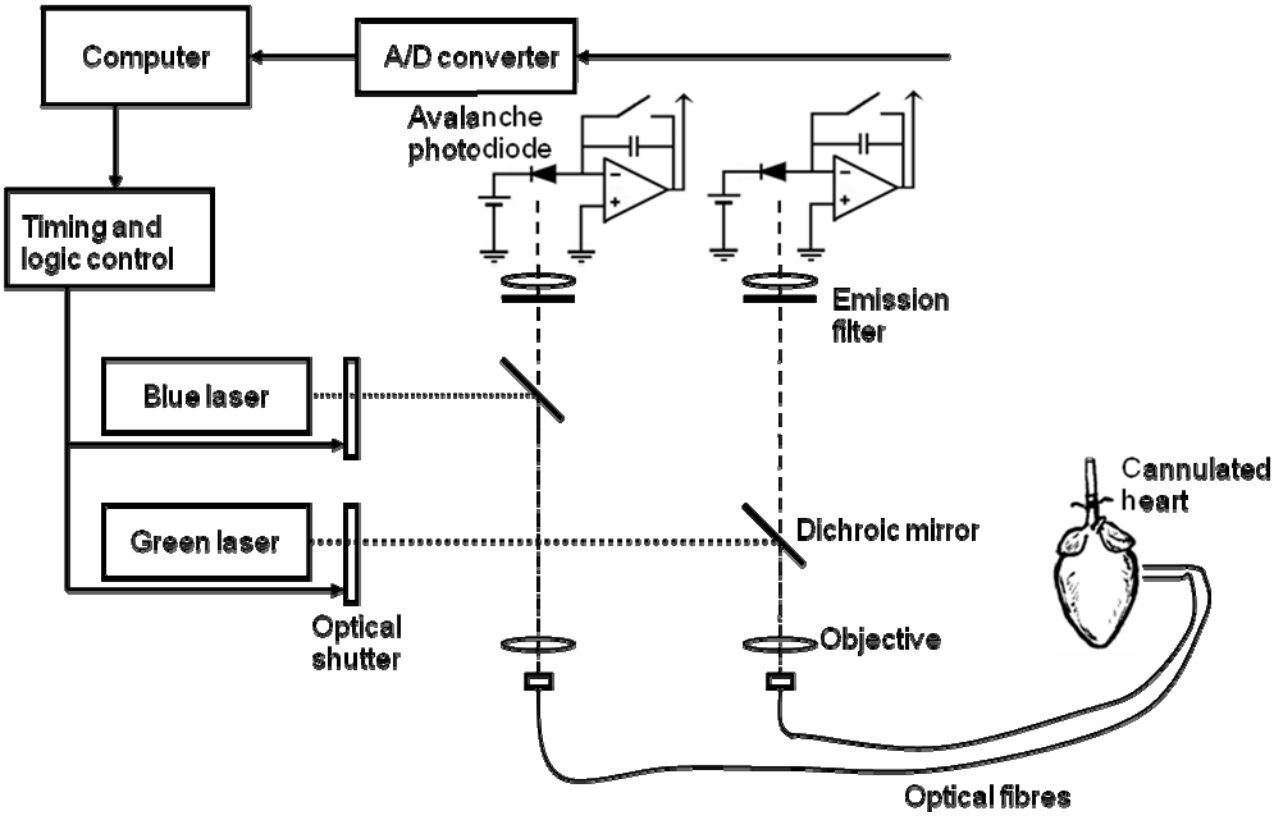
Kornyeyev D, Reyes M, Escobar AL

Luminal Ca^{2+} content regulates intracellular Ca^{2+} release in subepicardial myocytes of intact beating mouse hearts: Effect of exogenous buffers

DATA SUPPLEMENT

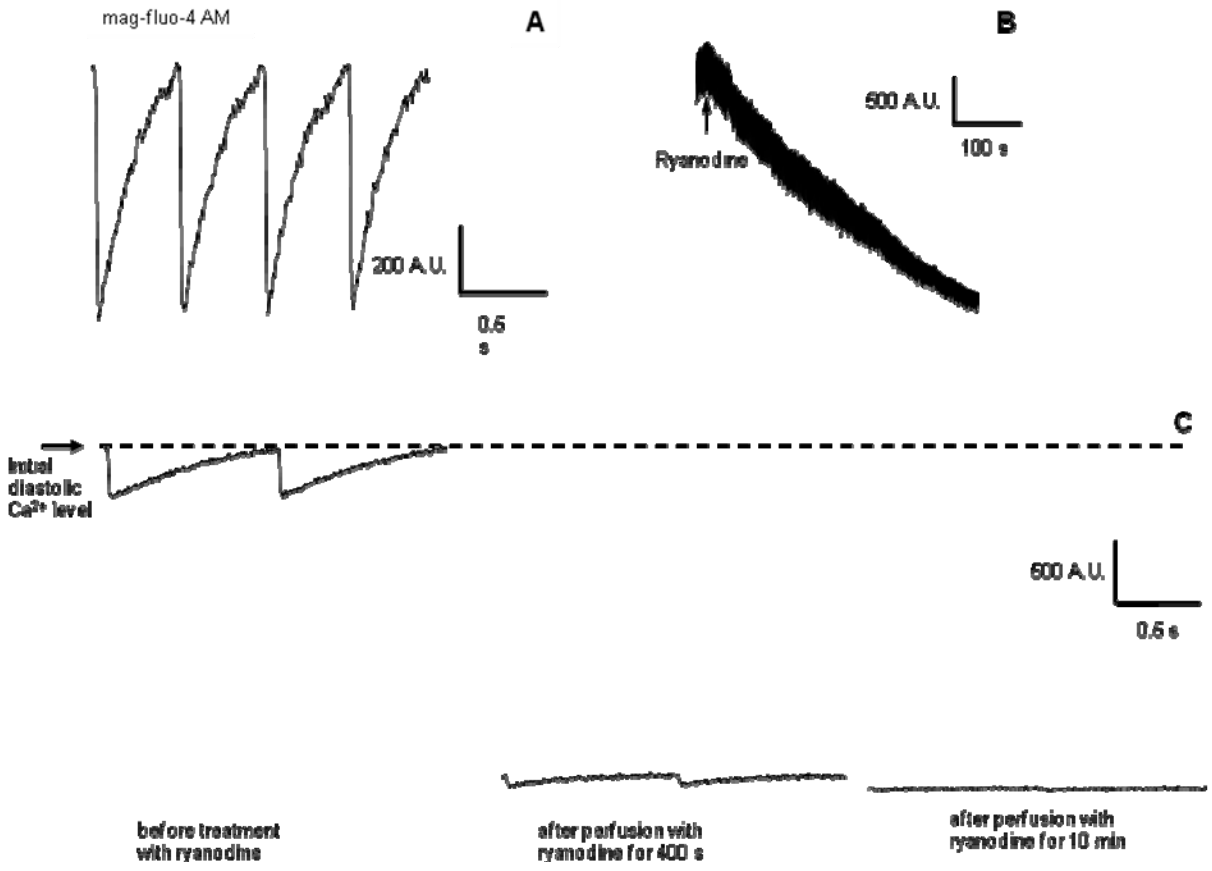
METHODS



Supplementary figure 1. Scheme of the experimental setup for detecting whole heart fluorescent signals. A solid state blue laser (473 nm) was used to excite mag-fluo-4 and a solid state green laser (532 nm) was used to excite rhod-2, X-rhod-5F, and Di-8-ANEPPS. Both lasers were time-multiplexed by two ferroelectric modulators (optical shutters). The fluorescence

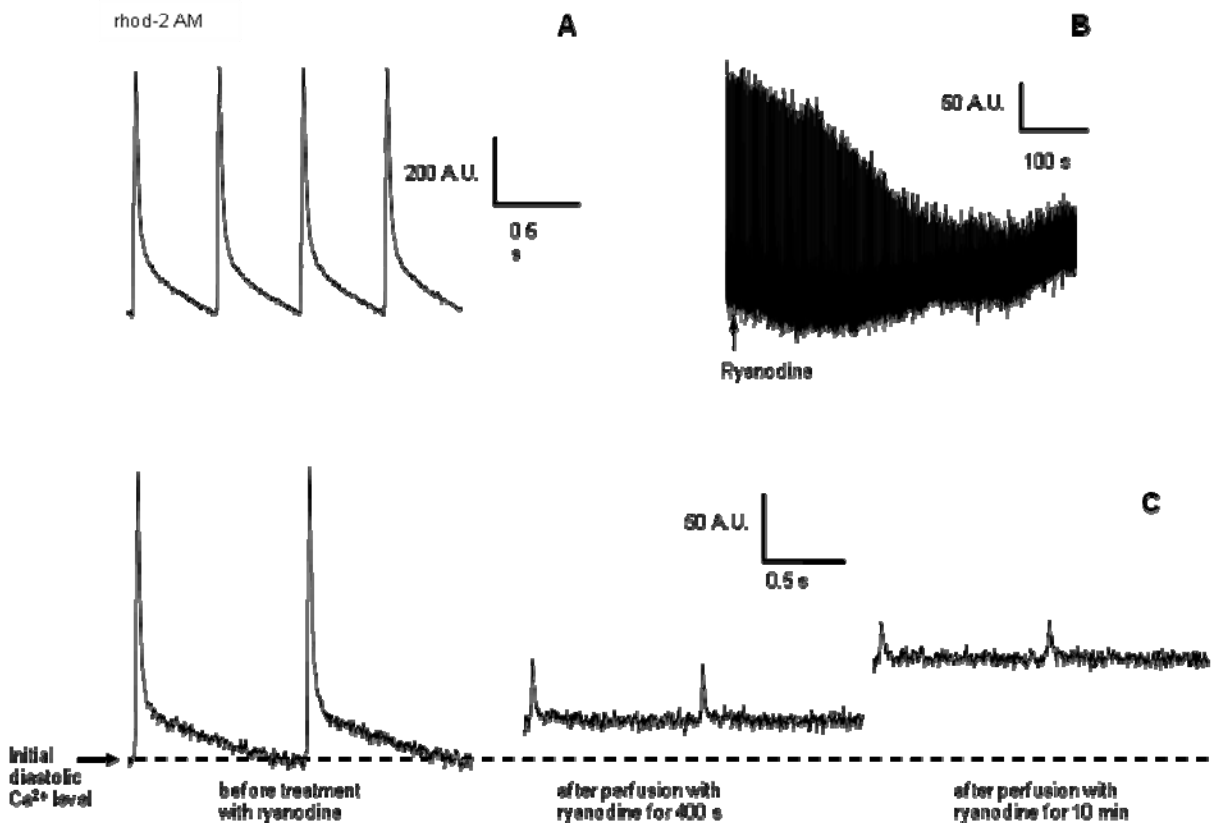
was detected with avalanche photodiodes that were connected to an integrating current-to-voltage converter controlled by a Digital Signal Processor (TI DSP 320, Texas Instruments, TX, USA).

RESULTS



Supplementary figure 2. Changes in fluorescence from the SR lumen that were measured with the fluorescent dye mag-fluo-4 AM. Mouse hearts were loaded with the dye in the presence of 211 μM EGTA AM. (A) A representative trace of luminal Ca^{2+} transients obtained at 21^{o}C at a pacing frequency of 2 Hz. (B) Time course of the fluorescence during perfusion of a mouse

heart with Tyrode solution containing 25 μM ryanodine. The vertical arrow indicates the beginning of the application of ryanodine. (C) Luminal Ca^{2+} transients recorded before ryanodine treatment (left trace), at 400 s (middle trace), and at 10 min (right trace) during the treatment. The horizontal arrow and the stroke line identify the diastolic level of SR Ca^{2+} prior to the perfusion with ryanodine. The experiments with ryanodine (B, C) were conducted at 21°C and at a pacing frequency of 1 Hz.



Supplementary figure 3. Changes in fluorescence from the cytosol measured with the fluorescent dye rhod-2 AM. Mouse hearts were loaded with the dye in the presence of 211 μM

EGTA AM. (A) A typical recording of cytosolic Ca^{2+} transients obtained at 21°C and a pacing frequency of 2 Hz. (B) Time course of fluorescence signal during perfusion of a mouse heart with Tyrode solution containing 25 μM ryanodine. Ryanodine application is indicated by a vertical arrow. (C) Cytosolic Ca^{2+} transients recorded before ryanodine treatment (left trace), at 400 s (middle trace) and at 10 min (right trace) during the treatment. The horizontal arrow and the stroke line identify diastolic level of cytosolic [Ca^{2+}] prior to the perfusion with ryanodine. The experiments with ryanodine (B, C) were conducted at 21°C and at a pacing frequency of 1 Hz.

Mathematical model

Ca^{2+} distribution in the myoplasm

Changes in Ca^{2+} concentration in the myoplasm were evaluated by dividing the cytosolic space into four regions: a diadic compartment where the release channels are present (Ryanodine Receptors (RyR) and L type Channels(L)); a region composed of *l* intermediate compartments where diffusion, binding to diffusional buffers and a Ca^{2+} pump were present; *m* central compartments where fixed buffers and a transport mechanism to the sarcoplasmic reticulum were also present; and *n* final compartments where just binding to diffusional buffers and diffusion were the sole mechanism for Ca^{2+} and cytosolic buffers translocation.

a) [Ca^{2+}] changes at the dyadic space (compartment 0):

The absolute changes in Ca^{2+} concentration in this compartment were given by the difference between influx and efflux of Ca^{2+} as follows:

$$\frac{\partial [\mathrm{Ca}^{2+}](t,0)}{\partial t} = J_{in}^{Ca}(t,0) - J_{out}^{Ca}(t,0)$$

where $J_{in}^{Ca}(t,0)$ is the net Ca^{2+} influx and $J_{out}^{Ca}(t,0)$ is the net Ca^{2+} efflux from compartment 0 at all times. The net Ca^{2+} influx was given by:

$$J_{in}^{Ca}(t,0) = J_{in}^{Ca}(t,0)_L + J_{in}^{Ca}(t,0)_{RyR} + J_{in}^{Ca}(t,0)_B + J_{in}^{Ca}(t,0)_D + J_{in}^{Ca}(t,0)_{Diff}$$

where $J_{in}^{Ca}(t,0)_{RyR}$ represents Ca^{2+} influx from sarcoplasmic reticulum (SR) through Ryanodine receptor channels, $J_{in}^{Ca}(t,0)_L$ represents Ca^{2+} influx from extracellular space through L type channels, $J_{in}^{Ca}(t,0)_B$ is the Ca^{2+} released from mobile intracellular buffers located in compartment 0, $J_{in}^{Ca}(t,0)_D$ is the Ca^{2+} unbound from the Ca^{2+} indicator at compartment 0 and $J_{in}^{Ca}(t,0)_{Diff}$ is the Ca^{2+} influx from the cytosolic compartment 1 into compartment 0. These components were defined as follows:

$$J_{in}^{Ca}(t,0)_{RyR} = P_{RyR}(t) \bullet ([Ca^{2+}]_{SR}(t,0) - [Ca^{2+}](t,0))$$
$$J_{in}^{Ca}(t,0)_B = k_{offB} \bullet CaB(t,0)$$
$$J_{in}^{Ca}(t,0)_D = k_{offD} \bullet CaD(t,0)$$
$$J_{in}^{Ca}(t,0)_{Diff} = \frac{D_{Ca}}{dx^2}[Ca^{2+}](t,1)$$
$$J_{in}^{Ca}(t,0)_L = \frac{G_L(t) \bullet (V_m(t) - Eion(t))}{2 \bullet F \bullet Vol}$$

$CaB(t,0)$ and $CaD(t,0)$ are the concentration of Ca^{2+} bound to mobile buffers and the Ca^{2+} indicator located in compartment 0, k_{offB} and k_{offD} are the dissociation rate constant of Ca^{2+} from the mobile buffers and the Ca^{2+} indicator, D_{Ca} is the Ca^{2+} diffusion coefficient, dx is the thickness of compartment 0 and $[Ca^{2+}](t,1)$ is the Ca^{2+} concentration in the first non-diadic compartment. $P_{RyR}(t)$ is the Ca^{2+} permeability of the sarcoplasmic reticulum through Ryanodine receptors, $[Ca^{2+}]_{SR}(t,0)$ is the local free Ca^{2+} concentration at the SR that is seen by the Ryanodine receptors and $[Ca^{2+}](t,0)$ represents the cytosolic Ca^{2+} in the compartment 0 at all times. $G_L(t)$ is the macroscopic Ca^{2+} conductance through L type channels, $Eion(t)$ is the

equilibrium potential for Ca, $Vm(t)$ is the potential difference across the cellular membrane, which was changed from a resting potential V_{rest} to an excitation potential V_{ex} during a T_L amount of time in order to depolarized the plasma membrane. F is the Faraday constant and Vol is the volume of the compartment 0.

The RyR was modeled using a ten-state Markov Model with six closed states, one inhibited state and three open states. The model was defined by the following equations.

$$\frac{d[C1_{RyR}(t)]}{dt} = -k_{C1C2} \bullet [Ca^{2+}](t,0) \bullet C1_{RyR}(t) + l_{C2C1} \bullet C2_{RyR}(t)$$

$$\frac{d[C2_{RyR}(t)]}{dt} = k_{C1C2} \bullet [Ca^{2+}](t,0) \bullet C1_{RyR}(t) - (l_{C2C1} + k_{C2C3} \bullet [Ca^{2+}](t,0)) \bullet C2_{RyR}(t) + l_{C3C2} \bullet C3_{RyR}(t)$$

$$\frac{d[C3_{RyR}(t)]}{dt} = k_{C2C3} \bullet [Ca^{2+}](t,0) \bullet C2_{RyR}(t) - (l_{C3C2} + k_{C3C4} \bullet [Ca^{2+}](t,0)) \bullet C3_{RyR}(t) + l_{C4C3} \bullet C4_{RyR}(t)$$

$$\frac{d[C4_{RyR}(t)]}{dt} = k_{C3C4} \bullet [Ca^{2+}](t,0) \bullet C3_{RyR}(t) - (l_{C4C3} + k_{C4O1} \bullet [Ca^{2+}](t,0) + k_{C4O2}) \bullet C4_{RyR}(t) +$$
$$+ l_{O1C4} \bullet O1_{RyR}(t) + l_{O2C4} \bullet O2_{RyR}(t)$$

$$\frac{d[I1_{RyR}(t)]}{dt} = -l_{I1O1} \bullet I1_{RyR}(t) + k_{O1I1} \bullet [Ca^{2+}](t,0) \bullet O1_{RyR}(t)$$

$$\frac{d[C5_{RyR}(t)]}{dt} = -(l_{C5C6} + k_{C5O2} + k_{C5O1}) \bullet C5_{RyR}(t) + k_{C6C5} \bullet C6_{RyR}(t) + l_{O1C5} \bullet O1_{RyR}(t) + l_{O2C5} \bullet O2_{RyR}(t)$$

$$\frac{d[C6_{RyR}(t)]}{dt} = l_{C5C6} \bullet C5_{RyR}(t) - k_{C6C5} \bullet C6_{RyR}(t)$$

$$\frac{d[O1_{RyR}(t)]}{dt} = k_{C4O1} \bullet [Ca^{2+}](t,0) \bullet C4_{RyR} + k_{C5O1} \bullet C5_{RyR}(t) + l_{O1I1} \bullet I1_{RyR}(t) -$$
$$- (l_{O1C4} + l_{O1C5} + k_{O1I1} \bullet [Ca^{2+}](t,0) + k_{O1O3} \bullet A1_{Cseq}(t,0)) \bullet O1_{RyR}(t) + l_{O3O1} \bullet O3_{RyR}(t)$$

$$\frac{d[O2_{RyR}(t)]}{dt} = k_{C4O2} \bullet C4_{RyR} + k_{C5O2} \bullet C5_{RyR}(t) - (l_{O2C4} + l_{O2C5}) \bullet O2_{RyR}(t)$$

$$\frac{d[O3_{RyR}(t)]}{dt} = k_{O1O3} \bullet A1_{Cseq}(t,0) \bullet O1_{RyR}(t) - l_{O3O1} \bullet O3_{RyR}(t)$$

Where $O1_{RyR}$, $O2_{RyR}$ and $O3_{RyR}$ represent the three RyR's open states, $C1_{RyR}$, $C2_{RyR}$, …. , $C7_{RyR}$ represent the six RyR's closed states, $I1_{RyR}$ represents the inhibited state and where k and l are the association and dissociation constants between different states. $A1_{Qseq}(t,0)$ is the

interaction between the local Calsequestrin and the RyR and will be described later. The $P_{RyR}(t)$ was

$$P_{RyR}(t) = Pe_{RyR} \bullet (O1_{RyR}(t) + O2_{RyR}(t) + O3_{RyR}(t))$$

where Pe_{RyR} was the permeability of the RyR.

The L type Channel was modeled using a Markov Model with three closed states, two inactive states and one open state. The L type channel was defined by the following equations.

$$\begin{aligned}
&S(t) = \alpha_0 - (Vm(t) + 60)/K_\alpha \\
&\alpha(t) = S(t)/(\exp(S(t)) - 1) \\
&\beta(t) = \beta_0 \bullet \exp(-(Vm(t) + 60)/K_\beta) \\
&\frac{d[C1_L(t)]}{dt} = -3 \bullet \alpha(t) \bullet C1_L(t) + \beta(t) \bullet C2_L(t) \\
&\frac{d[C2_L(t)]}{dt} = 3 \bullet \alpha(t) \bullet C1_L(t) - (2 \bullet \alpha(t) + \beta(t)) \bullet C2_L(t) + 2 \bullet \beta(t) \bullet C3_L(t) \\
&\frac{d[C3_L(t)]}{dt} = 2 \bullet \alpha(t) \bullet C2_L(t) - (2 \bullet \beta(t) + \alpha(t)) \bullet C3_L(t) + 3 \bullet \beta(t) \bullet O1_L(t) \\
&\frac{d[O1_L(t)]}{dt} = \alpha(t) \bullet C3_L - (3 \bullet \beta(t) + \delta + k \bullet [Ca^{2+}](t,0)) \bullet O1_L(t) + \gamma \bullet Iv_L(t) + l \bullet ICa_L(t) \\
&\frac{d[Iv_L(t)]}{dt} = \delta \bullet O1_L(t) - \gamma \bullet Iv_L(t) \\
&\frac{d[ICa_L(t)]}{dt} = k \bullet [Ca^{2+}](t,0) \bullet O1_L(t) - l \bullet ICa_L(t)
\end{aligned}$$

where $C1_L$, $C2_L$ and $C3_L$ represent the three L type channel closed states, $O1_L$ represents the open state, Iv_L represents the voltage inactivated state , ICa_L represents the Ca^{2+} inactivated state and where α_0, β_0, δ, γ, k, and l are the association and dissociation constants between different states; and K_α and K_β are the voltage sensitivities of α and β. The $P_L(t)$ is

$$P_L(t) = Pe_L \bullet O1_L(t)$$

where Pe_L is the permeability of the L type channel.

The net Ca^{2+} efflux from compartment 0, $J_{out}(t,0)$ is given by three components. The unbinding from mobile buffers and the indicator, and Ca^{2+} movement by diffusion:

$$J_{out}^{Ca}(t,0) = J_{out}^{Ca}(t,0)_B + J_{out}^{Ca}(t,0)_D + J_{out}^{Ca}(t,0)_{Diff}$$

Ca^{2+} efflux from compartment 0 by Ca^{2+} binding to mobile buffers and the Ca^{2+} indicator is defined as:

$$J_{out}^{Ca}(t,0)_B = k_{onB} \bullet [Ca^{2+}](t,0) \bullet [B_{Tot}(t,0) - CaB(t,0)]$$

and

$$J_{out}^{Ca}(t,0)_D = k_{onD} \bullet [Ca^{2+}](t,0) \bullet [D_{Tot}(t,0) - CaD(t,0)]$$

where $B_{Tot}(t,0)$ and $D_{Tot}(t,0)$ are the total mobile buffer concentration and the total Ca^{2+} indicator in compartment 0; k_{onB} and k_{onD} are the association rate constants for the mobile buffer and the Ca^{2+} indicator. The Ca^{2+} diffusional efflux component is given by:

$$J_{out}^{Ca}(t,0)_{Diff} = \frac{D_{Ca}}{dx^2} \bullet [Ca^{2+}](t,0)$$

On the other hand, both the mobile buffers and the Ca^{2+} indicator are able to diffuse from and to the diadic space. This factor made the fraction of the mobile buffer bound to Ca^{2+} and the indicator bound to Ca^{2+} in this compartment change not only because of kinetics of this reaction but also because of diffusion. Formally,

$$\frac{\partial [\mathrm{CaB}](t,0)}{\partial t} = J_{in}^{CaB}(t,0) - J_{out}^{CaB}(t,0)$$

and

$$\frac{\partial [\mathrm{CaD}](t,0)}{\partial t} = J_{in}^{CaD}(t,0) - J_{out}^{CaD}(t,0)$$

where

$$J_{in}^{CaB}(t,0) = J_{in}^{CaB}(t,0)_B + J_{in}^{CaB}(t,0)_{Diff}$$
$$J_{out}^{CaB}(t,0) = J_{out}^{CaB}(t,0)_B + J_{out}^{CaB}(t,0)_{Diff}$$

and

$$J_{in}^{CaD}(t,0) = J_{in}^{CaD}(t,0)_D + J_{in}^{CaD}(t,0)_{Diff}$$
$$J_{out}^{CaD}(t,0) = J_{out}^{CaD}(t,0)_D + J_{out}^{CaD}(t,0)_{Diff}$$

Specifically,

$$J_{in}^{CaB}(t,0)_B = k_{onB} \bullet \left[B_{Tot}(t,0) - CaB(t,0)\right] = J_{out}^{Ca}(t,0)_B$$
$$J_{in}^{CaD}(t,0)_D = k_{onD} \bullet \left[D_{Tot}(t,0) - CaD(t,0)\right] = J_{out}^{Ca}(t,0)_D$$
$$J_{in}^{CaB}(t,0)_{Diff} = \frac{D_{CaB}}{dx^2}[CaB](t,1)$$
$$J_{in}^{CaD}(t,0)_{Diff} = \frac{D_{CaD}}{dx^2}[CaD](t,1)$$

and

$$J_{out}^{CaB}(t,0)_B = k_{offB} \bullet CaB(t,0) = J_{in}^{Ca}(t,0)_B$$
$$J_{out}^{CaD}(t,0)_D = k_{offD} \bullet CaD(t,0) = J_{in}^{Ca}(t,0)_D$$
$$J_{out}^{CaB}(t,0)_{Diff} = \frac{D_{CaB}}{dx^2}[CaB](t,0)$$
$$J_{out}^{CaD}(t,0)_{Diff} = \frac{D_{CaD}}{dx^2}[CaD](t,0)$$

In this case, D_{CaB} and D_{CaD} are the diffusion coefficients for the mobile buffers and the indicator bound to Ca^{2+}. Assuming that the free and bound form of the mobile buffers and the indicator can diffuse with the same speed, the total concentration of the mobile buffer and the indicator will be constant as function of time in these compartments.

$$B_{Tot}(t,0) = B_{free}(t,0) - CaB(t,0) = B_{Tot-initial}(0) = \text{constant}$$
$$D_{Tot}(t,0) = D_{free}(t,0) - CaD(t,0) = D_{Tot-initial}(0) = \text{constant}$$

b) [Ca $^{2+}$] changes in the intermediate compartments (*1 to I+1*):

The change of Ca^{2+} concentration in any of these compartments was given by:

$$\left.\frac{\partial[Ca^{2+}](t,j)}{\partial t}\right|_{j=1}^{j=l+1} = J_{in}^{Ca}(t,j) - J_{out}^{Ca}(t,j)$$

Ca^{2+} influx to these compartments was given by:

$$J_{in}^{Ca}(t,j) = J_{in}^{Ca}(t,j)_B + J_{in}^{Ca}(t,j)_D + J_{in}^{Ca}(t,j)_{Diff}$$

Particularly,

$$J_{in}^{Ca}(t,j)_B = k_{offB} \bullet CaB(t,j)$$
$$J_{in}^{Ca}(t,j)_D = k_{offD} \bullet CaD(t,j)$$
$$J_{in}^{Ca}(t,j)_{Diff} = \frac{D_{Ca}}{dx^2}[Ca^{2+}](t,j-1) + \frac{D_{Ca}}{dx^2}[Ca^{2+}](t,j+1)$$

Where $[Ca^{2+}](t,j-1)$ and $[Ca^{2+}](t,j+1)$ were the Ca^{2+} concentrations in the compartments contiguous to compartment *j.* Efflux from each intermediate compartment was given by Ca^{2+} binding, Ca^{2+} diffusion and Ca^{2+} extrusion to the extracellular compartment by a plasma membrane extrusion system (PMES):

$$J_{out}^{Ca}(t,j) = J_{out}^{Ca}(t,j)_B + J_{out}^{Ca}(t,j)_D + J_{out}^{Ca}(t,j)_{Diff} + J_{out}^{Ca}(t,j)_{PMES}$$

Specifically,

$$J_{out}^{Ca}(t,j)_B = k_{onB} \bullet [Ca^{2+}](t,j) \bullet [B_{Tot}(j) - CaB(t,j)]$$
$$J_{out}^{Ca}(t,j)_D = k_{onD} \bullet [Ca^{2+}](t,j) \bullet [D_{Tot}(j) - CaD(t,j)]$$
$$J_{out}^{Ca}(t,j)_{Diff} = 2 \bullet \frac{D_{Ca}}{dx^2} \bullet [Ca^{2+}](t,j)$$
$$J_{out}^{Ca}(t,j)_{PMES} = P_{PMES}(t,j) \bullet [Ca^{2+}](t,j)$$

$P_{CPump}(t,j)$ was the rate at which the pump extrudes Ca^{2+} to the extracellular space. The PMES (Na/Ca Exchanger) was modeled using a Markov Model with two closed states and one open state. The PMES was defined by the following equations.

$$\frac{d[C1_{PMES}(t)]}{dt} = -2 \bullet k_{PMES} \bullet [Ca^{2+}](t,j) \bullet C1_{PMES}(t) + l_{PMES} \bullet C2_{PMES}(t)$$

$$\frac{d[C2_{PMES}(t)]}{dt} = 2 \bullet k_{PMES} \bullet [Ca^{2+}](t,j) \bullet C1_{PMES}(t) - (l_{PMES} + k_{PMES} \bullet [Ca^{2+}](t,j)) \bullet C2_{PMES}(t) +$$
$$+ 2 \bullet l_{PMES} \bullet O1_{PMES}(t)$$

$$\frac{d[O1_{PMES}(t)]}{dt} = k_{PMES} \bullet [Ca^{2+}](t,j) \bullet C2_{PMES}(t) - 2 \bullet l_{PMES} \bullet O1_{PMES}(t)$$

Where $C1_{PMES}$ and $C2_{PMES}$ represent the two closed states of the PMES, $O1_{PMES}$ represents the open state, and where k_{PMES} and l_{PMES} were the association and dissociation constants between the different states. The $P_{PMES}(t)$ was

$$P_{PMES}(t) = R_{PMES} \bullet N_{PMES} \bullet O1_{PMES}(t)$$

where R_{PMES} represents the maximum rate of the PMES and N_{PMES} is the number of PMES in each compartment.

Analogous to what was shown for the first compartment, the indicator and the mobile buffers can react and diffuse.

$$\frac{\partial[\mathrm{CaB}](t,j)}{\partial t} = \left\{k_{onB} \bullet \left[B_{Tot}(t,j) - CaB(t,j)\right] - k_{offB} \bullet CaB(t,j)\right\} +$$
$$\frac{D_{CaB}}{dx^2}\left\{CaB(t,j-1) - 2 \bullet CaB(t,j) + CaB(t,j+1)\right\}$$

and

$$\frac{\partial[\mathrm{CaD}](t,0)}{\partial t} = \left\{k_{onD} \bullet \left[D_{Tot}(t,j) - CaD(t,j)\right] - k_{offD} \bullet CaD(t,j)\right\} +$$
$$\frac{D_{CaD}}{dx^2}\left\{CaD(t,j-1) - 2 \bullet CaD(t,j) + CaD(t,j+1)\right\}$$

Showing a conservation condition

$$B_{Tot}(t,j) = B_{Tot-initial}(j) = \text{constant}$$
$$D_{Tot}(t,j) = D_{Tot-initial}(j) = \text{constant}$$

c) [Ca^{2+}] changes in the central compartments (compartment *l+1* to *l+n+1*):

Once more, the change in Ca^{2+} concentration in the compartments from *l+1* to *n* was computed as the difference between Ca^{2+} influx and Ca^{2+} efflux as follows:

$$\left.\frac{\partial [Ca^{2+}](t,j)}{\partial t}\right|_{l+1}^{l+n+1} = J_{in}^{Ca}(t,j) - J_{out}^{Ca}(t,j)$$

Ca^{2+} influx into these compartments comprises four terms:

$$J_{in}^{Ca}(t,j) = J_{in}^{Ca}(t,j)_B + J_{in}^{Ca}(t,j)_D + J_{in}^{Ca}(t,j)_T + J_{in}^{Ca}(t,j)_{Diff}$$

The expressions for calculating $J_{in}^{Ca}(t,j)_B$, $J_{in}^{Ca}(t,j)_D$ and $J_{in}^{Ca}(t,j)_{Diff}$ were identical to the ones described in section b. Additionally, $J_{in}^{Ca}(t,j)_T$ is the Ca^{2+} released from the Ca^{2+} binding protein Troponin, represented as a immobile buffer located in the central compartments.

$$J_{in}^{Ca}(t,j)_T = k_{offT} \bullet CaT(t,j)$$

On the other hand, Ca^{2+} efflux from the central compartments involved five components due to Ca^{2+} extrusion by the sarcoplasmic reticulum Ca^{2+} pump, buffer binding, and Ca^{2+} diffusion to neighboring compartments:

$$J_{out}^{Ca}(t,j) = J_{out}^{Ca}(t,j)_B + J_{out}^{Ca}(t,j)_D + J_{out}^{Ca}(t,j)_T + J_{out}^{Ca}(t,j)_{Diff} + J_{out}^{Ca}(t,j)_{SERCA}$$

In particular,

$$J_{out}^{Ca}(t,j)_{SERCA} = P_{SERCA}(t,j) \bullet [Ca^{2+}](t,j) \bullet \frac{1}{1 + \left(\frac{[Ca^{2+}]_{SR}(t,j)}{kd_{SR}}\right)^2}$$

In this expression, $J_{out}^{Ca}(t,j)_{SERCA}$ is the magnitude of the Ca^{2+} efflux produced by the sarcoplasmic reticulum Ca^{2+} pump. The flux is proportional to the concentration of myoplasmic Ca^{2+} and is inactivated by the luminal Ca^{2+} concentration. $P_{SERCA}(t,j)$ is the rate at which the pump extrudes Ca^{2+} to the SR. The Pump was modeled using a Markov model with two closed states and one open state. The Pump was defined by the following equations.

$$\frac{d[C1_{SERCA}(t)]}{dt} = -2 \bullet k_{SERCA} \bullet [Ca^{2+}](t,j) \bullet C1_{SERCA}(t) + l_{SERCA} \bullet C2_{SERCA}(t)$$

$$\frac{d[C2_{SERCA}(t)]}{dt} = 2 \bullet k_{SERCA} \bullet [Ca^{2+}](t,j) \bullet C1_{SERCA}(t) - (l_{SERCA} + k_{SERCA} \bullet [Ca^{2+}](t,j)) \bullet C2_{SERCA}(t) +$$

$$+ 2 \bullet l_{SERCA} \bullet O1_{SERCA}(t)$$

$$\frac{d[O1_{SERCA}(t)]}{dt} = k_{SERCA} \bullet [Ca^{2+}](t,j) \bullet C2_{SERCA}(t) - 2 \bullet l_{SERCA} \bullet O1_{SERCA}(t)$$

where $C1_{SERCA}$ and $C2_{SERCA}$ represent the two closed states of the pump, $O1_{SERCA}$ represents the open state, and where k_{SERCA} and l_{SERCA} were the association and dissociation constants between the different states. The $P_{SERCA}(t)$ was

$$P_{CPump}(t) = R_{\mathrm{SERCA}} \bullet N_{SERCA} \bullet O1_{SERCA}(t)$$

where R_{SERCA} represents the maximum rate of the pump and N_{SERCA} is the number of pumps in each compartment.

Finally, in the central compartments Ca^{2+} can decrease by binding to Troponin.

$$J_{out}^{Ca}(t,j)_T = k_{onT} \bullet [Ca^{2+}](t,j) \bullet [T_{Tot}(j) - CaT(t,j)]$$

Similarly to what happen in the intermediate compartments the mobile buffers and the indicator were able to diffuse between the compartments in its free and bound forms.

d) [Ca^{2+}] changes in the final compartments (from *l+m+1* to *l+m+n+1*):

The Ca^{2+} dynamics, mobile buffers, and the indicator were the same as described for the intermediate compartments (b). In the last compartment a reflection condition was set in to order integrate the model and the model was assumed to be symmetric with respect to the release sites.

Ca^{2+} distribution in the Sarcoplasmic reticulum .

In this model the sarcoplasmic reticulum consists of *l+m+n+1* diffusionally connected compartments. All the compartments have the fix Ca^{2+} binding protein calsequestrin (*Q*). The general expression for calculating the free Ca^{2+} concentrations is given by:

$$\left.\frac{\partial[\mathrm{Ca}^{2+}]_{SR}(t,j)}{\partial t}\right|_{0}^{l+m+n+1} = J_{in}^{Ca}(t,j)_{SR} - J_{out}^{Ca}(t,j)_{SR}$$

$$= \left\{J_{in}^{Ca}(t,j)_{Q} - J_{out}^{Ca}(t,j)_{Q}\right\}$$

$$+ \left\{J_{in}^{Ca}(t,j)_{diff}^{SR} - J_{out}^{Ca}(t,j)_{diff}^{SR}\right\} + J_{in}^{Ca}(t,j)_{SERCA}^{SR} - J_{out}^{Ca}(t,0)_{RyR}^{SR}$$

and

$$J_{out}^{Ca}(t,0)_{RyR}^{SR} = P_{RyR}(t) \bullet \left\{[Ca^{2+}]_{SR}(t,0) - [Ca^{2+}](t,0)\right\} \bullet vol_{myo/SR}$$

$$J_{in}^{Ca}(t,j)_{diff}^{SR} - J_{out}^{Ca}(t,j)_{diff}^{SR} = \frac{D_{Ca}}{dx^2} \bullet \left\{[Ca^{2+}]_{SR}(t,j-1) - 2 \bullet [Ca^{2+}]_{SR}(t,j) + [Ca^{2+}]_{SR}(t,j+1)\right\}$$

$$J_{in}^{Ca}(t,j)_{Pump}^{SR} = P_{SERCA}(t,j) \bullet [Ca^{2+}](t,j) \bullet \frac{1}{1+\left(\frac{[Ca^{2+}]_{SR}(t,j)}{kd_{SR}}\right)^2} \bullet vol_{myo/SR}$$

In these expressions, $vol_{myo/SR}$ is the ratio between the myoplasmic volume and the sarcoplasmic reticulum volume. The sarcoplasmic reticulum Ca^{2+} efflux through ryanodine receptors, $J_{out}^{Ca}(t,0)_{RyR}^{SR}$ is only present at the first compartment and the uptake to the SR, $J_{in}^{Ca}(t,0)_{SERCA}^{SR}$ was located on the central compartments (from *l* to *l+m*).

Calsequestrin was modeled with a binding reaction scheme in which only the fully bound protein can undergo a transition to the active state. The model was a Markov model with 34 states (32 binding sites and 1 active state) and was able to interact with the RyR. The equations that represent the model are the following:

$$\frac{d[C1_{Cseq}(t,j)]}{dt} = -k \bullet [Ca^{2+}]_{SR}(t,j) \bullet C1_{Cseq}(t,j) + l \bullet C2_{Cseq}(t,j)$$

$$\frac{d[C33_{Cseq}(t,j)]}{dt} = k \bullet [Ca^{2+}]_{SR}(t,j) \bullet C32_{Cseq}(t,j) - (l + k^*) \bullet C33_{Cseq}(t,j) + l^* \bullet O1_{Cseq}(t,j)$$

$$\frac{d[O1_{Cseq}(t,j)]}{dt} = k^* \bullet C33_{Cseq}(t,j) - l^* \bullet O1_{Cseq}(t,j)$$

and for i=2 to 32

$$\frac{d[Ci_{Cseq}(t,j)]}{dt} = k \bullet [Ca^{2+}]_{SR}(t,j) \bullet C[i-1]_{Cseq}(t,j) - (l + k \bullet [Ca^{2+}]_{SR}(t,j)) \bullet Ci_{Cseq}(t,j) +$$
$$+ l \bullet C[i+1]_{Cseq}(t,j)$$

The concentration of calsequestrin and calcium bound to calsequestrin (*CaQ*) were given by:

$$CaQ(t,j) = \left[\frac{\left(\sum_{i=1}^{32} (i-1) \bullet Ci_{Cseq}(t,j) + 32 \bullet (C33_{Cseq}(t,j) + O1_{Cseq}(t,j)) \right)}{32} \right] \bullet Q_{total}$$

$$Q(t,j) = Q_{total} - CaQ(t,j)$$

where Q_{total} is the total calsequestrin concentration.

The equations were numerically integrated using a finite difference approximation (Euler method). The initial values for the state variables were calculated using the inversion of the Q-matrix procedure (Colquhoun and Hawks, 1981). The code was written in G language, LabView 8.2 (National Instruments, Austin,TX, USA).

e) Addition of exogenous EGTA:

Total exogenous EGTA was added homogenously in all the cytosolic compartments at a given time The Ca^{2+} dynamics, binding and diffusion of EGTA was calculated as follows. The net Ca^{2+} efflux from compartment 0, $J_{out}(t,0)$ was given by two components. Namely, the unbinding of EGTA and Ca^{2+} and the movement by diffusion:

$$J_{out}^{Ca}(t,0) = J_{out}^{Ca}(t,0)_B + J_{out}^{Ca}(t,0)_D + J_{out}^{Ca}(t,0)_{EGTA} + J_{out}^{Ca}(t,0)_{Diff}$$

The Ca^{2+} efflux from compartment 0 by Ca^{2+} binding to mobile buffers and the Ca^{2+} indicator was defined as:

$$J_{out}^{Ca}(t,0)_B = k_{onB} \bullet [Ca^{2+}](t,0) \bullet [B_{Tot}(t,0) - CaB(t,0)]$$
$$J_{out}^{Ca}(t,0)_D = k_{onD} \bullet [Ca^{2+}](t,0) \bullet [D_{Tot}(t,0) - CaD(t,0)]$$
and
$$J_{out}^{Ca}(t,0)_{EGTA} = k_{onEGTA} \bullet [Ca^{2+}](t,0) \bullet [EGTA_{Tot}(t,0) - CaEGTA(t,0)]$$

where $B_{Tot}(t,0)$, $D_{Tot}(t,0)$ and $EGTA_{Tot}(t,0)$ were the total mobile buffer concentrations and the total Ca^{2+} indicator in compartment 0; k_{onB}, k_{onD} and k_{onEGTA} were the association rate constants for the mobile buffer, the Ca^{2+} indicator and EGTA. The Ca^{2+} diffusional efflux component was given by:

$$J_{out}^{Ca}(t,0)_{Diff} = \frac{D_{Ca}}{dx^2} \bullet [Ca^{2+}](t,0)$$

Both the mobile buffers and the Ca^{2+} indicator were able to diffuse from and to the diadic space. A mentioned above, this factor made the fraction of mobile buffer bound to Ca^{2+}, EGTA, and the indicator bound to Ca^{2+} in this compartment change not only because of kinetics of this reaction but also because of diffusion.

$$\frac{\partial [\mathrm{CaB}](t,0)}{\partial t} = J_{in}^{CaB}(t,0) - J_{out}^{CaB}(t,0)$$

$$\frac{\partial [\mathrm{CaD}](t,0)}{\partial t} = J_{in}^{CaD}(t,0) - J_{out}^{CaD}(t,0)$$
and
$$\frac{\partial [\mathrm{CaEGTA}](t,0)}{\partial t} = J_{in}^{CaEGTA}(t,0) - J_{out}^{CaEGTA}(t,0)$$

where

$$J_{in}^{CaB}(t,0) = J_{in}^{CaB}(t,0)_B + J_{in}^{CaB}(t,0)_{Diff}$$
$$J_{\text{int}}^{CaD}(t,0) = J_{in}^{CaD}(t,0)_D + J_{in}^{CaD}(t,0)_{Diff}$$
$$J_{in}^{CaEGTA}(t,0) = J_{in}^{CaEGTA}(t,0)_{EGTA} + J_{in}^{CaEGAT}(t,0)_{Diff}$$

and

$$J_{out}^{CaB}(t,0) = J_{out}^{CaB}(t,0)_B + J_{out}^{CaB}(t,0)_{Diff}$$
$$J_{out}^{CaD}(t,0) = J_{out}^{CaD}(t,0)_D + J_{out}^{CaD}(t,0)_{Diff}$$
$$J_{out}^{CaEGTA}(t,0) = J_{out}^{CaEGTA}(t,0)_{EGTA} + J_{out}^{CaEGAT}(t,0)_{Diff}$$

Specifically,

$$J_{in}^{CaB}(t,0)_B = k_{onB} \bullet \left[B_{Tot}(t,0) - CaB(t,0)\right] = J_{out}^{Ca}(t,0)_B$$
$$J_{in}^{CaD}(t,0)_D = k_{onD} \bullet \left[D_{Tot}(t,0) - CaD(t,0)\right] = J_{out}^{Ca}(t,0)_D$$
$$J_{in}^{CaEGTA}(t,0)_D = k_{onEGTA} \bullet \left[EGTA_{Tot}(t,0) - CaEGTA(t,0)\right] = J_{out}^{Ca}(t,0)_{EGTA}$$
$$J_{in}^{CaB}(t,0)_{Diff} = \frac{D_{CaB}}{dx^2}[CaB](t,1)$$
$$J_{in}^{CaD}(t,0)_{Diff} = \frac{D_{CaD}}{dx^2}[CaD](t,1)$$
$$J_{in}^{CaEGTA}(t,0)_{Diff} = \frac{D_{CaEGTA}}{dx^2}[CaEGTA](t,1)$$

and

$$J_{out}^{CaB}(t,0)_B = k_{offB} \bullet CaB(t,0) = J_{in}^{Ca}(t,0)_B$$
$$J_{out}^{CaD}(t,0)_D = k_{offD} \bullet CaD(t,0) = J_{in}^{Ca}(t,0)_D$$
$$J_{out}^{CaEGTA}(t,0)_{EGTA} = k_{offEGTA} \bullet CaEGTA(t,0) = J_{in}^{Ca}(t,0)_{EGTA}$$

In this case, D_{CaEGTA} is the diffusion coefficient for the mobile EGTA bound to Ca^{2+}. Assuming that the free and bound form of the mobile EGTA can diffuse with the same speed, the total concentration of the mobile buffer will be constant as function of time in these compartments.

$$EGTA_{Tot}(t,0) = EGTA_{free}(t,0) - CaEGTA(t,0) = EGTA_{Tot-initial}(0) = \text{constant}$$

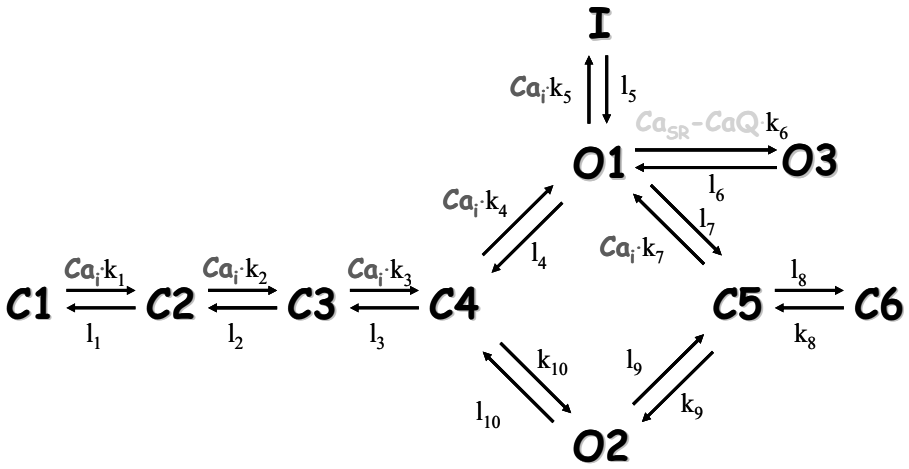
Finally, this approach for the addition of EGTA was not only used for the first compartment but also for all of the cytosolic compartments as well.

Supplemental table 1 - General Parameters

Parameter	Description	Value	Unit
$[Ca^{2+}]_i$	Resting myoplasmic [Ca^{2+}]	0.08	μM
$D_{Ca\ Cy}$	Ca^{2+} diffusion coefficient (Cytosol)	0.25	$\mu m^2/ms$
$D_{Ca\ SR}$	Ca^{2+} diffusion coefficient (SR)	0.08	$\mu m^2/ms$
D_{Tot}	Total indicator concentration	80	μM
k_{onD}	Indicator association rate constant	0.13	$\mu M^{-1}ms^{-1}$
k_{offD}	Indicator dissociation rate constant	0.069	ms^{-1}
D_{CaD}	Ca^{2+}-Indicator diffusion coefficient	0.05	$\mu m^2/ms$
B_{Tot}	Total cytosolic Buffer concentration	600	μM
k_{onB}	Cytosolic Buffer association rate constant	0.002	$\mu M^{-1}ms^{-1}$
k_{offB}	Cytosolic Buffer association rate constant	0.002	ms^{-1}
D_{CaB}	Ca^{2+}- Cytosolic Buffer diffusion coefficient	0.02	$\mu m^2/ms$
T_{Tot}	Total Troponin concentration	40	μM
k_{onT}	Troponin association rate constant	0.005	$\mu M^{-1}ms^{-1}$
k_{offT}	Troponin dissociation rate constant	0.005	ms^{-1}
$[Ca^{2+}]_{SR}$	Resting sarcoplasmic reticulum [Ca^{2+}]	1000	μM
$EGTA_{Tot}$	Total cytosolic EGTA concentration	400	μM
k_{onEGTA}	Cytosolic EGTA association rate constant	0.005	$\mu M^{-1}ms^{-1}$
$k_{offEGTA}$	Cytosolic EGTA dissociation rate constant	0.00025	ms^{-1}
D_{CaEGTA}	Ca^{2+}- Cytosolic EGTA diffusion coefficient	0.1	$\mu m^2/ms$
Q_{Tot}	Total Calsequestrine concentration	1000	μM
dx	Compartment length	0.2	μM
$vol_{myo/SR}$	Myoplasm/SR volume ratio	200	
n	Total number of compartments	10	
l	Number of compartments in intermediate region	2	
m	Number of compartments in central region	6	
n	Number of compartments in final region	2	
δt	Integration time step	0.004	ms
N_{PMES}	# of P. Membrane Extrusion Systems per compartment	10	
R_{PMES}	P. Membrane Extrusion Systems transfer rate	20	ms^{-1}
R_{SERCA}	SERCA transfer rate	12	ms^{-1}
Pe_{RyR}	Permeability of the RyR	20	ms^{-1}
Pe_L	Permeability of the L type Channel	5	ms^{-1}
$[Ca^{2+}]_{Ext}$	Extra cellular [Ca^{2+}]	1000	μM
V_{rest}	Resting membrane Potential	- 90	mV
V_E	Excitation Potential	110	mV
T_L	Excitation Time	1	Ms

Supplemental table 2 - RyR Model's Parameters Table

Parameter	Value	Unit
k_{C1C2}	0.05	$\mu M^{-1}ms^{-1}$
l_{C2C1}	0.002	ms^{-1}
k_{C2C3}	0.17	$\mu M^{-1}ms^{-1}$
l_{C3C2}	1.42	ms^{-1}
k_{C3C4}	0.17	$\mu M^{-1}ms^{-1}$
l_{C4C3}	4.44	ms^{-1}
k_{C4O1}	0.17	$\mu M^{-1}ms^{-1}$
l_{O1C4}	25	ms^{-1}
k_{O1I1}	1e-5	$\mu M^{-1}ms^{-1}$
l_{I1O1}	0.001	ms^{-1}
k_{O1O3}	0.02	$\mu M^{-1}ms^{-1}$
l_{O3O1}	0.1	ms^{-1}
k_{C5O1}	0.01	$\mu M^{-1}ms^{-1}$
l_{O1C5}	0.0025	ms^{-1}
k_{C6C5}	0.02	ms^{-1}
l_{C5C6}	0.5	ms^{-1}
k_{C5O2}	1	ms^{-1}
k_{O2C5}	2	ms^{-1}
k_{C4O2}	0.001	ms^{-1}
l_{O2C4}	0.001	ms^{-1}



Supplementary figure 4. Markovian scheme of modal RyR2 channel gating.

Supplemental table 3 - PMES (Na/Ca Exchanger) Model's Parameters Table

Parameter	Value	Unit
k	0.01	$\mu M^{-1}ms^{-1}$
I	0.01	ms^{-1}

Supplemental table 4 - SERCA Pump Model's Parameters Table

Parameter	Value	Unit
k	0.01	$\mu M^{-1}ms^{-1}$
I	0.01	ms^{-1}

Supplemetal table 5 - Calsequestrin Parameters Table

Parameter	Value	Unit
k	100	$\mu M^{-1}ms^{-1}$
I	8.5	ms^{-1}
k^*	100	ms^{-1}
I^*	1	ms^{-1}