Biophysical Journal, Volume 100

Supporting Material

Synchronization of stochastic Ca2+ release units creates a rhythmic Ca2+ clock in cardiac pacemaker cells

Anna V Maltsev, Victor A Maltsev, Maxim Mikheev, Larissa A Maltseva, Syevda G Sirenko, Edward G Lakatta, and Michael Stern

Online Supplement

For paper by Anna V. Maltsev et al.

Numerical model of local Ca^{2+} dynamics in SANC: Description and formulations

We explored Ca^{2+} dynamics in submembrane space in rabbit SANC, in which all surface membrane currents are set to zero, thus approximating experimental conditions in depolarized, electrically inactive SANC. For continuity and simplicity of Ca dynamics, we model SANC as a torus with length 100 μm (cell length) and radius 4 μm. The model has three adjacent compartments: submembrane space, cytosol, and SR. Ca^{2+} is released from SR by Ca^{2+} release units (CRUs) and pumped into SR form cytosol by SERCA. The model uses a 2D array of diffusively coupled Ca^{2+} release units (CRUs) that stochastically release Ca^{2+} after a fixed refractory time, with a fixed rate of I_{spark}, for a fixed duration of time of 10 ms. Ca^{2+} is buffered by calmodulin in the submembrane space. Ca^{2+} diffusion occurs within the submembrane space, from the submembrane space to the cytosol, and within SR (main text Figure 3B). Thus, Ca cycling occur in the direction from SR to subspace via local Ca releases of CRUs, then Ca diffuses within subspace and simultaneously to cytosol, then Ca is pumped from cytosol to SR, Ca diffuses within SR and is released locally by CRUs, and so on (main text Fig.3B). **The probability of Ca release from a given CRU is sensitive to local Ca.** The CRUs are diffusively coupled by the following mechanism. When a neighboring CRU releases Ca, the neighboring local Ca levels increase via diffusion, thus affecting the probability of Ca release by adjacent CRUs. Different CRUs are coupled only via Ca diffusion and the dependence of the probability of CRU firing function on local Ca^{2+}. This is in accordance with the well known process of CICR (1).

Submembrane space, cytosol, and SR

We model the submembrane space of SANC as a very thin torus with the cell length L_{cell} = 100 μm and radius r_{cell}=4 μm (by 25.13 μm cross section perimeter) and having a depth of 0.02 μm as suggested for submembrane depth in previously numerical studies of rabbit SANC (2, 3). Since the torus depth is negligible the local Ca^{2+} concentration dynamics Ca_{sub} are described in the model in two dimensions only, i.e. a function $Ca_{sub}(t,x,y)$ of x (position along cell length) and y (position along the perimeter of transverse cross-section area of the cell). The continuous submembrane space is modeled as a set of discrete adjacent elementary spaces/volumes with dimensions 0.33 x 0.35 μm. Each elementary space (of total 285 x 72 = 20520, see main text Fig.3C) is described by its own "local" Ca^{2+} concentration. Thus our simulated Ca^{2+} signals $Ca_{sub}(t,x,y)$ are described by a dynamic array of 285 x 72 elements. The signals are illustrated simply in a rectangle, as shown in main text Fig.3C and Suppl. Movie #4.

SR volume V_{SR} was set as suggested for rabbit SANC elsewhere (2) to 0.0116 part of the cell volume $V_{cell} = \pi\, r_{cell}^2\, L_{cell} = 5026.5482\ \mu m^3$. In turn, the cytosol occupies almost the entire cell volume (except relatively small compartments of SR and submembrane space). Continuous cytosol and SR were modeled (sampled) similar to the submembrane space, so that each elementary submembrane space compartment has its adjacent cytosol compartment and elementary adjacent SR compartment. The respective elementary volumes of each compartment v_{sub} , v_{cyt} and v_{SR} were estimated as follows: v_{sub} = 0.33 x 0.35 x 0.02 = 0.00231 μm^3, $v_{cyt} = V_{cell}$ /

20520 = 0.24495849 μm^3, and v_{SR} = 0.0116 ·V_{cell}/20520 =0.0028415185 μm^3. The respective local cytosol and SR Ca^{2+} concentrations, $Ca_{cyt} = Ca_{cyt}(t,x,y)$ and $Ca_{SR} = Ca_{SR}(t,x,y)$, were also modeled as dynamic arrays with 285 x 72 elements, similar to [Ca^{2+}] in submembrane space. The initial Ca^{2+} concentrations were set to 200 nM in the submembrane space, 150 nM in cytosol, and 0.9 mM in SR.

Approximation of CRUs

The CRUs are set in a square grid within the submembrane space (75 * 18 = 1350 CRUs, in *x* and *y* directions, respectively). The CRU-to-CRU distances are 1.33 μm along *x* axis and 1.4 μm along *y* axis. CRUs in our model are stochastic, i.e. they sometimes open and release Ca^{2+}. The probability *p* of a given CRU at the location *(x,y)* to release Ca^{2+} is 0, if it opened recently or a power function of the local [Ca^{2+}] in submembrane space $Ca_{sub}(t,x,y)$:

$$p = ProbConst \cdot (Ca_{sub}/Ca_{thresh})^{ProbPower} \cdot TimeTick \quad (1a)$$

where $ProbConst = 5 \cdot 10^{-4}$ ms/mM, $Ca_{thresh} = 150 \cdot 10^{-6}$ mM (150 nM), $ProbPower = 2.5$.
The time when the probability of CRU opening is 0 is called the refractory period. We fix the refractory period to be a constant 300 ms in our model. The non-linear dependence of opening probability on Ca^{2+} makes CRU interactions possible. When a CRU releases Ca^{2+}, the probability of opening increases for nearby CRUs. In this study we model the time course of Ca^{2+} release to be rectangular, i.e. when a CRU opens it generates a constant current I_{spark} for some length of time, which is constant throughout the model. We call it spark duration and we set it equal to 10 ms. We fix all the model parameters, and explore CRU interactions and related Ca^{2+} dynamics by simply varying I_{spark} from 0.5 pA to 2 pA. Thus, at any moment each CRU can be in one of three functional states: firing state, refractory state, and ready to fire state (see main text Fig.3C inset). The local Ca release flux $j_{rel}(t, x, y)$ in the submembrane space as a function of time and space can be summarized as follows:

(1b)

j_{rel} = 0 for any submembrane location *(x,y)* lacking CRU at any time *t* and
for any submembrane location *(x,y)* with a CRU when the CRU is not firing
$j_{rel} = I_{spark} / (2F v_{sub})$ for any submembrane location *(x,y)* with a CRU when the CRU is firing

F is the Faraday constant and v_{sub} is elementary subspace volume. Initial states for CRUs for simulations shown in man text Fig.4-6 were as follows: no CRU in firing state, but "ready to fire" state and refractory state were equally and randomly distributed among all CRUs. Ca^{2+} cycling quickly reaches a steady state in the model in about 100,000 model time ticks, i.e. in 5 seconds. All LCR parameters in the main text Figs 4-6 and Supplemental movie 4 were evaluated at the steady state. To insure history independence, we also tested other initial conditions with all CRU in the refractory states or all CRUs in the ready to fire state. In either case the system comes to the same steady state in about 100,000 ticks. The fact that the system operates at steady state (i.e. without a drift) was also insured by comparison of average Ca levels in different 1.7 second periods in each Ca compartment, i.e. subspace, cytosol, and SR. In contrast, in simulations of the system transitions (main text Fig.8), all CRUs were initially set into the beginning of the refractory state, and the local Ca^{2+} dynamics were recorded and analyzed immediately after the simulation onset.

Local SR Ca^{2+} uptake (pumping)

The local instant flux rate $j_{SRCaP}(t,x,y)$ of Ca^{2+} pumping by the local SR fragment with coordinates (x,y) from the adjacent elementary cytosol volume was adopted from Shannon et al. (4) as a function of respective local concentrations of Ca in cytosol, $Ca_{cyt}(t,x,y)$ and in SR, $Ca_{SR}(t,x,y)$:

$$j_{SRCaP} = P_{up} \frac{V_{\max} \cdot \left(\frac{Ca_{cyt}}{K_{mf}}\right)^H - V_{\max} \cdot \left(\frac{Ca_{SR}}{K_{mr}}\right)^H}{1 + \left(\frac{[Ca]_{cyt}}{K_{mf}}\right)^H + \left(\frac{[Ca]_{SR}}{K_{mr}}\right)^H} \quad (2)$$

where P_{up} = 0.012 mM/ms, K_{mf} = 0.000246 mM, K_{mr} = 1.7 mM, and H = 1.787.

Approximation of Ca^{2+} diffusion in submembrane space using Green's functions

Ca^{2+} diffusion in the submembrane space is a fast process which requires special care. Conventionally used algorithms for modeling diffusion move some fraction of the Ca^{2+} in every space element into the immediately neighboring space elements at every time tick. This is too slow for our model. In one time tick of our model (0.05 ms) Ca^{2+} diffuses farther than the neighboring elementary spatial compartments. To find the spatial distribution of the Ca one tick later, we take the convolution of the current Ca^{2+} spatial distribution with the appropriate Green's function. The Green's function is the solution of the diffusion equation with delta function as the initial condition, which in this case is given explicitly by

$$G(x,y) = \frac{\exp[-(x^2+y^2)/4 \cdot TimeTick \cdot DiffCoeff]}{4 \cdot \pi \cdot TimeTick \cdot DiffCoeff} \quad (3)$$

The diffusion coefficient in the submembrane space (*DiffCoeff*) was set to 0.6 μm^2/ms. While Equation 3 includes theoretical normalization coefficients $1/(4 \cdot TimeTick \cdot DiffCoeff)$, they are valid only for continuous integration with convolution radius $\rightarrow \infty$, i.e. for the entire 2D space. Since our model is discrete, we replace the continuous convolution with a sum and the continuous Green's function with a discrete approximation. We use a convolution radius of 3 elementary spaces (i.e. our Green's matrix size is 7 x 7, as 3*2+1=7) and neglect more distant contributions ($<10^{-5}$). In order to avoid losing Ca^{2+} in the system, we did not use the theoretical normalization coefficients but simply normalized our Green's matrix to the sum of all its elements. In other words, the sum of all its elements becomes exactly 1 and our convolution summation simply redistributes Ca^{2+} with its total amount being preserved. Since the Green's function matrix is time-independent, it is calculated only once at the beginning of each simulation.

Local Ca^{2+} diffusion to cytosol

Local Ca^{2+} diffusion flux, $j_{Ca\_dif\_cyt}(t,x,y)$ from a subspace element (x,y) into its adjacent cytosol element is described simply as previously suggested by Kurata et al. (2):

$$j_{Ca\_dif\_cyt} = (Ca_{\text{sub}} - Ca_{cyt})/\tau_{difCa} \qquad (4)$$

where $Ca_{sub}(t,x,y)$ and $Ca_{cyt}(t,x,y)$ are instant local Ca^{2+} concentrations in the submembrane space and in the cytosol, respectively, and τ_{difCa} is a diffusion time constant of 0.15 ms. This time constant, which is longer than that used by Kurata, takes into account the possible recirculation of recently entered cytosolic Ca^{2+} just below the subspace into adjacent volume elements of the subspace. The value of τ_{difCa} was determined by matching the amount and timing of arrival of released Ca^{2+} from one CRU to the next to the value it would have in a full 3D diffusion model (calculated analytically).

Local Intra-SR diffusion

For our luminal diffusion we use the native Repast algorithm, with diffusion Constant (*diffusionConstant*) of 0.8. For a given elementary volume at each time tick, we get newValue = ownValue + *diffusionConstant* *(nghAvg - ownValue) where nghAvg is the weighted average of eight neighbors of the elementary SR volume, and own Value is the current value for the current elementary SR volume. For more details please see the repast web link: http://repast.sourceforge.net/api/uchicago/src/sim/space/Diffuse2D.html#diffuse()

To evaluate the respective diffusion coefficient (described by the *diffusionConstant* of 0.8 in repast) in physical units, this repast diffusion can be compared with that approximated by Green's matrix (described above). The repast diffusion is actually similar to diffusion described by a normalized smallest Green's matrix (3 x 3, i.e. a center +eight its neighbors =9, similar to the repast algorithm) with *DiffCoeff* ~ 1 μm^2/ms, because the Green's matrix element with a maximum value (in the matrix center) is calculated to be ~0.2 (i.e. 1-0.8 as in the repast diffusion). Thus, the Ca diffusion in SR in our model is faster than that in submembrane space (*DiffCoeff*=0.6 μm^2/ms) and does not limit release as Ca always present in SR during release. Since we simulated a CRU release simply by fixing release current Ispark for a fixed time of 10 ms, described above, the quantitative concentration of Ca^{2+} in the SR does not play an important role in regulating release in the model, but only serves an "accounting" function in overall Ca^{2+} balance. The fact that intra-SR diffusion indeed does not limit Ca^{2+} SR release in rabbit cardiac cells has been shown experimentally by Shannon et al. 2003 (5).

Numerical integration

Our numerical model was implemented based on the cross-platform SPARK (Simple Platform for Agent-based Representation of Knowledge) that is free software for multi-scale agent-based modeling (ABM) developed at University of Pittsburgh (see details at the website http://www.pitt.edu/~cirm/spark/). We applied the Java Parallel Arrays library (jsr166y) to implement parallelization on a single multi-core computer. This library implements the Open Multi-Processing (OpenMP) API, which supports multi-platform, shared memory, and multiprocessing programming. Both agent actions and data layer computations were parallelized. To implement parallelized SPARK ABM's on a cluster, we divided the space into smaller parts and assigned each part to a particular machine in a cluster. Because agents occupy a particular

part of the space, we distributed the agents among machines in a spatial manner. For cluster synchronization, we used the P2P-MPI implementation of the Message Passing Interface (MPI) protocol. Our model simulations were performed by 6 Hewlett Packard xw8400 workstation computers, each of which having eight processors (two Quad-Core Intel® Xeon® 5355 processors 2.66 GHz). The model was integrated with a constant time tick of 0.05 ms. We also tested our model with a smaller time tick of 0.02. The phase transition between sparks and waves is only slightly (insignificantly) shifted towards larger I_{spark}. That is also expected because Ca^{2+} buffering in subspace and diffusion into cytosol becomes better approximated and stronger, so that CICR becomes slightly less pronounced at each particular I_{spark}. Since the dynamics of the CRU itself are modeled phenomenalogically, the slight loss of precision due to the longer time-tick is not important for purposes of this study.

Summary of differential equations for Ca^{2+} dynamics in the model

$$\frac{\partial Ca_{sub}}{\partial t} = D_{sub}\nabla^2 Ca_{sub} + j_{rel} - j_{Ca\_dif\_cyt} - [CM]_{tot} \cdot \partial f_{CMs} / \partial t \quad (6)$$

$$\frac{\partial Ca_{cyt}}{\partial t} = j_{Ca\_dif\_cyt} - j_{SRCaP} \quad (7)$$

$$\frac{\partial Ca_{SR}}{\partial t} = D_{SR}\nabla^2 Ca_{SR} - j_{rel} \cdot \frac{v_{sub}}{v_{SR}} + j_{SRCaP} \quad (8)$$

$$\partial f_{CMs} / \partial t = k_{fCM} \cdot Ca_{sub} \cdot (1 - f_{CMs}) - k_{bCM} \cdot f_{CMs} \quad (9)$$

F is the Faraday constant and v_{sub} and v_{SR} are elementary subspace and SR volumes, respecctively. The Equation 9 describes local Ca^{2+} buffering by calmodulin in submembrane space. Local fractional occupancy of calmodulin by Ca^{2+} in submembrane space, variable $f_{CMs}(t,x,y)$, was adopted from Kurata et al. model (2), where k_{fCM}=227.7 $mM^{-1} \cdot ms^{-1}$ is Ca^{2+} association constant for calmodulin; k_{bCM}=0.542 ms^{-1}: Ca^{2+} dissociation constant for calmodulin; total calmodulin concentration $[CM]_{tot}$ was set to 0.045 mM. All initial f_{CMs} values were set to 0.09 as suggested in (3). For accuracy, we used 3 additional steps within each time tick to integrate Ca^{2+} buffering by calmodulin in Equation 6. Also, for each time tick diffusion from submembrane space location (x,y) to adjacent cytosol location ($j_{Ca\_dif\_cyt}$ in Equations 6 and 7) was solved simply and explicitly as an exponential with the time constant τ_{difCa} (see Equation 4).

References:

1. Fabiato, A., and F. Fabiato. 1978. Calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cells from adult human, dog, cat, rabbit, rat, and frog hearts and from fetal and new-born rat ventricles. Ann N Y Acad Sci 307:491-522.
2. Kurata, Y., I. Hisatome, S. Imanishi, and T. Shibamoto. 2002. Dynamical description of sinoatrial node pacemaking: improved mathematical model for primary pacemaker cell. Am J Physiol 283:H2074-2101.
3. Maltsev, V. A., and E. G. Lakatta. 2009. Synergism of coupled subsarcolemmal Ca^{2+} clocks and sarcolemmal voltage clocks confers robust and flexible pacemaker function in a novel pacemaker cell model. Am J Physiol Heart Circ Physiol 296:H594-H615.

4. Shannon, T. R., F. Wang, J. Puglisi, C. Weber, and D. M. Bers. 2004. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. Biophys J 87:3351-3371.
5. Shannon, T. R., T. Guo, and D. M. Bers. 2003. Ca^{2+} scraps: local depletions of free [Ca^{2+}] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca^{2+} reserve. Circ Res 93:40-45.