

# **Multi-Scale Modeling in Rodent Ventricular Myocytes: Contributions of structural and functional heterogeneities to excitation-contraction coupling**

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## Introduction

There is a growing body of experimental evidence suggesting that the  $\text{Ca}^{2+}$  signaling in ventricular myocytes is characterized by high gradient near the cell membrane and a more uniform  $\text{Ca}^{2+}$  distribution in the cell interior [1]-[7]. An important reason for this phenomenon might be that in these cells the t-tubular system forms a network of extracellular space, extending deep into the cell interior. This allows the electrical signal, that propagates rapidly along the cell membrane, to reach the vicinity of the sarcoplasmic reticulum (SR) where intracellular  $\text{Ca}^{2+}$  required for myofilament activation is stored [1], [8]-[11]. Early studies of cardiac muscle showed that the t-tubules are found at intervals of  $\sim 2 \mu\text{m}$  along the longitudinal cell axis in close proximity to the Z-disks of the sarcomeres [12]. Subsequent studies have demonstrated that the t-tubular system has also longitudinal extensions [9]-[11], [13].

The SR is an entirely intracellular, membrane-bounded compartment that abuts but is not continuous with the sarcolemma. The junctions where SR approaches the sarcolemma contain specialized proteins [1], [4], [14]. The sarcolemmal L-type  $\text{Ca}^{2+}$  channels (LCC) are located primarily at the SR junctions where the  $\text{Ca}^{2+}$  release channels in the SR, the ryanodine receptors (RyRs), exist [2], [3], [11], [15]-[17]. The RyRs are arranged in organized arrays of hundreds of receptors up to 200 nm in diameter.

The concept that the LCCs and RyRs form a local functional unit (release unit, RU) is supported by the observations of  $\text{Ca}^{2+}$  sparks.  $\text{Ca}^{2+}$  sparks reflect the nearly synchronous activation of a cluster of about 6-20 RyRs at a single junction.  $\text{Ca}^{2+}$  sparks are the fundamental units of the SR  $\text{Ca}^{2+}$  release both at rest and during cell excitation [1], [3]-[7]. Thus, the microanatomy of t-tubules and SR permits spatially homogeneous

and synchronized SR  $\text{Ca}^{2+}$  release throughout the cell. During physiologically normal excitation-contraction coupling (EC-coupling) a several thousand  $\text{Ca}^{2+}$  sparks in each cell are synchronized in time by the action potential to achieve a spatially homogeneous  $\text{Ca}^{2+}$  transient [1], [4]-[6], [8], [14]. It has also been observed that the spatially uniform  $\text{Ca}^{2+}$  transient might be achieved if the SR  $\text{Ca}^{2+}$  release and uptake are abolished [8]. However the mechanisms underlying cell activation synchrony and  $\text{Ca}^{2+}$  homogeneous distribution still remains unclear.

Recent immunohistochemical studies but one [17] have demonstrated also that marked variations in the distribution of  $\text{Ca}^{2+}$ -handling proteins (L-type  $\text{Ca}^{2+}$  channel,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, sarcolemmal  $\text{Ca}^{2+}$  ATPase) along the cell membrane probably exist [10], [11], [15], [16]. The analysis suggests that most of the L-type  $\text{Ca}^{2+}$  channels are concentrated in the t-tubules (from 3 to 9 times more in the t-tubule membrane than on the surface sarcolemma) and that the concentration of LCC along the t-tubule increases toward the center of the cell [10], [16].

Studies on the distribution of the main  $\text{Ca}^{2+}$  efflux pathway, the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX), are more controversial. All studies but one [18] have reported NCX to localize both to the surface and t-tubule membrane, and most studies suggest that the NCX is 1.7 to 3.5 times more concentrated in the t-tubule membrane, [15], [18]-[20]. However, Kieval *et al.* data [21] indicate the NCX is more evenly distributed. The distribution of the sarcolemmal  $\text{Ca}^{2+}$  ATPase is also unclear [10]. Only one study reports that in hamster and canine ventricular cells this  $\text{Ca}^{2+}$  efflux pathway is located predominantly in the surface membrane [22]. In summary, the observed differences in the spatial distribution and molecular architecture of  $\text{Ca}^{2+}$  microdomains suggest that

significant differences in the EC-coupling between the cell surface and cell interior may exist. However how the localization of  $\text{Ca}^{2+}$ -handling proteins along the sarcolemma regulates the intracellular  $\text{Ca}^{2+}$  signaling still remains uncertain.

Taken together above studies demonstrate that remarkable amount of fundamental quantitative data on the ventricular cell structure and function has been accumulated. Recently it has been also emphasized that biophysically realistic computational models, incorporating transverse-axial t-tubular system and considering geometric irregularities and inhomogeneities in the distribution of ion-transporting proteins, are missing and needed [11], [23]. For this reason, our main goal here was to develop a detailed 3-D model at the sub-cellular level that would allow us to examine how the distribution of  $\text{Ca}^{2+}$  fluxes via t-tubule and surface membrane may affect  $\text{Ca}^{2+}$ -entry, diffusion and buffering. Thus, SR  $\text{Ca}^{2+}$  uptake and release was not included here. The current model of the rat ventricular myocyte includes: (1) a simplified 3-D geometry of a single t-tubule and its surrounding half-sarcomeres; (2) the spatially distributed L-type  $\text{Ca}^{2+}$  channel,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, sarcolemmal  $\text{Ca}^{2+}$  pump and background  $\text{Ca}^{2+}$  leak along the sarcolemma; and (3) the stationary and mobile endogenous  $\text{Ca}^{2+}$  buffers (troponin C, ATP, calmodulin) and the exogenous mobile  $\text{Ca}^{2+}$  buffer, Fluo-3.

The results suggest that, in the presence of 100  $\mu\text{M}$  Fluo-3, the model is able to predict a uniform  $\text{Ca}^{2+}$  distribution inside the cell if  $\text{Ca}^{2+}$  microdomains are distributed heterogeneously along the cell membrane. In the absence of  $\text{Ca}^{2+}$  indicator the model predicts non-uniform  $\text{Ca}^{2+}$  distribution in the cytoplasm and high  $\text{Ca}^{2+}$  gradient near the cell edges when the  $\text{Ca}^{2+}$  flux pathways were distributed heterogeneously. We concluded that the distribution of  $\text{Ca}^{2+}$  handling proteins along the cell membrane might be another

important mechanism regulating ventricular EC-coupling. These model predictions are in qualitative agreement with published experimental data in rat ventricular myocytes [8]. Preliminary results of this work have been presented to the Biophysical Society in abstract form [24].

It is important to mention here that this 3-D sub-cellular model of single t-tubule and surrounding structures also yields insights across two other scales of biological organization: a microscopic scale of individual  $\text{Ca}^{2+}$  RU and a whole-cell scale. It allows us not only to extend the analysis further to integrate models of individual  $\text{Ca}^{2+}$  RU, SR  $\text{Ca}^{2+}$  pump or leak but also to examine how experimentally suggested spatial distributions of these  $\text{Ca}^{2+}$  transporters [2], [3], [11], [15]-[17] may affect the behavior of a single RU or the mechanisms underlying a synchronized SR  $\text{Ca}^{2+}$  release. Future modeling efforts will be focused on replacing the idealistic t-tubule and surrounding structures geometries with more realistic or to include several surrounding t-tubules and other sub-cellular organelles that might help to understand better normal and pathophysiological mechanisms.

## **Materials and Methods**

### ***Model cell geometry and spatial protein distribution along the cell membrane***

The model geometry was derived from the published structural data [9], [11], [23]. The cell model contains one repeating unit inside the ventricular myocyte, including single t-tubule and its surrounding half-sarcomeres (Fig. 1A and Table 1). The t-tubule was assumed to be cylinder, with a diameter of 0.25  $\mu\text{m}$  and a depth of 6.87  $\mu\text{m}$ . The surrounding half-sarcomeres were modeled as a cube-shaped box enclosing the t-tubule

with dimensions  $2 \mu\text{m} \times 2 \mu\text{m}$  in the plane of the cell surface and  $7 \mu\text{m}$  in depth. The volume of the model compartment was estimated to be  $\sim 27.68 \mu\text{m}^3$ . The compartment membrane area was  $\sim 9.34 \mu\text{m}^2$  where the percentage of cell membrane within t-tubule was 58% ( $\sim 5.42 \mu\text{m}^2$ ) and within the surface membrane 42% ( $\sim 3.92 \mu\text{m}^2$ ), [9], [11], [23], [25].

**Figure 1A-B and Table 1 - near here**

The accessible volume for  $\text{Ca}^{2+}$  was estimated from reported measurements in adult rat ventricular myocytes [1], [26]. These data, suggest that myofilaments occupy 47-48 % of the cell volume, mitochondria 34-36%, nucleus 0-2%, t-tubule system 0-1.2% and SR lumen 3.5%. The experiments also suggest that about 50% of the myofilament space is accessible for  $\text{Ca}^{2+}$  ions (i.e. contains water) and that mitochondria and nuclei are not rapidly accessible for  $\text{Ca}^{2+}$  [1], [27]. In this study we also assume that the SR lumen is not accessible to  $\text{Ca}^{2+}$  in the presence of ryanodine and thapsigargin. Thus, from the experimental data and above assumptions, the accessible volume for  $\text{Ca}^{2+}$  in adult rat ventricular cells was estimated to be  $\sim 35\text{-}37\%$  of the total cytosolic volume ( $V_{acc}$ )  $\sim 12.9\text{-}13.6 \text{ pL}$ .

The depth of the cleft between the sarcolemmal and SR membranes, where the LCC and RyR localize, has been reported to be 12-20 nm [27] and the sub-sarcolemma space between cellular membrane and myofibrils  $\sim 45 \text{ nm}$  [1]. In this study, the size of both spaces was considered infinitely thin on the scale of the continuum model. This assumption allowed: (1) not to explicitly define a different diffusion coefficients for  $\text{Ca}^{2+}$  and mobile buffers (Fluo-3, ATP, Cal) in the cleft, sub-sarcolemmal and myofibril spaces

as in Michailova *et al.* [27], (2) to simplify the model and to improve the model stability and efficiency.

In agreement with reported experimental data,  $\text{Ca}^{2+}$  transporters were distributed heterogeneously along the model cell surface (Fig. 1B and 2A). The concentration of LCC in the t-tubule membrane was 6 times of that in the surface membrane and increased 1.7-fold along the length of the t-tubule. The concentration of NCX in the t-tubule membrane was three times that in the surface membrane. The sarcolemmal  $\text{Ca}^{2+}$  ATPase was located only in the surface membrane. The background  $\text{Ca}^{2+}$  leak was assumed to be present throughout the whole sarcolemma because no data were available of how this  $\text{Ca}^{2+}$  channel is distributed.

In the axial t-tubule direction, the distribution of LCC current was computed by combining the cluster density and fluorescent intensity plots [16]. The data were then scaled and fitted by a cubic polynomial:

$$f(x) = p_1x^3 + p_2x^2 + p_3x + p_4 \quad (1)$$

where:  $x$  is the distance from the cell surface.

The parameter values of the polynomial ( $p_j$ ,  $j=1-4$ ) are shown in Table 2. This polynomial was further scaled by a single factor  $C$  (see Table 2) such that the total  $\text{Ca}^{2+}$  flux along the t-tubule membrane remained unchanged by redistributing the  $\text{Ca}^{2+}$  fluxes.

**Figure 2A-B and Table 2 - near here**

### ***Reaction-diffusion equations***

The effects of four exogenous and endogenous  $\text{Ca}^{2+}$  buffers (Fluo-3, ATP, calmodulin, troponin C) were considered (Fig. 1B). The endogenous stationary buffer troponin C (TN) was distributed uniformly throughout the cytosol but not on the cell membrane. The

free  $\text{Ca}^{2+}$  and mobile buffers (Fluo-3, ATP, calmodulin) diffuse and react throughout the cytoplasm and cell membrane subject to reflective boundary conditions at the cell surfaces. The nonlinear reaction-diffusion equations describing  $\text{Ca}^{2+}$  and buffers dynamics inside the cell are:

$$\frac{\partial [C a^{2+}]_i}{\partial t} = D_{C a} \nabla^2 [C a^{2+}]_i - \sum_{m=1}^3 R_{B_m} - R_{B_s} + J_{C a_{flux}} \quad (2)$$

$$\frac{\partial [C a B_m]}{\partial t} = D_{C a B_m} \nabla^2 [C a B_m] + R_{B_m} \quad (3)$$

$$\frac{\partial [C a B_s]}{\partial t} = R_{B_s} \quad (4)$$

$$R_{B_m} = k_+^m ([B_m] - [C a B_m])[C a^{2+}]_i - k_-^m [C a B_m] \quad (5)$$

$$R_{B_s} = k_+^s ([B_s] - [C a B_s])[C a^{2+}]_i - k_-^s [C a B_s] \quad (6)$$

where:  $[B_m]$  mobile buffer Fluo-3, calmodulin or ATP;  $[B_s]$  stationary buffer troponin C.

The diffusion coefficients for  $\text{Ca}^{2+}$ , CaATP, CaCal and CaFluo as well as the total buffer concentrations and rate buffer constants used in the model are shown in Table 3. In the model we also assume: (1)  $\text{Ca}^{2+}$  binds to Fluo-3, calmodulin, ATP, and TN without cooperativity; (2) the initial total concentrations of the mobile buffers are spatially uniform; (3) the diffusion coefficients of Fluo-3, ATP or calmodulin with bound  $\text{Ca}^{2+}$  are equal to the diffusion coefficients of free Fluo-3, ATP or calmodulin.

### Table 3 - near here

The total  $\text{Ca}^{2+}$  flux ( $J_{Ca_{flux}}$ ) throughout the t-tubule and surface membrane is:

$$J_{C a_{flux}} = J_{C a} - J_{N C X} - J_{p C a} + J_{C a b} \quad (7)$$



where:  $J_{Ca}$  - total LCC  $Ca^{2+}$  influx;  $J_{NCX}$  - total NCX  $Ca^{2+}$  efflux;  $J_{pCa}$  - total  $Ca^{2+}$  pump efflux;  $J_{Cab}$  - total background  $Ca^{2+}$  leak influx.

To fit the whole-cell LCC current density to reported data in rat myocytes with SR release inhibited [28] we used a MATLAB implementation of Hinch *et al.* model [29], (Fig. 2B). To describe the  $Na^+/Ca^{2+}$  exchanger, membrane  $Ca^{2+}$  pump and leak current densities we used expressions from Hinch *et al.*:

$$I_{NCX} = g_{NCX} \frac{e^{\eta VF/RT} [Na^+]_i^3 [Ca^{2+}]_e - e^{(\eta-1)VF/RT} [Na^+]_e^3 [Ca^{2+}]_i}{(k_{m,Na}^3 + [Na^+]_e^3)(k_{m,Ca} + [Ca^{2+}]_e)(1 + k_{sat} e^{(\eta-1)VF/RT})} \quad (8)$$

$$I_{pCa} = \frac{g_{pCa} [Ca^{2+}]_i}{[Ca^{2+}]_i + k_{m,pCa}} \quad (9)$$

$$I_{Cab} = g_{Cab} \left[ \frac{RT}{2F} \ln \left( \frac{[Ca^{2+}]_e}{[Ca^{2+}]_i} \right) - V \right] \quad (10)$$

Flux parameter values were estimated or taken from the literature (see Table 4). At rest the  $Ca^{2+}$  influx via background  $Ca^{2+}$  leak was adjusted to match the  $Ca^{2+}$  efflux via NCX and  $Ca^{2+}$  ATPase so that no net movement across the cell membrane occurred.

#### Table 4 - near here

In the model, each current density ( $I_i$ ) was converted to  $Ca^{2+}$  flux ( $J_i$ ) by using the experimentally suggested surface to volume ratio ( $\frac{C_m}{V_{cell}} \sim 8.8$  pF/pL) in adult rat ventricular myocytes [1], [25]:

$$J_i = \left( \frac{1}{2F} \frac{C_m}{V_{cell}} \right) I_i \quad (11)$$

Then, the total compartment  $\text{Ca}^{2+}$  flux ( $J_{\text{Ca}_{flux}}$ ) was computed by multiplying each total  $J_i$  with the model cell volume ( $V_{mc}$ ), and distributing  $J_{\text{Ca}_{flux}}$  to the surface membrane and t-tubule membrane according to the prescribed  $\text{Ca}^{2+}$  handling protein concentration ratio.

### **Software**

The nonlinear reaction diffusion system was solved using a finite difference method in time and finite element method in space. Here we developed a distributed finite element software package, to be used on a Linux cluster for parallel computations. Computations took ~18 minutes to simulate 400 ms of one  $\text{Ca}^{2+}$  cycle on 10 processors of an Intel Xeon-based cluster (see Fig 3B). A discrete time stepping of 4ms was used during the simulations.

The software was built using several established software packages. Mesh generators included Netgen4.3.1 and TetGen1.3 authored by Schöberl and Si respectively [30]. The parallel finite element assembler was based on libMesh0.4.3-rc2 by Kirk *et al.* [31]. The discrete linear system was solved using PETSc-2.2.1 [32]. An operator splitting method was used to de-couple the reaction-diffusion system [33]. The nonlinear ordinary differential equations were solved using an A-stable and implicit Runge-Kutta method of order 5. The simulation results were visualized using GMV3.4 [34]. Post-processing and data analysis were performed using customized Python and MATLAB version 7.1 (The MathWorks, Natick, MA) scripts.

In designing the software, the portability and reusability were emphasized over computational efficiency. The implementation was based on object-oriented programming. Therefore the resulting software contains a set of loosely connected

application tools, which focused on both flexibility and functionality. The structure of the software followed that of a finite element simulation package (Fig. 3A).

**Figure 3A-B – near here**

## Results

### ***Ca<sup>2+</sup> concentration changes in the presence of 100 μM Fluo-3 and inhibited SR release and uptake***

Model results in Fig. 4 were computed for conditions approximating those of experiments by Cheng *et al.* [8] (see Fig. 4M), who examined Ca<sup>2+</sup> signals in rat ventricular myocytes in the presence of 100 μM Fluo-3 and pharmacological blockade of the SR. The voltage-clamp protocol (holding potential –50mV, electric pulse of 10mV for 70ms) and whole-cell L-type Ca<sup>2+</sup> current were derived from the study of Zahradnikova *et al.* [28] with blocked SR (see Fig.2B and Figs. 4A-B).

**Figure 4A-M - near here**

Consistent with the experimental study [8] in the model the scanned line was located at 200nm from the surface of the t-tubule (*see red line* in Fig. 5A). The line-scan images and local Ca<sup>2+</sup> time-courses are shown in Figs. 4G-I and Figs. 4J-L, respectively. These results suggest that the model was able to predict uniform Ca<sup>2+</sup> distribution inside the cell when LCC and NCX current densities were heterogeneously and Ca<sup>2+</sup> leak homogeneously distributed along the sarcolemma and Ca<sup>2+</sup> pump was located on the surface membrane. Furthermore, when the LCC flux density was 6 times higher and uniform in the t-tubule, the Ca<sup>2+</sup> concentration profiles were less evenly distributed (Figs. 4H and 4K) but the predicted variations in [Ca<sup>2+</sup>]<sub>i</sub> seem to be within the range of

experimental noise in Fig. 4M. Finally, these studies revealed that heterogeneous  $\text{Ca}^{2+}$  transients might occur if the LCC current density was uniformly distributed throughout the whole cell surface (Figs. 4I and 4L). The model also demonstrated that distributing NCX flux homogeneously along the sarcolemma did not significantly affect  $\text{Ca}^{2+}$  uniformity (*data not shown*). The distribution of  $\text{Ca}^{2+}$  pump and leak pathways also did not seem to have a significant effect. Blockade of these fluxes reduced the control  $[\text{Ca}^{2+}]_i$  peak in Fig. 4J by less than 0.4% (*data not shown*). Figures 4F and 4C-E show the global  $[\text{Ca}^{2+}]_i$  transient,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger,  $\text{Ca}^{2+}$  pump and leak currents when  $\text{Ca}^{2+}$  pathways were distributed heterogeneously as in Fig. 4G. Figures 4F and 4J-L illustrate: (1) that the global and all local  $\text{Ca}^{2+}$  transients reached the peak after  $\sim 68$  ms; and (2) that the decay of the  $\text{Ca}^{2+}$  signals was extremely slow, since the reuptake of  $\text{Ca}^{2+}$  to the SR was blocked and  $[\text{Ca}^{2+}]_i$  was lowered solely by extrusion from the cell via the  $\text{Na}^+-\text{Ca}^{2+}$  exchanger and  $\text{Ca}^{2+}$  pump. Possible reasons for the predicted extremely slow NCX rate might be that in the model the intracellular  $\text{Na}^+$  concentration ( $[\text{Na}^+]_i$ ) was kept constant (in contrast to existing evidence for the presence of local sub-membrane  $[\text{Na}^+]_i$  gradients on the action potential time-scale [1], [35]) or that the realistic distribution of NCX flux density probably differs as assumed in the model. The 3-D  $\text{Ca}^{2+}$  concentration distributions at  $\text{Ca}^{2+}$  peak (line scan 200 nm away from the t-tubule) and the local  $\text{Ca}^{2+}$  transients (line scan 0 nm and 875 nm away from the t-tubule) are shown in Fig. 5.

### **Figure 5A-G - near here**

In summary, the results in Fig. 4 and Fig. 5 suggest that in the presence of 100  $\mu\text{M}$  Fluo-3 and with the SR blocked (1)  $\text{Ca}^{2+}$  concentration near the surface membrane decreased while  $[\text{Ca}^{2+}]_i$  in the cell interior increased when  $\text{Ca}^{2+}$  transporters were

uniformly distributed and after that heterogeneously redistributed, and (2) in each moment of the cell cycle the overall  $\text{Ca}^{2+}$  distribution remained almost uniform across the model compartment when  $\text{Ca}^{2+}$  transporters were heterogeneously distributed.

The good agreement between the model and experimental observations suggests that heterogeneous channel distributions may be another important mechanism regulating intracellular  $\text{Ca}^{2+}$  distribution and myofilament function. Moreover, it allows the model to be used to simulate experiments that cannot be performed because of technical reasons (for example in the absence of  $\text{Ca}^{2+}$  indicator).

***$\text{Ca}^{2+}$  concentration changes in the absence of Fluo-3, inhibited SR and heterogeneous distribution of  $\text{Ca}^{2+}$  membrane pathways***

Figure 6 shows membrane currents, and  $\text{Ca}^{2+}$  signals arising from the ionic influx via L-type  $\text{Ca}^{2+}$  channels at zero Fluo-3 with heterogeneously distributed membrane  $\text{Ca}^{2+}$  fluxes (as in Fig. 4G) during voltage-clamp stimulation.

**Figure 6A-J - near here**

Since it has been suggested [2], [36] that the dye does not affect  $\text{Ca}^{2+}$  entry via L-type channels, the same global LCC flux was used during this numerical experiment (Fig. 4B and Fig. 6B). In the absence of Fluo-3 the peak of average  $\text{Ca}^{2+}$  transient increased ~1.6-fold (from 0.19  $\mu\text{M}$  up to 0.3  $\mu\text{M}$ ), while the time to peak (~ 68 ms) remained unchanged (Fig. 6). Figures 6H and 6I-J demonstrate that local  $\text{Ca}^{2+}$  peaks also increased while the times to peak remained ~ 68 ms. In addition,  $[\text{Ca}^{2+}]_i$  decay at zero Fluo-3 was slow. The increase in local  $\text{Ca}^{2+}$  levels across the cell membrane affected  $\text{Na}^{2+}/\text{Ca}^{2+}$  exchange and  $\text{Ca}^{2+}$  pump activities more significantly than the  $\text{Ca}^{2+}$  background leak (see Figs. 6C-E and Figs. 4C-E). It is interesting that, under these conditions, the model predicts non-

uniform  $\text{Ca}^{2+}$  concentration distributions in and along the cell membrane and in the cell interior. Figures 6 I-K demonstrate that in the absence of Fluo-3  $[\text{Ca}^{2+}]_i$  in and near the t-tubule membrane was much more higher than in the cell interior. These results indicate that  $\text{Ca}^{2+}$  levels vary in the transverse direction too. Along the t-tubule,  $[\text{Ca}^{2+}]_i$  was higher near to the surface membrane and in the cytoplasmic t-tubule end while in the cell interior  $[\text{Ca}^{2+}]_i$  was higher only near the surface membrane.

## Discussion

In this study we developed a 3-D continuum model of  $\text{Ca}^{2+}$ -signaling, buffering and diffusion inside a small representative region of the rat ventricular muscle cell. The simplified model geometry, derived from published structural data [9], [11], [23], contained one repeating unit inside the myocyte, including single cylindrical t-tubule and its surrounding half-sarcomeres. Following the morphological studies on 3-D reconstruction of t-tubule system, ~58% of membrane surface was assumed to be within the t-tubule. On the basis of experimental data in rat myocytes [26] the aqueous sub-cellular volume, accessible to  $\text{Ca}^{2+}$ , was estimated to be ~ 35-37%. In addition, we assumed the cleft and sub-membrane sizes infinitely thin that allowed us not to explicitly define a deferent values for  $D_{Ca}$ ,  $D_{CaFluo}$ ,  $D_{CaATP}$  and  $D_{CaCal}$  in these near-membrane spaces and in myofibrils. To test the correctness of above assumption we examined how 2-fold increase in  $D_{Ca}$  or mobile buffer diffusion coefficients (values suggested in water) in the cleft and sub-membrane space or variations in the sub-membrane depth (from 12 nm to 45 nm) would affect local and global  $\text{Ca}^{2+}$  transients. We found that these changes had insignificant effects on the calculated  $\text{Ca}^{2+}$  signals (*data not shown*). We concluded

that a functional importance of these spaces lies in the immediate vicinity of the sarcolemma where all membrane proteins and sarcolemmal ion channels are located [1-7], [27]. In view of the fact that  $\text{Ca}^{2+}$  signaling in cells is largely governed by  $\text{Ca}^{2+}$  diffusion and binding to mobile and stationary  $\text{Ca}^{2+}$  buffers [3], [27], [37] the effect of four  $\text{Ca}^{2+}$  buffers (Fluo-3, ATP, calmodulin, TN) was considered. We validated the model against published experimental data on  $\text{Ca}^{2+}$  influx, membrane protein distributions and  $\text{Ca}^{2+}$  diffusion in rat ventricular myocytes treated with ryanodine and thapsigargin [8], [10], [15], [16], [18]-[22]. We used the model: (1) to examine how the distribution of L-type  $\text{Ca}^{2+}$  channels,  $\text{Na}^+/\text{Ca}^{2+}$  exchangers, membrane  $\text{Ca}^{2+}$  ATPases and membrane leaks regulates the spatio-temporal features of intracellular  $\text{Ca}^{2+}$  signals in the presence of fluorescent dye; and (2) to simulate and analyze the  $\text{Ca}^{2+}$  signals that are not accessible experimentally, i.e. in the absence of  $\text{Ca}^{2+}$  indicator. The parallel numerical software enabled us to solve the reaction-diffusion equations in a reasonable time and to test the model carefully.

An important model limitation is that we simplified the t-tubule and surrounding structures geometries assuming them cylindrical and cube-shaped box, respectively. However, several studies provide evidence that in normal ventricular cells the realistic t-tubule geometry is quite complex (with large local variations in the diameter and transverse-axial anatomies) and that its surrounding structures might form quite arbitrary shapes (see Fig.7) [9], [13], [38]. It has been also observed that in the failing hearts the t-tubules might be aberrantly shaped or dilated [13], [39]. Taken together above data strongly suggest that further extending of the current model toward more realistic geometries is needed. The use of idealistic shapes might change the diffusion distances in

plane and depth directions and consequently the predicted  $\text{Ca}^{2+}$  distributions.

**Figure 7 – near here**

*$\text{Ca}^{2+}$  signals in the presence of 100  $\mu\text{M}$  Fluo-3 and absence of SR activity Cheng et al.*

[8] examined the propagation of EC-coupling in isolated rat ventricular cells by using laser scanning confocal microscope and 100  $\mu\text{M}$  Fluo-3. They found that the depolarization-evoked  $[\text{Ca}^{2+}]_i$  transients in the presence or absence of SR release and uptake are activated synchronously near the cell surface and in the cell interior and that the time of  $[\text{Ca}^{2+}]_i$  rise does not depend on whether SR activity is abolished or not. They concluded that the lack of systematic differences in the  $\text{Ca}^{2+}$  fluorescence signal recorded from either the center or the edge of the cell indicates that sarcolemmal  $\text{Ca}^{2+}$  and the SR  $\text{Ca}^{2+}$ -release channels (RyRs) are distributed throughout the heart cell, and that ventricular EC-coupling is not limited by diffusion of the second messenger from the surface of the cell to the center.

In this study, to investigate further the mechanisms underlying EC-coupling propagation in rat ventricular myocytes, we used modeling approach. We examined how the distribution of the sarcolemmal  $\text{Ca}^{2+}$  influx and efflux transporters regulates  $\text{Ca}^{2+}$  movement from the cell surface to the cell interior when the SR was blocked. In agreement with experiment [10], [16], we found that in the presence of 100  $\mu\text{M}$  Fluo-3 model predicts a homogenous  $\text{Ca}^{2+}$  distribution inside the cell if L-type  $\text{Ca}^{2+}$  current density is  $\sim 6$  times higher in the t-tubule than in surface membrane and increases  $\sim 1.7$  fold along the t-tubule length. An interesting model observation was also that the uniform  $\text{Ca}^{2+}$  distribution might be achieved assuming LCC flux density 6-fold higher and



uniform along the t-tubule because the predicted  $[Ca^{2+}]_i$  fluctuations here were within the range of experimental noise [8]. New experiments should be performed to test this hypothesis.

In addition, our results revealed that the spatio-temporal features of local  $Ca^{2+}$  signals depended on the diffusion distances in the axial and cell surface directions. Thus, when the LCC were distributed uniformly the local  $Ca^{2+}$  peak in radial depth (7  $\mu m$ ) decreased from  $\sim 0.25 \mu M$  to  $\sim 0.15 \mu M$  while in the other cell directions (1  $\mu m \times 1 \mu m$ ) no significant changes were found. Redistributing the amount of  $Ca^{2+}$  pumped via the cell membrane (i.e. increasing LCC current density  $\sim 6$ -fold along the t-tubule) while keeping total  $Ca^{2+}$  flux unchanged, lowered  $Ca^{2+}$  gradients near the surface membrane and increased  $Ca^{2+}$  levels in the cell interior.

Other interesting model findings were that: (1) the global  $Ca^{2+}$  time-course and time to  $[Ca^{2+}]_i$  peak ( $\sim 68$  ms) do not depend on whether  $Ca^{2+}$  flux pathways are distributed homogeneously, uniformly in the t-tubule or heterogeneously (*data not shown*); (2) the changes in the local  $Ca^{2+}$  transients near the cell membrane when  $Ca^{2+}$  microdomains were distributed differently affected NCX and  $Ca^{2+}$  pump time-courses while  $Ca^{2+}$  leak current remained unchanged (*data not shown*); and (3) the NCX,  $Ca^{2+}$  pump or  $Ca^{2+}$  leak redistribution alone were not able to alter significantly the predicted  $Ca^{2+}$  uniformity (*data not shown*).

***Ca<sup>2+</sup> signals in the absence of Fluo-3 and SR activity and heterogeneous distribution of Ca<sup>2+</sup> membrane fluxes*** Another advantage of the model was its ability to predict  $Ca^{2+}$  signals that would occur in the absence of Fluo-3. The model simulations revealed that at zero Fluo-3 and with 260  $\mu M$  ATP and 24  $\mu M$  calmodulin as mobile buffers, the  $Ca^{2+}$

distribution in the cell interior would be non-uniform if LCC are distributed heterogeneously (as in Figs. 4G). Note in the absence of 100  $\mu\text{M}$  mobile Fluo-3 a local and global  $\text{Ca}^{2+}$  peaks increased while the time of  $\text{Ca}^{2+}$  rise remained almost unchanged. Furthermore, during the  $\text{Ca}^{2+}$  influx larger, steeper and heterogeneous  $\text{Ca}^{2+}$  concentration gradients were predicted between the cytosol and sub-membrane space while in the cell interior  $[\text{Ca}^{2+}]_i$  was more uniformly distributed. In addition, results suggest that the calculated sub-membrane  $[\text{Ca}^{2+}]_i$  levels were higher: (1) near the t-tubule mouth because a close topological proximity of this membrane to the surface sarcolemma additionally increased the relative amount of  $\text{Ca}^{2+}$  entering there; (2) near the cytosolic t-tubule end because the LCC flux density was assumed higher. The simulations also showed that removing Fluo-3 affected NCX and  $\text{Ca}^{2+}$  pump time-courses by increasing local free  $[\text{Ca}^{2+}]_i$ . Thus, these findings clearly reveal that (1) the exogenous Fluo-3 may act as a significant buffer and carrier for  $\text{Ca}^{2+}$ , and that (2) the use of 100  $\mu\text{M}$  Fluo-3 during the experiment may sensitively alter the realistic  $\text{Ca}^{2+}$  distribution. However the question arising here is: Based on the model what might be the underlying mechanism(s) for the predicted  $\text{Ca}^{2+}$  concentrations gradients in the absence of Fluo-3? A reasonable answer is that  $\text{Ca}^{2+}$  movement and distribution inside the cell also strongly relies on the presence of mobile and stationary  $\text{Ca}^{2+}$  buffers [27], [37]. Thus now in the absence of Fluo-3 (1) the stationary  $\text{Ca}^{2+}$  buffer troponin C (TN) imposed stronger diffusion barrier for  $\text{Ca}^{2+}$  that resulted in larger and steeper  $\text{Ca}^{2+}$  concentration gradients between the cytosol and narrow sub-membrane space, (2) the buffer capacity and spread of ATP and Cal alone via the t-tubule membrane were not able to mask the assumed heterogeneous  $\text{Ca}^{2+}$  entering via the sarcolemma, (3) in the cell interior, the overall  $\text{Ca}^{2+}$  distribution remained almost

uniform in each moment of the cell cycle (because TN, ATP and Cal buffering capacity dominated) and a little bit higher in and near the surface membrane (because [TN] was zero there).

Taken together, our studies suggest that in ventricular myocytes when the SR is pharmacologically inhibited: (1) intracellular  $\text{Ca}^{2+}$  concentration rapidly increases during  $\text{Ca}^{2+}$  entrance (0-70 ms) while the decay of  $[\text{Ca}^{2+}]_i$  is slow; (2) in the absence of fluorescent dye, large  $\text{Ca}^{2+}$  concentration gradients might develop near the cell membrane; and (3) intracellular  $\text{Ca}^{2+}$  distribution is tightly regulated by the localization of  $\text{Ca}^{2+}$  transporter proteins along the sarcolemma and strongly relies on the presence of mobile and stationary  $\text{Ca}^{2+}$  buffers. These studies also imply that in ventricular cells with intact and functional SR, the  $\text{Ca}^{2+}$  signal most likely would spread faster along the t-tubule and surface membrane than to the cell interior and that in the absence of  $\text{Ca}^{2+}$  dye high  $\text{Ca}^{2+}$  gradients under the surface membrane and more uniform  $\text{Ca}^{2+}$  distribution in the cell interior might be expected.

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## Figure Legends

**Fig. 1.** Panel A: Schematic drawing of the model geometry showing the single t-tubule and its surrounding half-sarcomeres. The top surface of the cube is the surface membrane for the model compartment. Panel B: A diagram illustrating  $\text{Ca}^{2+}$  entrance and extrusion along the t-tubule and surface membrane and  $\text{Ca}^{2+}$  buffering and diffusion inside the cell when the SR release and uptake are inhibited.

**Fig. 2.** Panel A: The distribution of L-type  $\text{Ca}^{2+}$  current was computed (*dashed line*) by multiplying the experimentally measured cluster density and fluorescent intensity plots (*solid line*), [8], [16]. Panel B: The L-type  $\text{Ca}^{2+}$  current density was fitted and plotted (*dashed line*) using shape preserving function of the data reported in rats with SR blocked (*solid line*), [28].

**Fig. 3.** Panel A: The finite element software package contained four components with data flowing from top to bottom. Panel B: Composite view of the subdomain-to-processor assignment used to partition the mesh on 10 processors.

**Fig. 4.** Model predictions in the presence of 100  $\mu\text{M}$  Fluo-3. The voltage-clamp protocol and the whole-cell L-type  $\text{Ca}^{2+}$  current used in this set of simulations are shown in panels A-B. Predicted global  $\text{Na}^+/\text{Ca}^{2+}$ ,  $\text{Ca}^{2+}$  pump and leak currents and global  $\text{Ca}^{2+}$  transient

when  $\text{Ca}^{2+}$  was uniformly distributed inside the cell are shown in panels C-F. Calcium concentrations visualized as line-scan images in transverse cell direction are shown in panels G-I. Panels J-L show the local  $\text{Ca}^{2+}$  transients taken at three featured spots along the scanning line ( $0.17\mu\text{m}$  – *blue lines*,  $3.09\mu\text{m}$  – *green lines*,  $6.65\mu\text{m}$  – *red lines*). In panels G and J the L-type  $\text{Ca}^{2+}$  current density followed heterogeneous distribution in the t-tubule that was 6 times higher than in surface membrane and increased 1.7 times along the length of t-tubule as shown in Fig.2A. In panels H and K the L-type  $\text{Ca}^{2+}$  current density was uniform along the t-tubule and 6 times higher than in surface membrane. In panels I and L the L-type  $\text{Ca}^{2+}$  current density was homogeneous throughout the cell surface. In this numerical experiment  $\text{Na}^+/\text{Ca}^{2+}$  current density was 3 times higher in the t-tubule membrane,  $\text{Ca}^{2+}$  pump was located only on the surface membrane, and  $\text{Ca}^{2+}$  leak was distributed homogeneously via the sarcolemma. Panel M illustrates local  $\text{Ca}^{2+}$  time-courses with re-plot from experimental data [8]. The re-plots were taken along the scanned line at  $0\mu\text{m}$  (*blue*),  $3.96\mu\text{m}$  (*green*) and  $7.57\mu\text{m}$  (*red*) from the near surface location. Panels J and M demonstrate that model predictions are in agreement with the experiment only when  $\text{Ca}^{2+}$  influx and efflux pathways were distributed heterogeneously. The scanned lines (*model and experiment*) were located at 200nm from the surface of the t-tubule (see *red line* in Fig. 5A).

**Fig. 5.** Predicted 3-D  $\text{Ca}^{2+}$  concentration distributions (*computed from the line-scan images in Figs. 4G-I*) at  $\text{Ca}^{2+}$  peak of 68 ms are shown in panels A-C. In panel D the spatial profiles at  $\text{Ca}^{2+}$  peak along the scanning line (*200nm from the surface of the t-tubule*) are compared: *gray line* -  $\text{Ca}^{2+}$  pathways heterogeneously distributed on the t-

tubule and surface membrane (see panel A); *violet line* -  $\text{Ca}^{2+}$  pathways uniformly distributed on the t-tubule (see panel B); *pink line* -  $\text{Ca}^{2+}$  pathways uniformly distributed via the sarcolemma (see panel C). Local  $\text{Ca}^{2+}$  concentration profiles at two different line-scan positions (0 nm - *solid lines*, 875 nm - '+' plots) are superimposed in panels E-G. Along the t-tubule the three featured spots were (0 $\mu\text{m}$  - *blue lines*, 3.48  $\mu\text{m}$  - *green lines*, 6.76  $\mu\text{m}$  - *red lines*) and in the cell interior (0 $\mu\text{m}$  - *blue lines*, 1.63  $\mu\text{m}$  - *green lines*, 6.51  $\mu\text{m}$  - *red lines*). In panels E-G the  $\text{Ca}^{2+}$  pathways were distributed heterogeneously or uniformly in the t-tubule membrane (as in panels A and B) or evenly along the cell surface (as in panel C).

**Fig. 6.** Model predictions in the absence of Fluo-3 with heterogeneous distribution of  $\text{Ca}^{2+}$  pathways via the cell membrane. Panels A and B show the voltage-clamp protocol and whole-cell L-type  $\text{Ca}^{2+}$  current used in this set of simulations. The predicted global  $\text{Na}^+/\text{Ca}^{2+}$ ,  $\text{Ca}^{2+}$  pump and leak currents are presented in panels C-E. The global  $\text{Ca}^{2+}$  transient and  $\text{Ca}^{2+}$  concentrations visualized as line-scan image in the transverse cell direction are shown in panels F and G. In panel H the local  $\text{Ca}^{2+}$  transients at three spots along the scanning line are superimposed (*blue line* - 0.17 $\mu\text{m}$ , *green line* - 3.09 $\mu\text{m}$ , *red line* - 6.65 $\mu\text{m}$ ). Panel K shows 3-D view of  $\text{Ca}^{2+}$  concentration distribution at  $\text{Ca}^{2+}$  peak (~68 ms). Scanning line in panels G-H and K was located at 200nm from the surface of the t-tubule. The local  $\text{Ca}^{2+}$  concentration profiles at two different line-scan positions (0 nm - *solid lines*, 875 nm - '+' plots) are superimposed in panel I. Panel J is an expanded view of panel I. On the t-tubule membrane and in the cell interior the featured spots were chosen to be the same as in Fig. 5E.

**Fig. 7.** T-tubular network complexity in adult mammalian ventricular myocytes. Cardiac sarcolemma including t-tubules was stained with Alexa-488-conjugated wheat germ agglutinin (WGA) in 80  $\mu\text{m}$  vibratome sections of adult mouse ventricular myocardium and visualized using a 2-photon microscopy (Radian 2000, BioRad, objective: 60x oil, NA=1.40, ex 800 nm, em 508-558, 59 nm/pixel). T-tubules invaginated from the surface membrane are shown to develop extensively branching in the middle of myocyte. Surface membrane (*blue arrows*), t-tubules (*white arrowheads*), nucleus (*Nuc*), bar 2  $\mu\text{m}$ . The image was kindly supplied by Masahiko Hoshijima and Takeharu Hayashi (*unpublished data*).



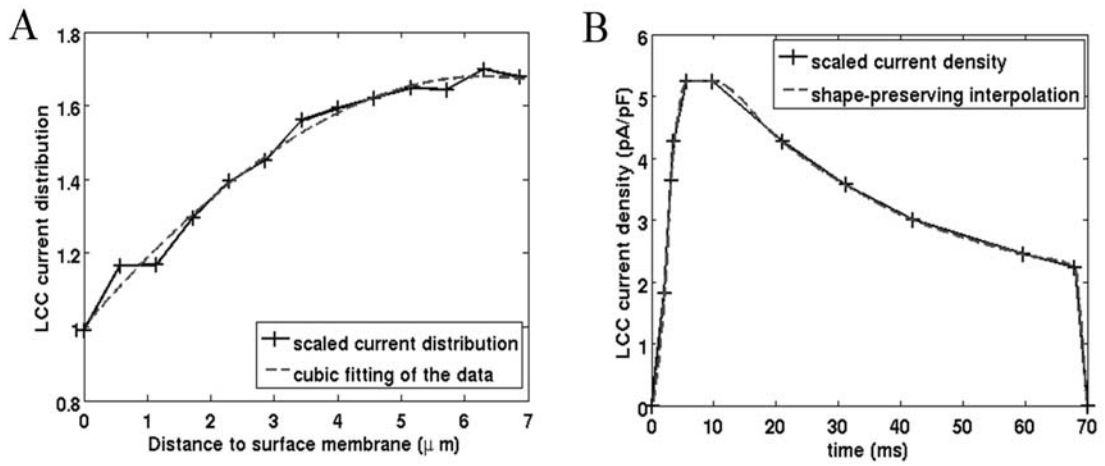
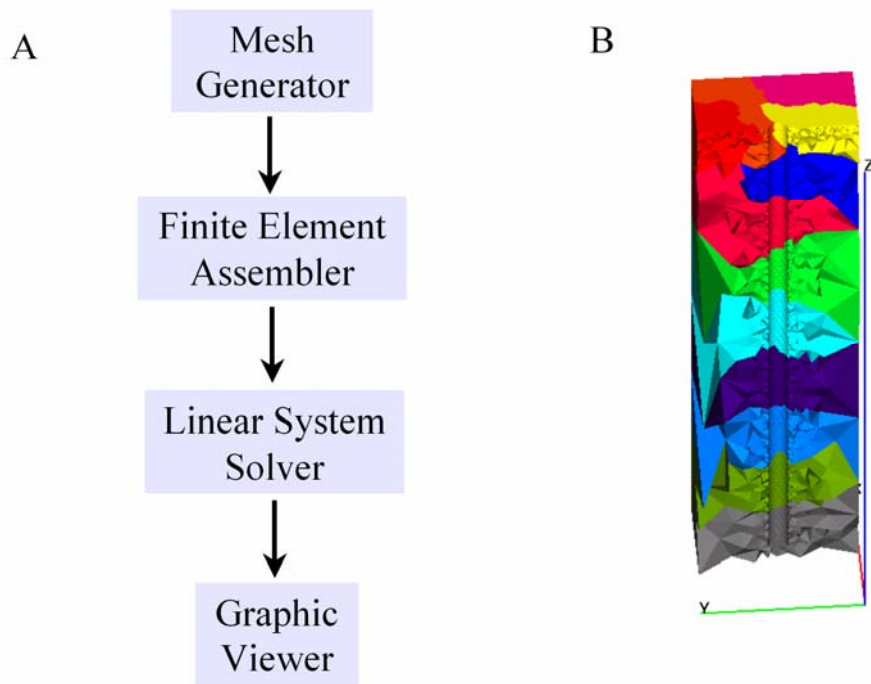


Figure 2



**Figure 3**

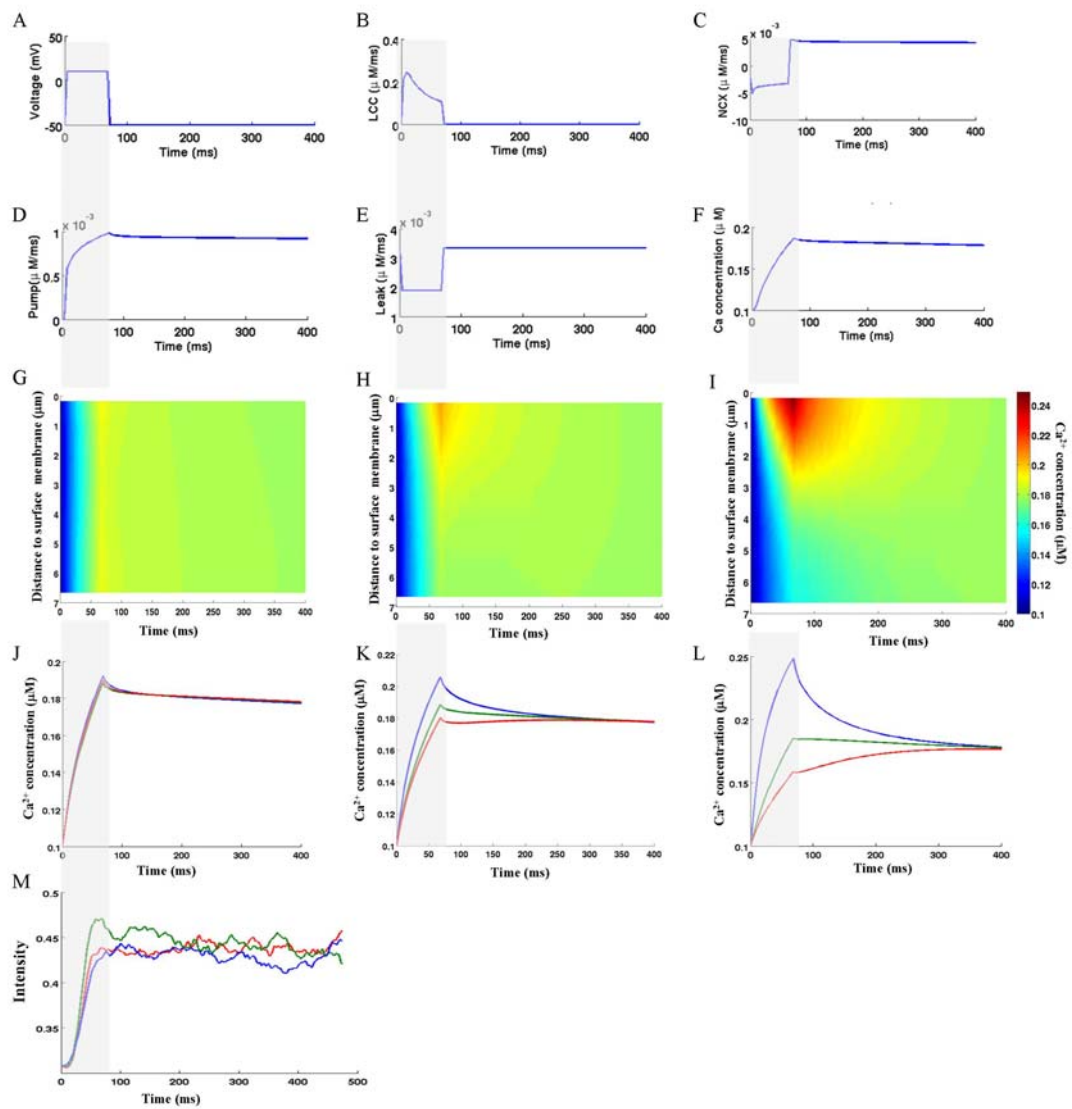


Figure 4



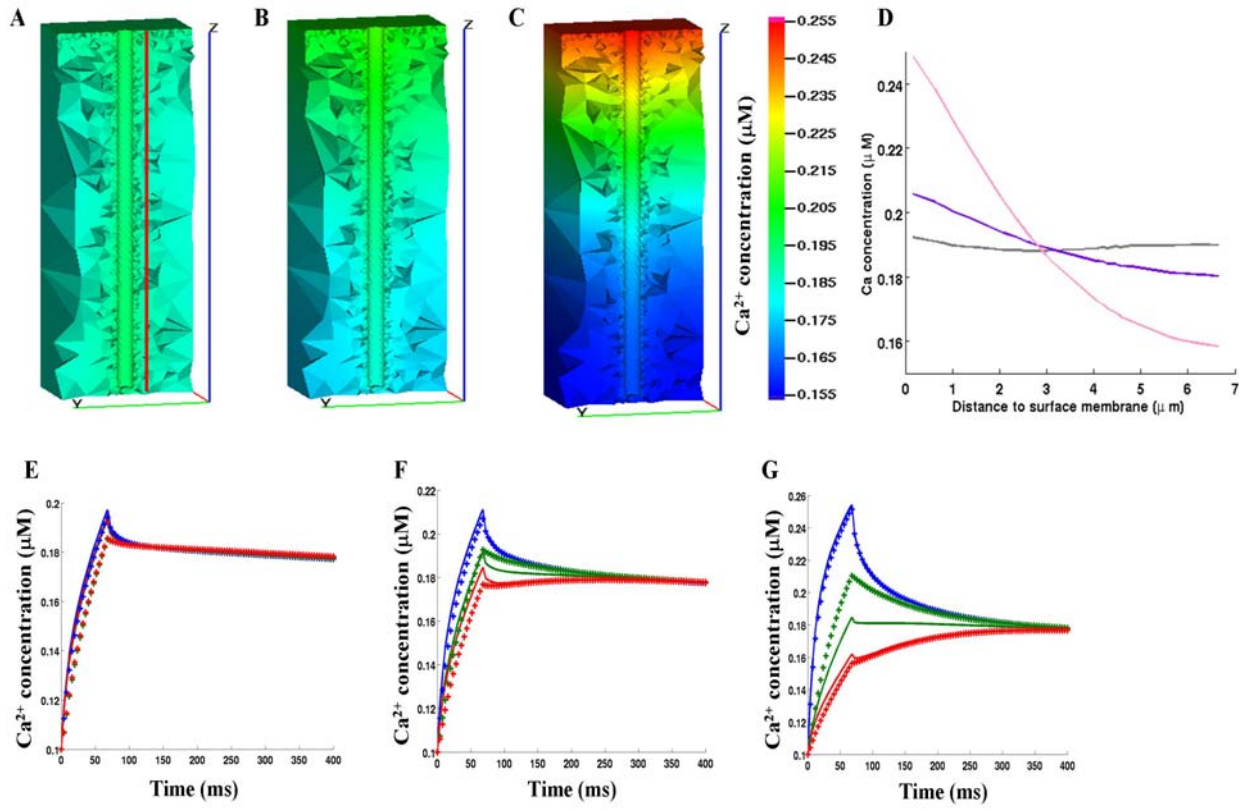


Figure 5

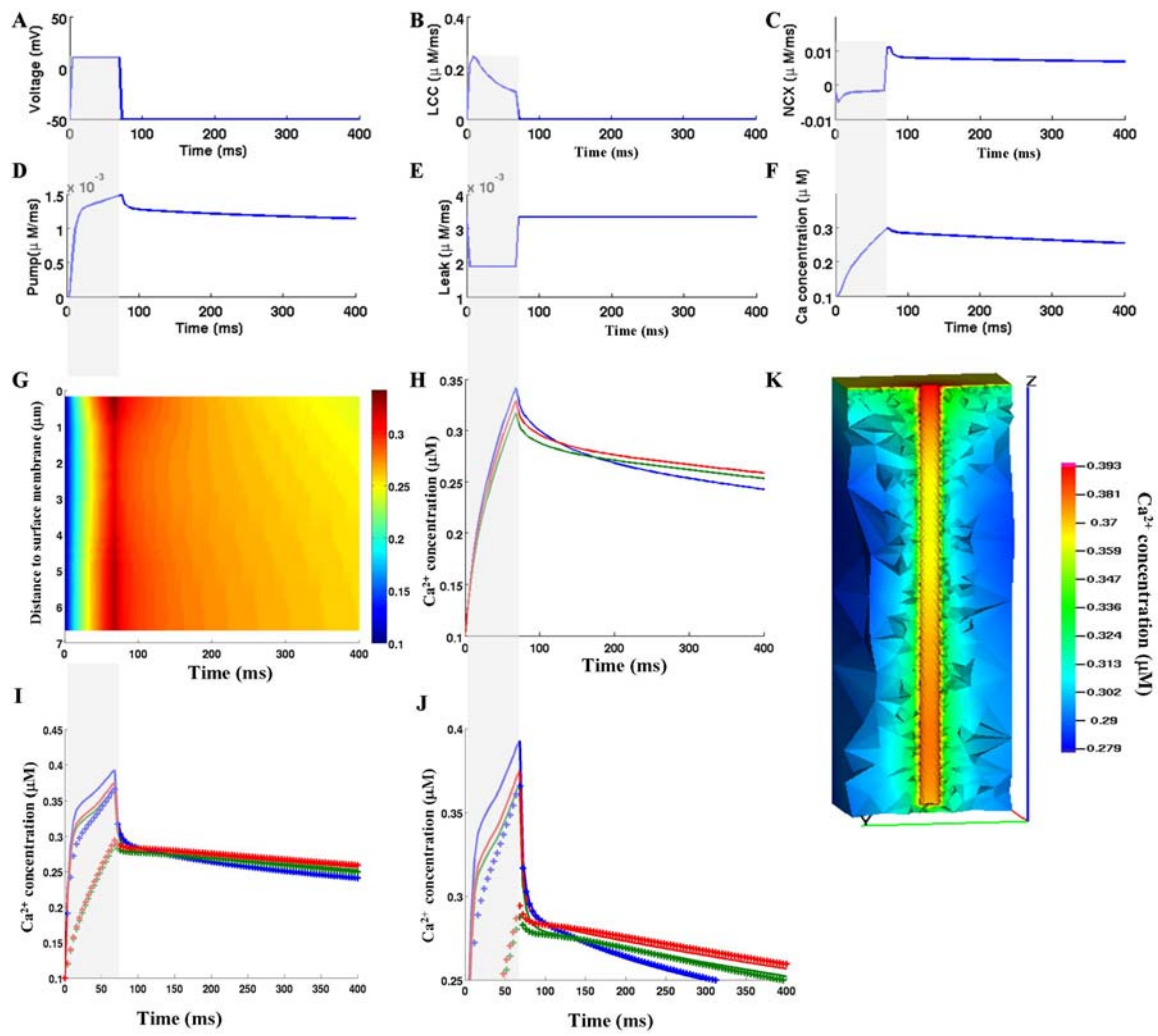
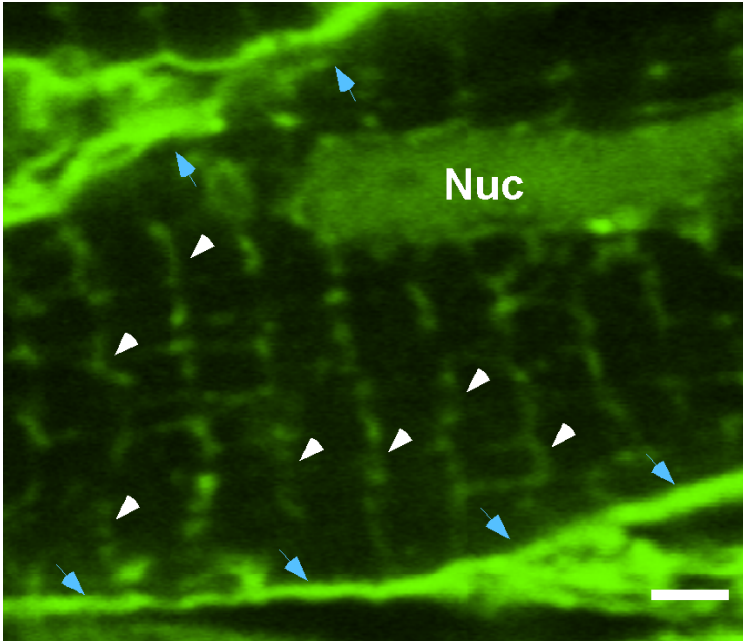


Figure 6



**Figure 7**

**Table 1.** Physical constants and cell geometry

Definition	Symbol	Value	Reference No.
Faraday constant	F	96.5 C mmol <sup>-1</sup>	Physical constant
Temperature	T	295 K	Physical constant
Universal gas constant	R	8.314 J mol <sup>-1</sup> K <sup>-1</sup>	Physical constant
Cell volume	$V_{cell}$	36.8 pL	[1]
Cell capacitance	$C_m$	324 pF	[1]
Accessible volume for Ca <sup>2+</sup> in the cell	$V_{acc}$	12.9-13.6 pL	Estimated
Compartment volume	$V_{mc}$	27.68 $\mu\text{m}^3$	Estimated
Compartment surface	$S_{mc}$	9.34 $\mu\text{m}^2$	Estimated
<b>T-tubule geometry</b>			
T-tubule radius	$r_{t-tub}$	0.125 $\mu\text{m}$	[9]
T-tubule depth	$dp_{t-tub}$	6.87 $\mu\text{m}$	[9]
<b>Cubic-shaped box geometry</b>			
Cell surface direction	$d_{box-1}$	2 $\mu\text{m}$	[9]
Cell surface direction	$d_{box-2}$	2 $\mu\text{m}$	[9]
Depth	$dp_{box}$	7 $\mu\text{m}$	[9]

**Table 2.** The parameter values of cubic polynomial describing the L-type Ca<sup>2+</sup> current distribution along the t-tubule

Symbols	C	p <sub>1</sub>	p <sub>2</sub>	p <sub>3</sub>	p <sub>4</sub>
L-type Ca <sup>2+</sup> channel	0.6827	-4.1379e-4	-1.1722e-2	1.978e-1	1.0033

**Table 3.** Ca<sup>2+</sup> and buffer reaction-diffusion parameters

Definition	Symbol	Value	Reference No.
<b>Ca<sup>2+</sup> and buffer concentrations</b>			
Extracellular Ca <sup>2+</sup> concentration	$[Ca^{2+}]_e$	1000 $\mu\text{M}$	[1]
Resting Ca <sup>2+</sup> concentration	$[Ca^{2+}]_{i0}$	0.1 $\mu\text{M}$	[1]
Total Fluo-3 concentration	$[Fluo]$	100 $\mu\text{M}$	[27]
Total free ATP concentration	$[ATP]$	260 $\mu\text{M}$	[27]
Total troponin concentration	$[TN]$	70 $\mu\text{M}$	[27]
Total calmodulin concentration	$[Cal]$	24 $\mu\text{M}$	[27]
<b>Diffusion coefficients (at 22°C)</b>			
Diffusion coefficient for Ca <sup>2+</sup>	$D_{Ca}$	0.39 $\mu\text{m}^2 \text{ms}^{-1}$	[27]
Diffusion coefficient for CaFluo	$D_{CaFluo}$	0.1 $\mu\text{m}^2 \text{ms}^{-1}$	[27]
Diffusion coefficient for CaATP	$D_{CaATP}$	0.168 $\mu\text{m}^2 \text{ms}^{-1}$	[27]
Diffusion coefficient for CaCal	$D_{CaCal}$	0.025 $\mu\text{m}^2 \text{ms}^{-1}$	[27]
<b>Rate coefficients (at 22°C)</b>			

Ca <sup>2+</sup> on-rate constant for TN	$k_+^{CaTN}$	0.04 $\mu\text{M}^{-1} \text{ms}^{-1}$	[27]
Ca <sup>2+</sup> off-rate constant for TN	$k_-^{CaTN}$	0.04 $\text{ms}^{-1}$	[27]
Ca <sup>2+</sup> on-rate constant for CaATP	$k_+^{CaATP}$	0.225 $\mu\text{M}^{-1} \text{ms}^{-1}$	[27]
Ca <sup>2+</sup> off-rate constant for CaATP	$k_-^{CaATP}$	45 $\text{ms}^{-1}$	[27]
Ca <sup>2+</sup> on-rate constant for CaFluo	$k_+^{CaFluo}$	0.23 $\mu\text{M}^{-1} \text{ms}^{-1}$	[27]
Ca <sup>2+</sup> off-rate constant for CaFluo	$k_-^{CaFluo}$	0.17 $\text{ms}^{-1}$	[27]
Ca <sup>2+</sup> on-rate constant for Cal	$k_+^{CaCal}$	0.125 $\mu\text{M}^{-1} \text{ms}^{-1}$	[27]
Ca <sup>2+</sup> off-rate constant for Cal	$k_-^{CaCal}$	0.2975 $\text{ms}^{-1}$	[27]

**Table 4.** Membrane Ca<sup>2+</sup> fluxes parameters

Definition	Symbol	Value	Reference No.
<b>Na<sup>+</sup>/Ca<sup>2+</sup> exchange current</b>			
Extracellular Na <sup>+</sup> concentration	$[Na^+]_e$	140 mM	[1]
Resting Na <sup>+</sup> concentration	$[Na^+]_i$	10 mM	[1]
Pump rate of NCX	$g_{NCX}$	38.5 $\mu\text{M} \text{ms}^{-1}$	[29]
Voltage dependence of NCX control	$\eta$	0.35	[29]
Na <sup>+</sup> half saturation of NCX	$k_{m,Na}$	87.5 mM	[29]
Ca <sup>2+</sup> half saturation of NCX	$k_{m,Ca}$	1380 $\mu\text{M}$	[29]
Low potential saturation factor of NCX	$k_{sat}$	0.1	[29]
<b>membrane Ca<sup>2+</sup> ATPase</b>			
Maximum Ca <sup>2+</sup> pump rate	$g_{pCa}$	0.0035 $\mu\text{M} \text{ms}^{-1}$	[29]
Half saturation of Ca <sup>2+</sup> pump	$k_{m,pCa}$	0.5 $\mu\text{M}$	[29]
<b>membrane Ca<sup>2+</sup> leak</b>			
Conductance of sarcolemmal Ca <sup>2+</sup> leak	$g_{Cab}$	1.65e-5 $\mu\text{M} \text{mV}^{-1} \text{ms}^{-1}$	Estimated