Computational Modelling of Local Calcium Handling in Cardiac Myocytes

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Abstract:

The goal of this project was to investigate local calcium dynamics during a single spark in a murine cardiomyocyte by using a general reaction-diffusion model. SubCell, a python based software developed by Dr. Johan Hake, was used to declare and solve the computational model on a previously reconstructed geometry. The geometry consisted of a Cytosolic domain, which was discretised using tetrahedrons and 12 interconnected sarcoplasmic reticulum (SR) compartments. Fluxes were declared on boundaries that connect the different domains together. The type of equations used in the model was not novel, but the level of subcellular details in the geometry was to our knowledge un-presidential. Nimrod, a parametric modelling system developed by the Message Lab at Monash University, was used to fit parameters in the model to numerous experimental data. The fitted model was run and properties related to release and refill of calcium from the sarcoplasmic reticulum (SR) were observed. The release, which was fitted to experimental data to last10 ms, was followed by a period of restoration of the calcium level in SR. The level was restored using two pathways: 1) passive diffusion from the rest of SR, and 2) active transport from Cytosol across the membrane through the sarco/endoplasmic reticulum calcium-ATPase (SERCA) pump. The refill period was fitted to last several hundred milliseconds. It was observed that the level of free calcium in SR continued to decrease up to 30 ms after the release gate was closed. This unexpected behaviour can be explained by a calcium buffer in SR, Calsequestrin, which continued to bind calcium after the release had terminated. It was also observed that the presence of TTflux increased the release rate during a calcium release.

Introduction:

Intracellular calcium movements dictate many vital functions in the human body. In this study, we focused on calcium's importance in triggering contraction in a cardiomyocyte,

the heart muscle cell. A heart beat originates in the Sino atrial node, the impulse generator of the heart, as an electric depolarization, an action potential (AP). The AP traverses the heart and excites myocytes along its way. Specialized structures called T-tubules, the tube like invaginations of the cell membrane, bring the AP into the cell. As a result of the depolarization, the Long Lasting (L-type) Calcium channels (LCCs) opens and calcium flows into the cell. The calcium enters a micro domain, the dyadic cleft, a region between a T-tubule and a junctional sarcoplasmic reticulum (jSR), and induces more calcium being released from SR into cytosol (the cytoplasmic matrix), causing a single calcium release to occur. Such release is called a spark. This entire chain of events is given a specific term-calcium induced calcium release. The calcium in cytosol then binds to Troponin C, a small protein residing in a complex of other proteins on the myofilaments, and triggers force generation. This is how the heart "beats". Calcium serves as an activator for this chain of events; a stronger calcium concentration gives rise to a stronger contraction. After the heart cell contracts, calcium is brought back into SR through the SERCA pump. Within SR, calcium binds to a buffer called Calsequestrin, which facilitates larger calcium content inside SR, and hence a larger reservoir of calcium is released. This entire process is known as the excitation contraction relaxation coupling, or ECR coupling [11].

Evidently, one small step that goes wrong in the process can lead to catastrophic consequences. It is currently known that a few severe heart diseases are linked to this process, such as arrhythmogenesis (heart failure) and catecholaminergic polymorphic ventricular tachycardia (CPVT), but little is known about their mechanisms and their spatiotemporal dynamics [10]. By understanding the local calcium signalling during ECR coupling in healthy and diseased cardiomyocytes, we are better equipped to address treatments for these diseases. The small spatial and temporal scales involved in these processes limit the ability to use experiments to understand them. Computational models have therefore emerged as an

alternative and complement to experiments to better understand details in the dynamics of the ECR coupling. Using pure mathematical analysis, the structural-based diffusion and reactions within a cardiac cell can be turned into a computational model of calcium movements within the myocyte. The model can be described in a python script, which serves as the input to a complicated numerical solver that solves the model.

The computational mesh used in this study has been generated using newly developed software tools. Subcellular features have been segmented from electron microscopy data. The quality of the resulting surface mesh was then improved, so it becomes suitable for finite element simulations. Particular boundaries were annotated; SR, T-Tubules, and mitochondria, so we can declare mathematical fluxes on different boundaries, each corresponding to a physiological equivalent. The mathematical model is declared and solved using SubCell, a python based software developed by Dr. Johan Hake. SubCell uses a finite element solver provided by FEniCS to solve the spatially varying partial differential equations, which a user declares on the geometry.

The first task of this project is to create a mathematical model that agrees with the past experimental data both qualitatively and quantitatively. Then we can use the model to investigate the following topics:

1) The pump that brings calcium back to SR, the SERCA pump, is believed to be situated only in the network SR. The effect of different locality and the distribution of the SERCA pump will be investigated in this paper.

2) The calcium diffusion within the network SR is slow. Whether this is dependent on pure geometrical features such as SR being thin, or it is dependent on an actual slower diffusion within the SR compared to bulk cytoplasm will be investigated.

3) Calcium extraction out of the cell is governed by proteins that are situated on the sarcolemma. The size of this calcium extraction is dependent on the local calcium concentration sensed by sarcolemmal proteins. The actual size of the local calcium concentration during release at the nearby sarcolemmal proteins will also be investigated.

Methods:

I. Model Making

The structures of the model were determined by the geometry segmented from the electron microscopy data of a mouse, originally acquired by Prof. Masahiko Hoshijima [11]. The data set is freely available from the web at http://ccdb.ucsd.edu project 3603. The mesh was loaded into Blender, and GAMer was used within Blender to improve the quality of the surface mesh and to annotate the mesh. The volumes of each SR compartment, and the area between them were determined using built in Blender tools. These data were then used to declare diffusion between the SR compartments in a Python script, in which SubCell used to solve diffusion equations. Data for ion types, buffers and their concentrations within each compartment, flux between the compartments, and other relevant constants were gathered from experiments done in Bondarenko et al in 2004 [9], Hayashi et al in 2009 [11], Bers et al in 2001 [12], and Bossen et al in 1981 [6]. The data were then used to further constrain the reaction diffusion model we declared using the Python script. To be able to use the model to predict local calcium concentration changes due to changes in SERCA level, the rest of the model had to be fitted with parameter sweeping using Nimrod.

II. Fitting the model

In the first experiment, the calcium release unit at the junctional SR was blocked and the local calcium concentration was set up to be 780 uM to mimic the calcium concentration in

SR after a spark is terminated. The passive refill rate of calcium in SR was then fitted to the experiments done by Zima et al in 2008 [1]. They found that the refill rate had a time constant of τ= 220 ms. We used this knowledge to fit the distance between SR Bulk and SR compartments in our model and the value of the diffusion constant of calcium within SR. The latter was described as the diffusion constant within cytosol scaled by a certain factor. The scaling factor was swept from 0.11 to 0.55 with the steps of 0.01, while the distance between the release unit on the SR and the SR bulk was swept from 100 to 2000 nm, with the steps of 100 nm. The simulation time was set to 85 ms.

In the second experiment, the calcium release unit at the junctional SR was unblocked and the calcium concentration in all of SR was set to be 1300 uM, which corresponds to the calcium concentration in SR before release. The experiment started with the release channel open and lasted until the calcium concentration in jSR was down to 60% of the initial concentration. The latter corresponds to data from Zima et al 2008 [1]. The release duration was fitted to data reported by Cheng et al in 2008; a little more than 10 ms [2]. The parameter which determined the length and size of the release flux is a scale parameter with nm as its unit. This parameter was determined in the experiment; the parametric sweep on Nimrod went from 4500 nm to 7500 nm with step size of 50 nm. The simulation time in this experiment was set to be 20 ms. The peak calcium current during the release was also checked to correspond to data obtained by Kettlun et al in 2003, which stated that the release flux through one open Ryanodine receptor was around 0.5 pA [8]. After the release current parameters were fitted, the cytosolic buffer Troponin C and T-tubule fluxes (NCX, IpCa, ICab) were added according to equations given by Bondarenko et al in 2004 [9].

In the third experiment, both release and refill of calcium was included in the model. This experiment was run to check if the refill after release would still match the theoretical value posted before, but using a more realistic SR calcium depletion level. The simulation

time was set to be 500 ms and the junctional release unit was set to close when the mean calcium concentration within SR went below 0.6 times its initial concentration. Another experiment was also run to check the differences between simulations with t-tubule fluxes and simulations without t-tubule fluxes.

The last experiment was run to determine the value of SERCAscale- the parameter used to scale the SERCA flux to match the experiment done by Zima et al 2008. While everything else was the same, SERCA pump would help pump calcium back into SR and shorten the refilling time. The time constant used to fit this part of the experiment was 160 ms.

Results:

The basic structure of the model was determined using Blender and GAMer, see Figure 1. The SR network was broken down into different regions; see Figure 2 for a schematic sketch of the connectivity. Since the calcium movements in the model was consistently too fast compared to experimental findings, three more intermediate compartments were added between jSR release and SR Bulk to slow down the refill and release

In the first refilling experiment with simulation time set to 85 ms, even though there were a few "good" hits by calculating the least square differences compared to the theoretical values, the experimental data didn't seem to follow the same trend (Figure 3). In order to fix this problem, the simulation was repeated with simulation time 500 ms, and the result was produced in Figure 4. The distance between the SR compartments and the Bulk SR was determined to be 1600 nm, and the ratio of the global Calcium flux to the local Calcium flux was determined to be 0.33. This combination yielded one of the best least-square values and was used in the rest of the experiments. As the experimental refilling line indicates in figure

4, the rise of the calcium concentration seemed a little slower compared to that from the theoretical refilling described by equation 1. This was because the outer compartments needed to be filled up before the junctional SR was filled up. By running the experiments from the release and allow the compartments to be filled in a more natural state, the difference between theoretical and experimental refilling rate was further reduced. The ratio between the diffusion constant in cytosol and SR, agreed with experimental data from Picht et al 2009.

The scale parameter that determined the size of the calcium release flux was determined to be 7100 nm after the first release experiment was run. Even though the gate did close when the calcium concentration in junctional SR depleted to 60%, the depletion time took slightly longer than expected (14 ms). At the same time, the average concentration of calcium within the SR network still decreased for roughly 20 more milliseconds until the calcium concentration hit its minimum. This phenomenon, that even though the release unit closed (no more calcium was leaving SR) and more calcium was being pumped into SR, the average calcium concentration in the SR was still dropped, can be explained by the presence of Calsequestrin in the SR network. Calsequestrin binds to the free flowing calcium in the SR and thereby decreasing the concentration, so SERCA pump can transport calcium against its gradient back into SR from cytosol more easily. However, this contradicted the original hypothesis that calcium concentration within SR reaches its minimum when the release gate in junctional SR closes, so the scale parameter determining the size of the release current had to be refit. In the refitting experiment, the scale parameter of calcium release was determined to be 6500 nm. The release gate at the junctional SR closed at between 10 ms while the calcium concentration within SR network reached the minimum at around 31 ms.

It was observed that after t-tubule fluxes were added to the model while everything else was the same, the release lasted slightly shorter and the average calcium concentration

within SR reached its minimum slightly faster. This made sense because t-tubule flux allowed the calcium concentration in the dyad to be lower, hence creating a larger calcium gradient for a larger calcium release flux. In addition, the minimum average calcium concentration within SR was also not as low compared to the simulation without t tubule fluxes (figure 5). Due to the drastic differences shown in figure 5, the scale parameter of calcium release at the junctional SR was set to be 6700 nm, which gave a nice fit shown in figure 6.The lowest concentration ended up at 781.54 uM at around 30 ms and the release channel closed at roughly 10 ms after the release started. At the same time, since there was no information about the distance between SR and the bulk of cytosol, few runs were executed to check its influence on the average calcium concentration in cytosol. The result (shown in figure 7) showed that the distance did not make a major impact in the calcium concentration in cytosol, thus the distance was arbitrarily picked to be 1000 nm.

The result obtained from the SERCA runs was unable to match the theoretical value at the time when this paper is due (results shown in figure 8). More simulations and further experiments will be executed to investigate the errors.

Error Analysis on the model and Future work:

The first implementation of the release stopper reported the close time at the time step after the SR calcium threshold was passed. This turned out to be inaccurate as t he concentration decreased too far between the time steps. The release stopper has to be reimplanted in order to increase the accuracy and produce good results. At the same time, SERCA pump was unable to stop pumping calcium back into SR after the compartment was saturated. This was probably the main reason that our model was unable to produce results that agree with the experimental value obtained in the past. The two problems have been fixed and the model will be refitted to the experimental values. Some of the distance

parameters were taken out and the flux from cytosol, t-tubules, and SERCA will be scaled to fit the new model. The effects of the SERCA location are currently being investigated.

Appendix:

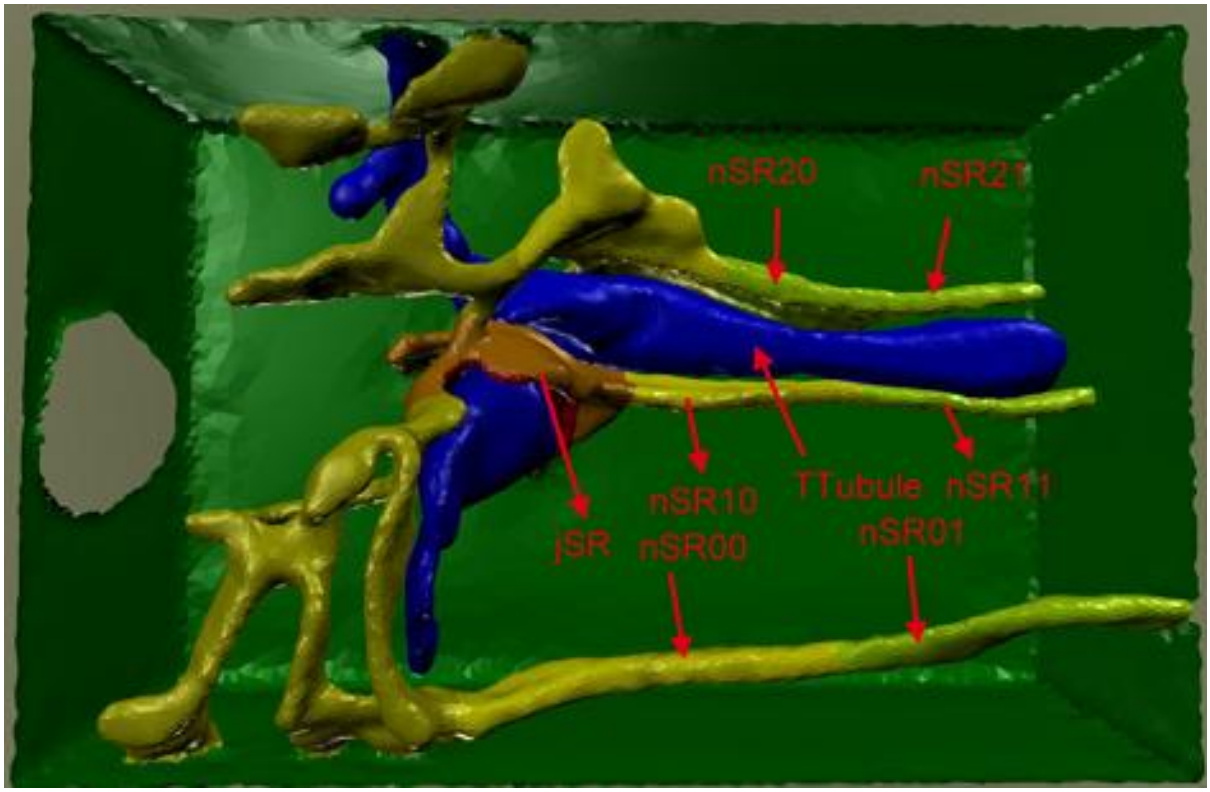

Figure 1. Calcium ions in the nSR compartments would flow into jSR and out to cytosol.

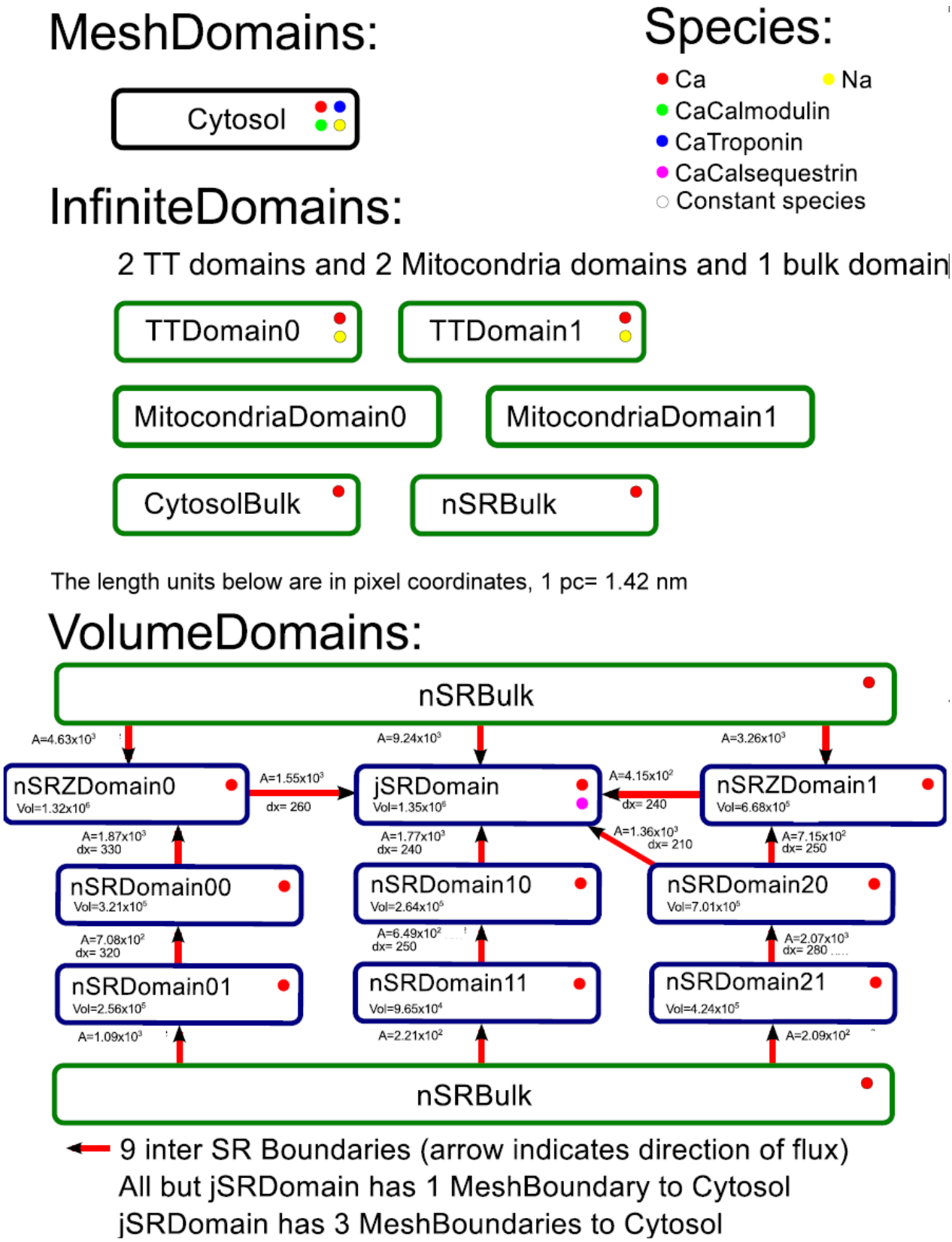

Figure 2. How the compartments are connected is demonstrated by the graph above. Three "inter" compartments were also added between jSR and cytosol for the model to fit the experimental value better.

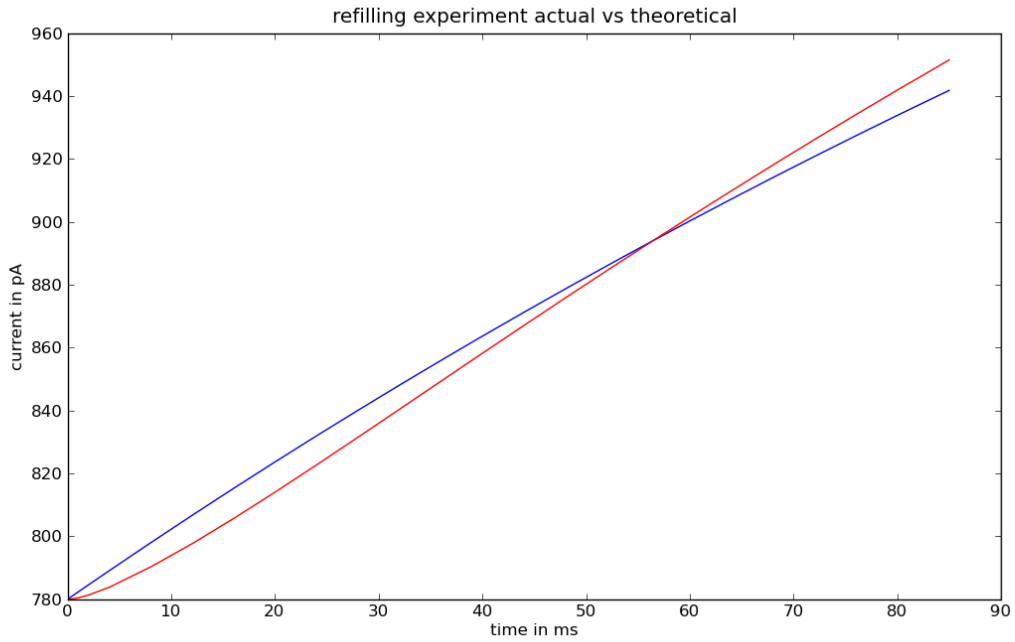

Figure 3. In the first refilling experiment, the run that yielded the least squared differences didn't seem to fit the theoretical (red) trend. The same experiment was set up again with simulation time = 500 ms.

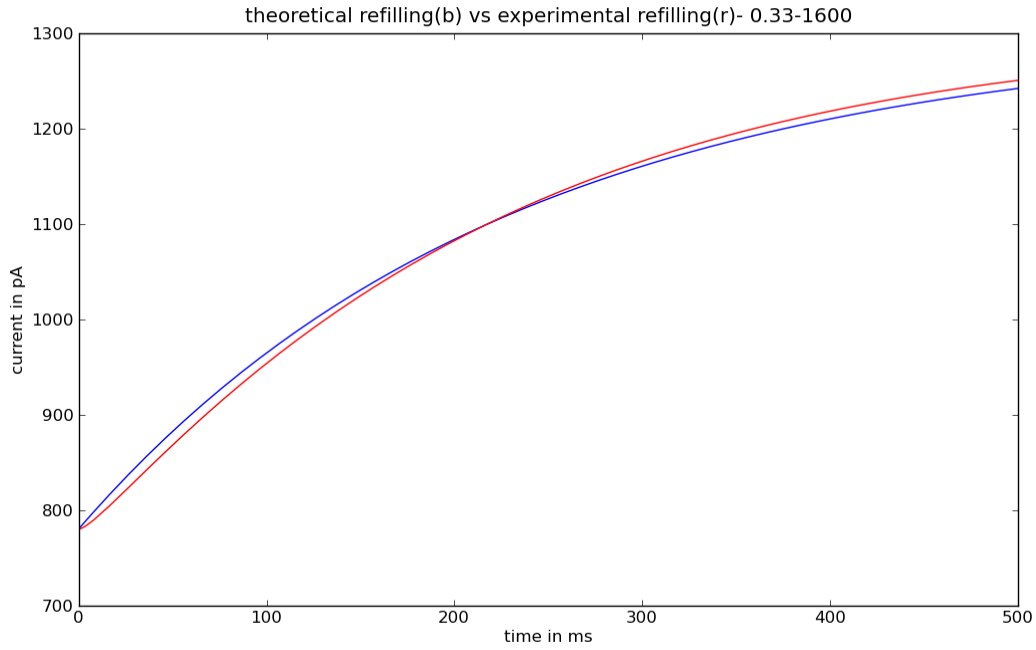

Figure 4. The second refilling experiment was allowed to run for 500 ms for the SR to fill up. The distance between the release unit on the SR and the bulk of SR was determined to be 1600 nm, whereas the ratio of the global Calcium flux to the local Calcium flux was determined to be 0.33

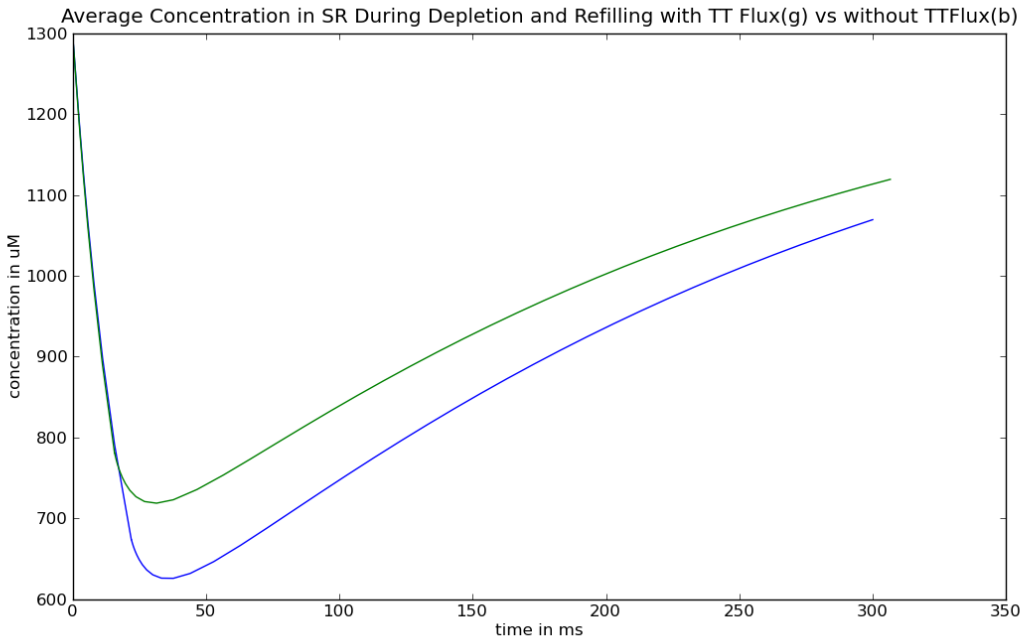

Figure 5. The minimum average calcium concentration within SR with the addition of t tubule fluxes. It also caused the release to terminate earlier and the average calcium concentration to reach its minimum faster.

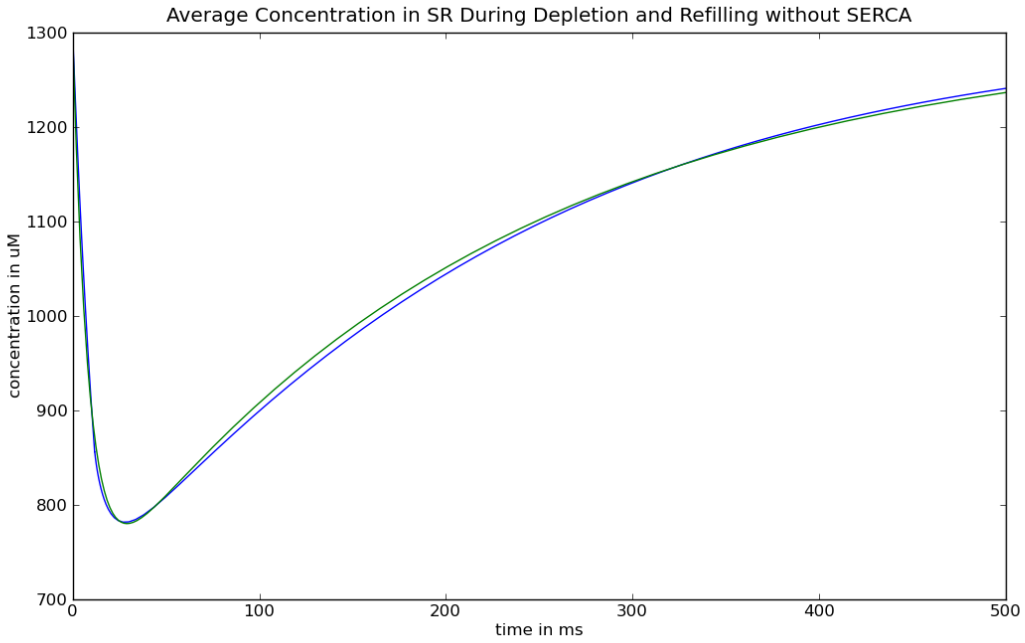

Figure 6. By setting the distance of calcium release at the junctional SR to be 6500 nm, the experimental value matched the theoretical value given in Zima et al 2008 almost perfectly.

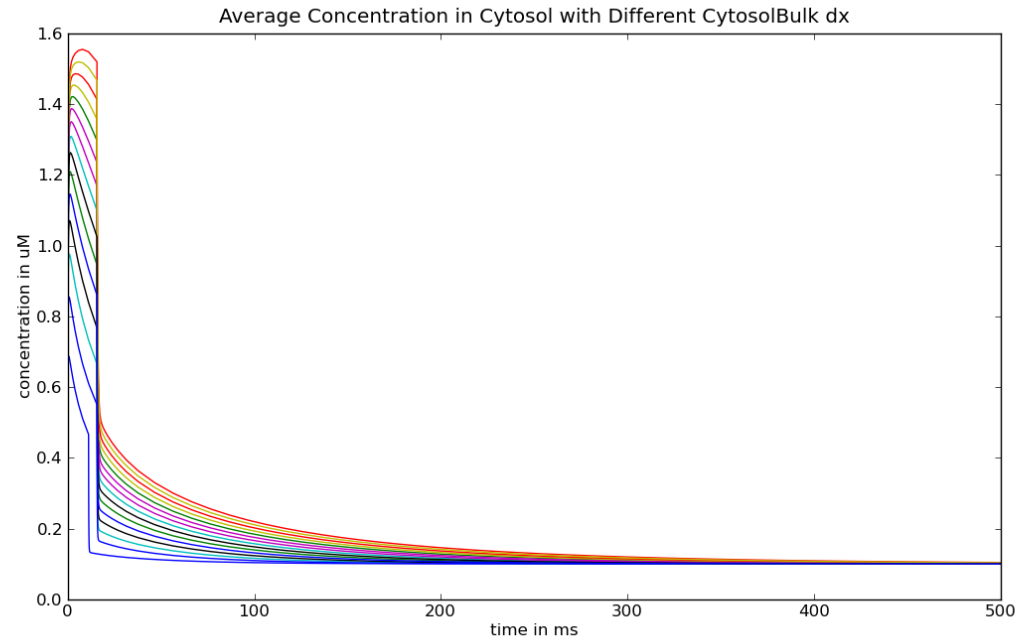

Figure 7. The average concentration in cytosol was plotted with different distances between the bulk of cytosol and SR network. It turned out that the distance didn't really affect the average concentration in cytosol significantly.

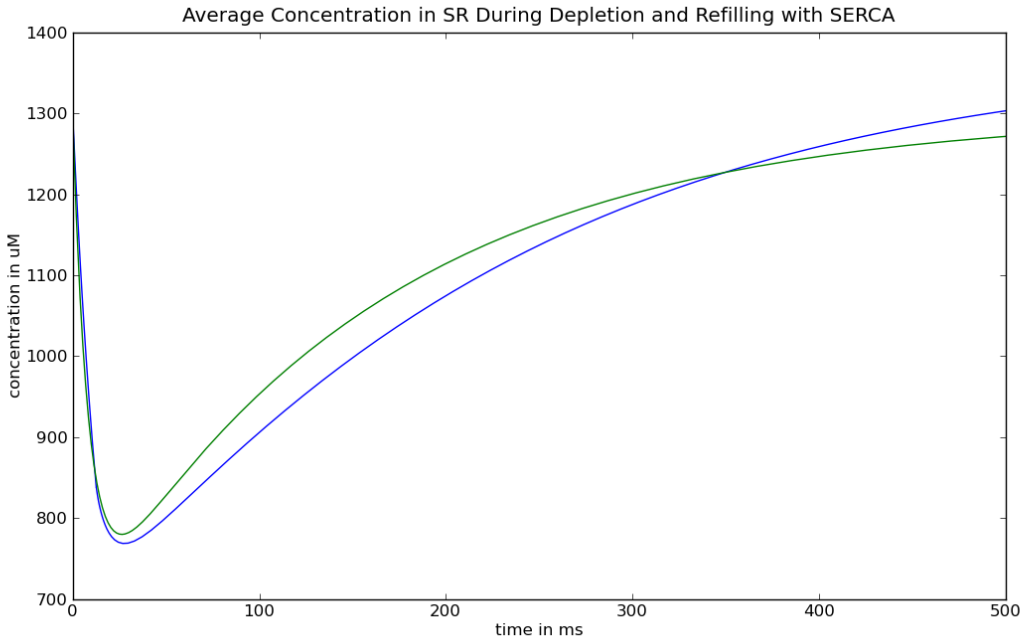

Figure 8. After the SERCA was added, the model was unable to fit its result to the theoretical value. It will be reconstructed and refitted. See future work.

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