NSCM005 - Mathematical Modelling in Biology and Medicine
Assessed Coursework 1

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Keizer-Levine 1996 Simplified Open-Cell Model

In 1996 Joel Keizer and Leslie Levine investigated the calcium dynamics of the ryanodine receptor (RyR), particularly, receptor adaption and its significance on calcium-induced calcium release-dependent calcium oscillations. To do this Keizer and Levine developed a simplified mechanism that mimics adaption and constructed open-cell and closed-cell models using parameter values which reproduce experimental data for the RyR from cardiac cells. In this report we will reproduce the simplified open-cell model and simulate the model forward in time for a variety of parameters to finally perform a dynamical analysis of model.

1 Reproducing the Model

Here we construct a simplified (not definite molecular mechanism) model of the regulation of the RyR by calcium at is cytosolic face ($\left[\mathrm{Ca}_i^{2+}\right]$) which reproduces semi-quantitatively the observations of adaption.

1.1 Adaption of the Ryanodine Receptor

The mechanism used to mimic adaption of the RyR is shown schematically in Figure 1. States C_1 and C_2 are closed states, with C_1 dominating at low $\left[\mathrm{Ca}_i^{2+}\right]$. States O_1 and O_2 are open state. We have taken these states to be the states of the entire RyR rather than states of the four sub-units for simplicity. Transitions from C_1 to O_1 and from O_1 to O_2 are assumed to be Ca^{2+} dependent and have been written as depending on the binding of n and m Ca^{2+} ions respectively. These steps correspond to calcium induced calcium release (CICR) phenomenon and without the O_1 to C_2 transition the mechanism would not give rise to adaption.

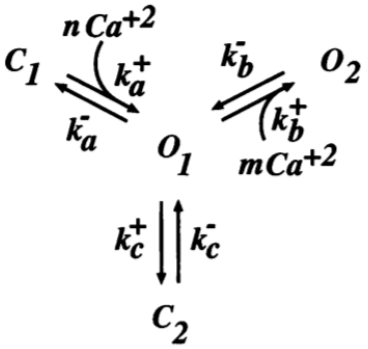


Figure 1: Schematic diagram of transitions among the four states of the RyR used to describe adaptation. States C_1, and C_2 are closed states, and O_1 and O_2 are open states, assumed to have the same single-channel conductance. The k are rate constants; only steps a and b are Ca^{2+} dependent. [KL96]

To analyze the mechanism in Figure 1 we translate the schematic diagram into kinetic equations, using the mass action law. Using the notation P_{C_1} to represent the fraction of RyR that is in state C_1, we produce the following set of differential equations:

$$\frac{dP_{C_1}}{dt} = -k_a^+ \left[\mathrm{Ca}_i^{2+}\right]_i^n P_{C_1} + k_a^- P_{O_1} \tag{1}$$

$$\frac{dP_{O_1}}{dt} = k_a^+ \left[\mathrm{Ca}_i^{2+}\right]_i^n P_{C_1} - k_a^- P_{O_1} - k_b^+ \left[\mathrm{Ca}_i^{2+}\right]_i^m P_{O_1} + k_b^- P_{O_2} - k_c^+ P_{O_1} + k_c^- P_{C_2} \tag{2}$$

$$\frac{dP_{O_1}}{dt} = k_b^+ \left[\mathrm{Ca}_i^{2+}\right]_i^m P_{O_1} - k_b^- P_{O_2} \tag{3}$$

$$\frac{dP_{C2}}{dt} = k_c^+ P_{O_1} - k_c^- P_{C_2} \tag{4}$$

Where subscripts a, b and c label the three kinetic steps in Figure 1. The $k_i^{\pm}$ are rate constant, which along with integers n and m are determined by data fitting. The fitting off these parameters relied on the data of Gyorke and Fill [GF93]. Values of the parameters which produce a semi-quantitative fit with experiments are given in Table 1.

Table 1: RyR kinetic constants (n = 4, m = 3)

Rate constant	Value
k_a^+	1500 $\mu M^{-4} s^{-1}$
k_a^-	28.8 s^{-1}
k_b^+	1500 $\mu M^{-3} s^{-1}$
k_b^-	385.9 s^{-1}
k_c^+	1.75 s^{-1}
k_c^-	0.1 s^{-1}

On a time scale longer than 10-20 ms "slow time scale" it is possible to approximate the values of P_{C_1}, P_{O_1} and P_{O_2} by taking advantage of the fact that the kinetic steps a and b in Figure 1 rapidly reach equilibrium compared with c. Hence, for any instantaneous value P_{C_2} this gives

$$P_{O_1} = \frac{w}{1 + \left(K_a / \left[\mathrm{Ca}_i^{2+}\right]\right)^4 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3} \tag{5}$$

Where $w = 1 - P_{C_2} = P_{C_1} + P_{O_1} + P_{O_2}$, $K_a^4 = k_a^- / k_a^+$, $K_b^3 = k_b^- / k_b^+$ and $K_c = k_c^- / k_c^+$ (defined for later).

Using Equation 5 combined with a comparable expression for P_{O_2} gives rise to the expression for $P_O = P_{O_1} + P_{O_2}$, the fraction of open channels on the slower time scale:

$$P_O^{\text{slow}} = \frac{w\left(1 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3\right)}{1 + \left(K_a / \left[\mathrm{Ca}_i^{2+}\right]\right)^4 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3} \tag{6}$$

Substituting Equation 5 and the definition of w into Equation 4 gives

$$\frac{dw}{dt} = -w \frac{k_c^+}{1 + \left(K_a / \left[\mathrm{Ca}_i^{2+}\right]\right)^4 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3} - k_c^-(w - 1) \tag{7}$$

With algebra, Equation 7 is rearranged into

$$\frac{dw}{dt} = \frac{-(w - w^\infty\left(\left[\mathrm{Ca}_i^{2+}\right]\right)}{\tau\left(\left[\mathrm{Ca}_i^{2+}\right]\right)} \tag{8}$$

With definitions

$$w^\infty\left(\left[\mathrm{Ca}_i^{2+}\right]\right) = \frac{1 + \left(K_a / \left[\mathrm{Ca}_i^{2+}\right]\right)^4 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3}{1 + (1/K_c) + \left(K_a / \left[\mathrm{Ca}_i^{2+}\right]\right)^4 + \left(\left[\mathrm{Ca}_i^{2+}\right] / K_b\right)^3} \tag{9}$$

$$\tau\left(\left[\mathrm{Ca}_i^{2+}\right]\right) = \frac{w^\infty\left(\left[\mathrm{Ca}_i^{2+}\right]\right)}{k_c^-} \tag{10}$$

Where w^∞ is the equilibrium value of w and τ is its relaxation time.

1.2 Closed-Cell Model

Using the above kinetic model of the RyR to describe CICR from an internal calcium store. This is combined with a passive leak and a sarco/endoplasmic reticulum Ca^{2+} ATPase- (SERCA-) type pump [LWB+92] that returns calcium to the store. The differential equations for the closed-cell model are the balance equations for $\left[\mathrm{Ca}_i^{2+}\right]$and the relaxation equation for w:

$$\begin{aligned} \frac{d\left[\mathrm{Ca}_i^{2+}\right]}{dt} &= f_i\left((\nu_1 + \nu_2)\left(\left[\mathrm{Ca}_s^{2+}\right] - \left[\mathrm{Ca}_i^{2+}\right]\right) - \nu_3 \frac{\left[\mathrm{Ca}_i^{2+}\right]^2}{\left[\mathrm{Ca}_i^{2+}\right]^2 + (K_3)^2}\right) \\ \frac{dw}{dt} &= \frac{-(w - w^\infty\left(\left[\mathrm{Ca}_i^{2+}\right]\right)}{\tau\left(\left[\mathrm{Ca}_i^{2+}\right]\right)} \end{aligned} \tag{11}$$

Where f_i is the fraction of calcium that is unbound (free) in the cytoplasm and $\left[\mathrm{Ca}_s^{2+}\right]$ is the calcium concentration in the store. ν_1 is the rate constant for RyR, ν_2 is the rate constant for store leak, ν_3 maximal rate of SERCA and k_3 is the dissociation constant for SERCA

Since the cell is assumed to be closed to calcium influx and efflux,

$$\left[\mathrm{Ca}_s^{2+}\right] = \left(C_0 - \left[\mathrm{Ca}_i^{2+}\right]\right) \frac{V_i f_s}{V_s f_i} = \frac{C_0 - \left[\mathrm{Ca}_i^{2+}\right]}{c_1} \tag{12}$$

Where V_i and V_s are the volumes of the cytoplasm and the internal store, respectively, f_s is the buffering factor for the store, and C_0 is the total amount of free calcium in the cell. The factor c_1 is often referred to as the ratio of effective volume of the store to that of the cytoplasm [Fri95]. The three terms on the right-hand side of Equation 11 are the contributions to the calcium balance in the cytoplasm that come from the RyR, the leak, and the SERCA pump, respectively.

1.3 Open-Cell Model

The simplest type of open cell contains voltage-activated Ca^{2+} channels for which one can fix the rate of influx by clamping the plasma membrane potential [Mil92]. to mimic this situation we consider an open-cell model with a constant influx of external Ca^{2+} , j_{in}, that can be controlled parametrically. Although both Ca^{2+} pumps and exchange mechanisms are prevalent in the plasma membrane of cells, we treat efflux as occurring via a plasma membrane Ca^{2+}-ATPase (PMCA pump) only [Car94]. Adding these terms to the closed-cell model described in the previous section gives us the open-cell $\left[Ca_i^{2+}\right]$balance equation:

$$\frac{d\left[Ca_i^{2+}\right]}{dt} = f_i\left((\nu_1+\nu_2)\left(\left[Ca_s^{2+}\right]-\left[Ca_i^{2+}\right]\right)-\nu_3\frac{\left[Ca_i^{2+}\right]^2}{\left[Ca_i^{2+}\right]^2+(K_3)^2}-\nu_{out}\frac{\left[Ca_i^{2+}\right]^2}{\left[Ca_i^{2+}\right]^2+(K_{out})^2}+j_{in}\right) \tag{13}$$

$$\tag{14}$$

where the final two terms represent the PMCA pump [Car94] and the Ca^{2+} influx. Because the cell is open to the external medium, the total free-Ca^{2+} concentration, C_0, is no longer fixed but varies according to the equation:

$$\frac{dC_0}{dt} = f_i\left(j_{in}-\nu_{out}\frac{\left[Ca_i^{2+}\right]^2}{\left[Ca_i^{2+}\right]^2+(K_{out})^2}\right) \tag{15}$$

Where j_{in} represents influx from a clamped membrane potential in an electrically excitable cell and K_{out} is the dissociation constant

1.4 Simplified Open-Cell Model

Keizer and Levine determined that adaption was not essential for oscillations in the open-cell model, this discovery allowed them to simplify the model further by requiring that:

$$w = w^{\infty}\left(\left[Ca_i^{2+}\right]\right) \tag{16}$$

Thus our simplified model has only two variables, $\left[Ca_i^{2+}\right]$and C_0 with the following parameters:

Table 2: **Parameters for simplified open-cell model**

Parameter	Meaning
f_i	Buffer factor in cytoplasm
ν_1	Rate constant RyR
ν_2	Rate constant for store leak
ν_3	Maximal rate of SERCA pump
ν_{out}	Maximal rate PMCA pump
k_3	Dissociation constant for SERCA
c_1	Weighted volume fraction
K_{out}	Dissociation constant for PMCA
j_{in}	Constant influx rate

2 Simulating Simplified Open-Cell Model

To simulate the model, I will be using the popular MATLABtoolbox, MatCont. This toolbox allows you to input a system of equations and define parameters and variables. We define our system as follows.

Name	test
Time	t
Coordinates	Ca_i, C0
Parameters	fi, v1, v2, v3, K3, c1, v_out, K_out, j_in
Derivatives	NNNNN
Userfunctions	(none)
Equations	ka_plus = 1500; ka_minus = 28.8;
	kb_plus = 1500; kb_minus = 385.9;
	kc_plus = 1.75; kc_minus = 0.1;
	Ka = (ka_minus/ka_plus)^(1/4);
	Kb = (kb_minus/kb_plus)^(1/3);
	Kc = (kc_minus/kc_plus);
	w = (1+(Ka/Ca_i)^4+(Ca_i/Kb)^3)/(1+(1/Kc)+(Ka/Ca_i)^4+(Ca_i/Kb)^3);
	P_slow = w*(1 + (Ca_i/Kb)^3) / (1 + (Ka/Ca_i)^4 + (Ca_i/Kb)^3);
	Ca_s = (C0 - Ca_i)/c1;
	Ca_i' = fi*((v1*P_slow+v2)*(Ca_s-Ca_i) - v3*(Ca_i^2/(Ca_i^2+K3^2)) - v_out*(Ca_i^2/(Ca_i^2 + K_out^2)));
	C0' = fi*(j_in - v_out*(Ca_i^2/(Ca_i^2 + K_out^2)));

Figure 2: A screenshot of the MatCont system used to simulate the simplified open-cell model.

Listing 1: System of Simplified Open-Cell Model

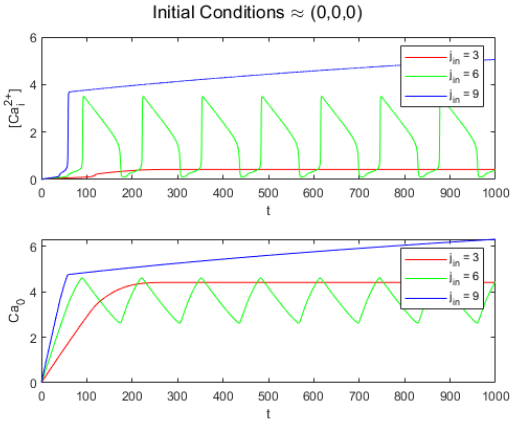
```
ka_plus = 1500; ka_minus = 28.8;
kb_plus = 1500; kb_minus = 385.9;
kc_plus = 1.75; kc_minus = 0.1;
Ka = (ka_minus/ka_plus)^(1/4);
Kb = (kb_minus/kb_plus)^(1/3);
Kc = (kc_minus/kc_plus);
w = (1+(Ka/Ca_i)^4+(Ca_i/Kb)^3)/(1+(1/Kc)+(Ka/Ca_i)^4+(Ca_i/Kb)^3);
P_slow = w*(1 + (Ca_i/Kb)^3) / (1 + (Ka/Ca_i)^4 + (Ca_i/Kb)^3);
Ca_s = (C0 - Ca_i)/c1;
Ca_i' = fi*( (v1*P_slow+v2)*(Ca_s-Ca_i) - v3*(Ca_i^2/(Ca_i^2+K3^2)) - v_out*(Ca_i^2/(Ca_i^2 + K_out
    ^2)) );
C0' = fi*(j_in - v_out*(Ca_i^2/(Ca_i^2 + K_out^2)));
```

Using the values from Table 3 we will simulate the model for 3 different values of $j_{in} = 3, 6, 9$. From 3 different initial conditions.

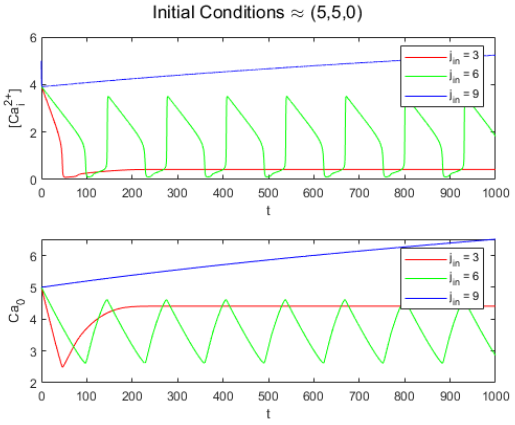
Table 3: **Parameters used for simulations**

Parameter	Value
f_i	0.01
ν_1	40 s^{-1}
ν_2	0.5 s^{-1}
ν_3	120 $\mu M s^{-1}$
ν_{out}	9.0 $\mu M s^{-1}$
k_3	0.30 μM
c_1	0.15
K_{out}	0.60 μM
j_{in}	3.0/6.0/9.0 μM

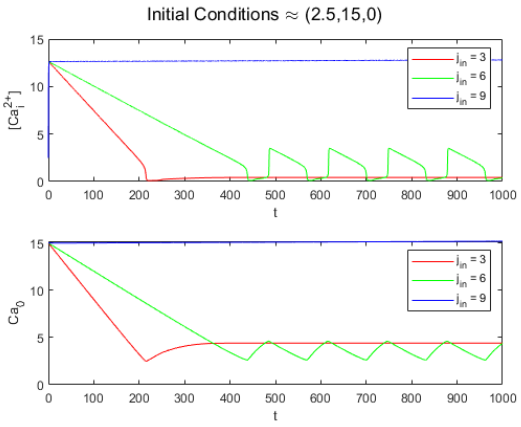
2.1 Varied Initial Conditions



(a) Initial Conditions ($[\mathrm{Ca}_i^{2+}]$,C_0,t) = (0,0,0)



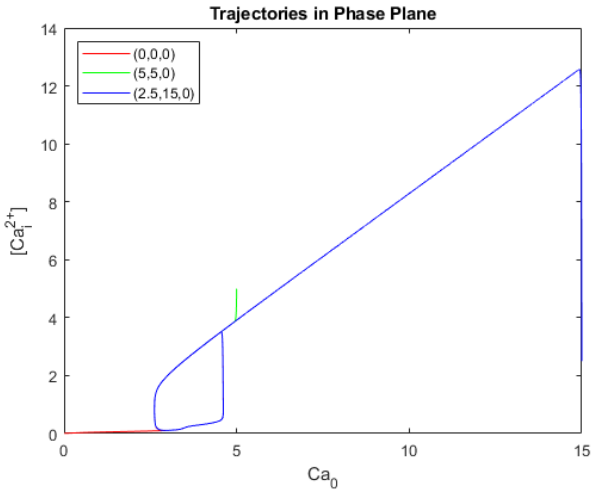
(b) Initial Conditions ($[\mathrm{Ca}_i^{2+}]$,C_0,t) = (5,5,0)



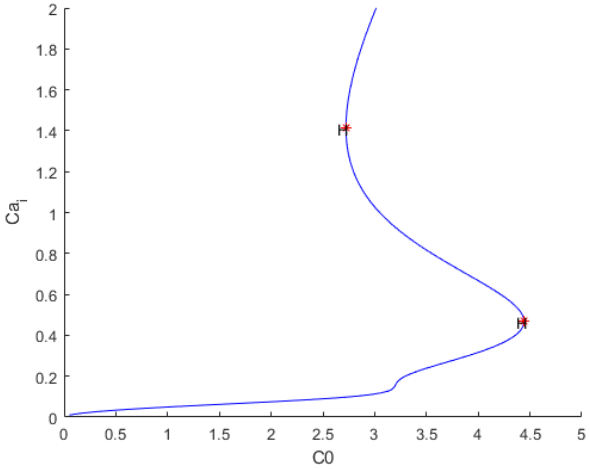
(c) Initial Conditions ($[\mathrm{Ca}_i^{2+}]$,C_0,t) = (2.5,15,0). Note the transient behaviour before the oscilations

Figure 3: Simulating the model for 3 different values of J_{in} showing that oscillations are dependent on Ca^{2+} influx.

3 Bifurcation Analysis



(a) A plot of the phase plane, showing three trajectories with varied initial conditions. We see that they all converged to the same periodic orbit.



(b) This plot shows a curve of equilibrium in the $\left[\mathrm{Ca}_i^{2+}\right]$and C_0 plane as J_{in} is allowed to varied. The two red crosses represent Hopf bifurcations.

Figure 4: Two figures of the ($\left[\mathrm{Ca}_i^{2+}\right]$,$\mathrm{Ca}_0$) phase plane

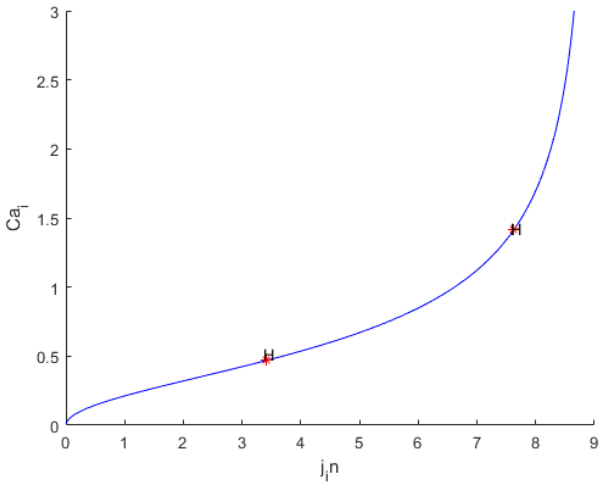


Figure 5: This plot shows a curve of equilibrium in the $\left[\mathrm{Ca}_i^{2+}\right]$and j_{in} plane with C_0 is allowed to varied. The two red crosses represent Hopf bifurcations. We can see this value range agrees with our earlier plots as oscillations will appear for j_{in} values between the 2 Hopf bifurcations.

References

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