The Modeling of Calcium Dynamics within the Dyadic Space using Random Walks

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Introduction

Heart disease is the world's number one cause of death. In order to understand more about the underlying mechanisms that drive ventricular fibrillation, a cardiac arrhythmia, we study calcium (Ca^{2+}) dynamics, which act as the messengers in muscle contraction. Ca^{2+} sparks play an important role in regulating the contraction and relaxation of cardiac myocytes. Ca^{2+} sparks occur due to a positive feedback process known as Ca^{2+}-induced-Ca^{2+}-release. The ryanodine receptor (RyR) is a Ca^{2+} sensitive channel and form a cluster within the dyadic space between the cell membrane and the sarcoplasmic reticulum (SR) membrane.

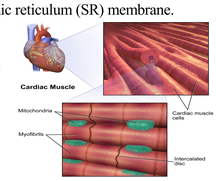

Figure 1: The heart, tissue and myocytes (source: Wikipedia)

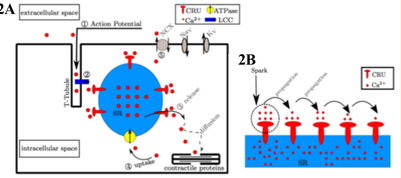

Figure 2: Schematic Diagrams- Ca^{2+} Cycling. A: Overview of Excitation-Contraction Coupling (ECC) Process. Action potential depolarizes cell membrane causing a release of Ca^{2+} through the L-type Ca^{2+} channels, which then induce Ca^{2+} release from the SR. The released Ca^{2+} ions diffuse and bind to contractile proteins causing contraction. Internal Ca^{2+} store is replenished by SERCA pumps, where Ca^{2+} ions are uptaken back into SR. **B**: During release, Ca^{2+} is very localized- forming Ca^{2+} sparks. These sparks can potentially propagate to its neighboring CRUs to form Ca^{2+} waves, which are the precursors of cardiac arrhythmias. In this study, we model Ca^{2+} sparks and waves to understand the detailed mechanisms.

Motivation

Ca^{2+} diffusion is often modeled by using a partial differential equation (PDE). However, since the dyadic space is extremely small and cytosolic Ca^{2+} concentration is relatively low during diastole, the continuum assumption may not provide a sufficiently accurate basis for describing Ca^{2+} leak and sparks as PDEs require larger samples of similar entities to generate approximations.

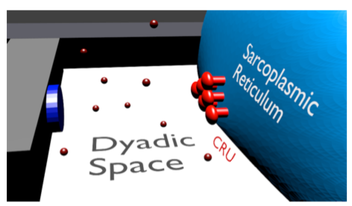

Figure 3: Dyadic Space–the crevice between the T-Tubule (in gray), cluster of RyRs (**red**), L-type Ca^{2+} channel (**dark blue**), and the SR (**light blue**). The volume of this space is around *0.0126 μm³* at times containing as little as 1~2 ions.

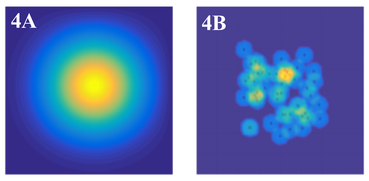

Figure 4: Comparison of PDE & Random Walks. A: Simulation of diffusion by solving the Diffusion Equation using the finite difference method. where the center (yellow) represents the highest concentration of ions. **B:** Simulation of diffusion using random walks for 50 ions, shown as the dark dots. This simulation, with a much lower number of ions, is not similar to the PDE approximation and therefore show that we can obtain very different and interesting behaviors.

Methods: Random Walks

In this study, we used random walks, which are the fundamental processes that underlie diffusion, to model Ca^{2+} dynamics within the dyadic space.

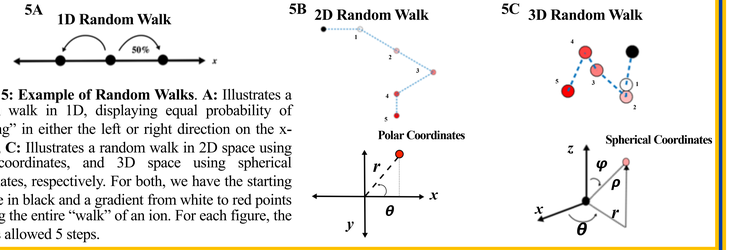

Figure 5: Example of Random Walks. **A:** Illustrates a random walk in 1D, displaying equal probability of "walking" in either the left or right direction on the x-axis. **B, C:** Illustrates a random walk in 2D space using polar coordinates, and 3D space using spherical coordinates, respectively. For both, we have the starting point be in black and a gradient from white to red points showing the entire "walk" of an ion. For each figure, the ion was allowed 5 steps.

Methods: Modeling of RyR & Dyadic Space

A single RyR is modeled with the following properties: a circle of radius of 15 *nm* with three states: ***Open***, ***Closed***, and ***Inactive***. The opening rate of the single RyR obeys the function $k[Ca^{2+}]^2$ for some constant k without its actual embedding in our model. Afterwards, by using that single RyR model, we modeled a single Ca^{2+} release unit (CRU, RyR cluster) and then, multiple CRUs.

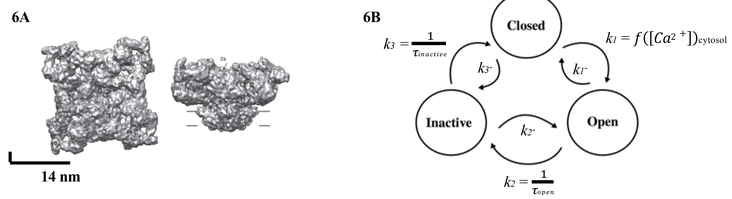

Figure 6: Single RyR Properties. A: Geometry of a single RyR obtained via electron microscopy from [2]. About 28 nm in length. We model our single RyRs as circles of 15 nm in radius for simplicity, which includes the size of the RyR and the distance around it of which Ca^{2+} ions would influence its change in state—what we call the radius of influence. **B:** 3-state stochastic RyR model. k_1 represents the rate from close to open. It depends on the Ca^{2+} concentration in the cytosol, and has a very small baseline firing probability. k_2 represents the rate from open to inactive where an open RyR will stay open for around τ_{open} (mean open time). During this time, Ca^{2+} will be released from the SR into the cytosol. k_3 represents the state change from inactive to closed. This inactive period can be considered a refractory period in which the previously open RyR must recover in order to be *induced* and release more calcium and is dependent on $\tau_{inactive}$. For our current model, k_{1^-}, k_{2^-}, & k_{3^-} are extremely small and considered 0 for our simulations.

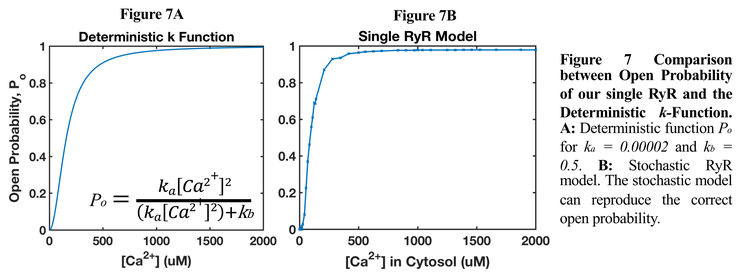

Figure 7 Comparison between Open Probability of our single RyR and the Deterministic *k*-Function. A: Deterministic function P_o for $k_a = 0.00002$ and $k_b = 0.5$. **B:** Stochastic RyR model. The stochastic model can reproduce the correct open probability.

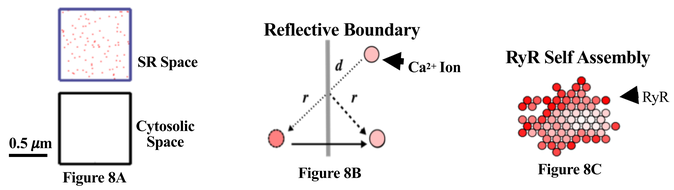

Figure 8: Different aspects of our simulation and model. A: Bi-domain set up for our simulation. **Red** dots represent Ca^{2+} ions in the SR, while **blue** ions represent ions in the cytosolic space. We focus more on the 2D aspect, as the *z*-axis is extremely small for our area of focus—the dyadic space—which is *1 μm x 1 μm x 12 nm*. This is due to the length of our random walk for each time step, based on the diffusion coefficient for a free Ca^{2+} ion in cytosolic space, $D = 223\ \mu m^2/s$, which leads to one ion moving across the z-axis multiple times in one time step. Also, we implemented an uptake mechanism where ions in the cytosol can be uptaken back into the SR at a rate of 0.3 μM/ms. In order to keep our ions within our domain, we implemented reflective boundaries as seen in **B** where a single calcium ion is represented by the pink circle, and the rose colored circle represents an ion that has gone out of the set boundaries by a distance of *r*. (One 'walk' in this case is a distance of $d + r$.) As mentioned before, a cluster of 10-100 RyRs form one CRU. Therefore, to initialize our CRUs, we used a self-assembly algorithm from [4] to place each RyR into hexagonal packaging and rearranges them into different and more realistic CRU structures. An example is seen in **C** where this is one of the many 70 RyR assemblies. The order of RyR is seen by the gradient from white to **red**, starting with the white circle as the starting point.

Results: Calcium Sparks and Non-Spark Leak

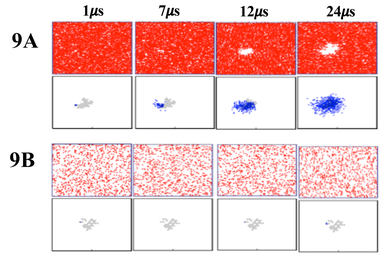

Figure 9: Ca^{2+} sparks and non-spark leaks in our simulation. A: Represents a Ca^{2+} spark, where the initially opened RyR successfully activates its neighboring RyRs, which occurs at a higher concentration of SR Ca^{2+} ions. **B:** Represents a non-spark leak, where the initially opened RyR does not activate its neighboring RyRs, which occurs at lower concentrations of SR Ca^{2+} ions.

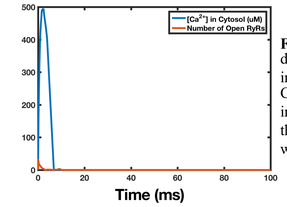

Figure 10: Cytosolic Ca^{2+} concentration during a Ca^{2+} spark event, which is shown in the blue line on the left. The peak of this Ca^{2+} spark is about 500 μM. Also, shown in red is the number of open RyRs during this spark event. A spark only happens when **5 or more** RyRs are open.

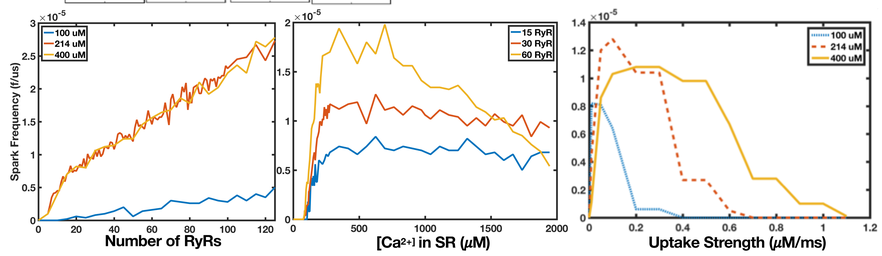

Figure 11: Spark Frequency over time with changes to the following parameters: SR Ca2+ load, the number of RyRs, and uptake strength. Further, we tested how those parameters could have been affected by another parameter of our model, explaining the three lines in each graph. **A:** shows how the increasing number of RyRs increase the frequency of Ca^{2+} sparks. We find that even increasing the number of $[Ca^{2+}]$ in the SR has a limit to increasing the the amount of sparks obtained. However, decreasing it can lead to significant decreases in spark activity. **B:** shows how the increase in SR $[Ca^{2+}]$ increases spark activity. However, after a certain point, it stabilizes. We find that by increasing the number of RyRs, it could actually hinder spark activity. **C:** shows how the increase in uptake strength will decrease spark activity. By increasing the SR $[Ca^{2+}]$, we obtain different peaks of spark activity.

Results: Calcium Waves

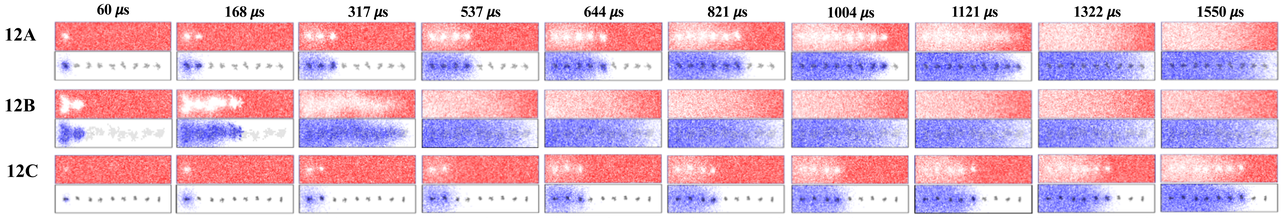

Figure 12: Simulation of Calcium Waves. Panel A: Ca^{2+} wave propagation. There are 9 clusters of 30 RyRs *uniformly spaced* from each other at 0.5 μm. By changing different parameters in our model, we are able to see differences in the wave propagation speed. Speed is calculated by calculating the distance between the first CRU and last CRU, divided by the time it took until the last CRU was activated. **Panel B** depict a *fast* wave compared to the regular paced one, while **Panel C** depicts a *slow* wave in comparison. These were taken at the same simulation time in order to accurately show the reader the difference. By the time the *fast* wave is finished, we are only able to obtain two fully opened RyR clusters. The *slow* wave ultimately finishes after 2500 μs in simulation time. These were recorded from changing the sensitivity of the RyRs, which are explained in the figures below.

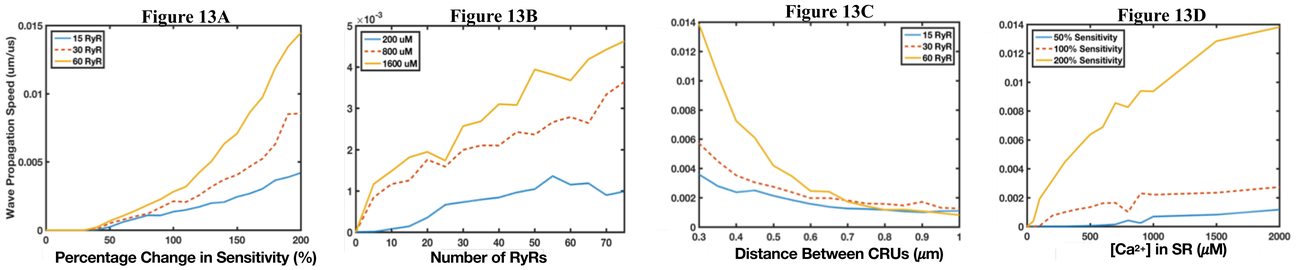

Figure 13 How Sensitivity of RyR (A), Number of RyRs per Cluster (B), Distance between CRUs (C), and $[Ca^{2+}]$ in SR (D) influence wave propagation speed. We changed the sensitivity of the RyRs—meaning that we changed the radius of which the RyRs are influenced by the surrounding Ca^{2+} ions, the number of RyRs per cluster, the distance between individual clusters, as well as SR Ca^{2+} load. Again, there will be three lines representing additional parameter changes to each plot. **A:** shows that wave propagation speed increases exponentially as we increase the sensitivity. By increasing the number of RyRs, we also notice higher wave propagation speed. **B:** shows that as we increase the number of RyRs per cluster, the wave propagation speed also increases. With increasing SR Ca2+ load, we find that the wave propagation speed will also increase. By changing the distance between RyRs, we find that as the distance increases, wave propagation speed exponentially decreases as seen in **C.** Interestingly, we find that increasing the number of RyRs may help increase wave speed for smaller distances between CRUs, however may affect it negatively for larger distances. **D:** As we increase the Ca^{2+} concentration in the SR, wave propagation speed also increases. By increasing sensitivity of individual RyRs, we also find a significant increase in wave speed.

Summary

In this study, we modeled Ca^{2+} cycling using random walks. The model of the single CRU could reproduce the transition from non-spark leak to Ca^{2+} sparks as SR Ca^{2+} load becomes high. We also observed Ca^{2+} spark wave propagations when RyR is sensitized / the spacing between Ca^{2+} release units is small / SR Ca^{2+} load is high. These results are consistent with experimental observations. Our study provides a more detailed mathematical description of intracellular Ca^{2+} cycling in order to improve our understanding of the dynamics involving Ca^{2+} release, Ca^{2+} waves, delayed after depolarizations, and triggered activities.

Future Directions

Although our model could reproduce many key phenomena, the model needs to be more realistic. Currently, we are planning to

- Incorporate Ca^{2+} buffers
- Include luminal Ca^{2+} regulation
- Simulate slower Ca^{2+} waves
- Simulate with more detailed geometries of RyR structures and the dyadic space

References

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