N_{RyR}, there are presented $n = 100$ samples. In contrast, there are presented also the output values for the deterministic model (black line).

50 microdomains results in a high degree of subcellular variance. In this case, whole-cell behavior reflects the average of thousands of independent and highly stochastic units. Conversely, if diffusion coupling can synchronize around 100 microdomains, we begin to see distinct domains of deterministic subcellular behavior. Thus, whole-cell calcium cycling can be understood in terms of the coupling of deterministic systems, both in relation to spark and wave activity.

This opens an important avenue for future research. Specifically, the levels of coupling among microdomains within a heterogeneous spatial grid become very important, since the average cell outcome can depend crucially on them. The simulation of spark-induced spark activation and wave propagation in heterogeneous grids should be the next step in our analysis. The key insight from this contribution is that if deterministic behavior emerges spontaneously at around 100 channels, then whether the observed level of coupling crosses this threshold is key to understanding the framework of subcellular calcium cycling and how to transition to proper whole-cell behavior.

Acknowledgments

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