Intracellular Calcium

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References: Chapter 6 of the Sterratt text

Signaling Models and Pathways

As we know, information is coded in neurons as electrical impulses, which are then passed from one neuron to others in the network. This is intercellular signaling. In addition to this, a great deal of intracellular signaling takes place in neurons and other electrically excitable cells. This often occurs as a result of neurotransmitters or hormones binding to receptors in the cell's plasma membrane, which triggers the production or activation of molecules inside the cell. This ultimately can result in gene expression of new proteins, a change in the secretion level from the cell, or the activation or inactivation of ion channels. So intracellular signaling is an extremely important aspect of how the cell behaves, and thus how it affects the neural network(s) that it is a part of.

The entry point to many signaling pathways is the plasma membrane-bound receptor. One type is associated with proteins called G-proteins. There are several types of G-proteins, each of which starts a cascade of actions within the cell. The majority of pharmaceutical drugs target G-protein receptors. Other receptor types are composed of tyrosine kinases or activate the JAK-STAT pathway. Activation of any one pathway can have several effects that operate on time scales from seconds to hours, and

several pathways can be operational at the same time.

There are many signaling molecules in cells. We will focus on one of these, Ca^{2+}, which also happens to be the most ubiquitous of the signaling molecules. Intracellular Ca^{2+} also activates several types of K^+ channels, linking it to a cell's electrical activity.

Calcium: the Universal Second Messenger

Intracellular Ca^{2+} activates enzymes such as protein kinase C (PKC), gene transcription factors such as cAMP response element-binding protein (CREB), and a family of K^+ channels called **Ca^{2+}-activated K^+ channels** (K(Ca) channels). It can also cause the cell to enter into apoptosis, i.e., programmed cell death, if it remains elevated for too long. The latter seems reasonable given the many roles played by Ca^{2+}; if elevated for too long then the cell becomes hyper-activated, which is usually not a good thing. For this reason, there are many buffering proteins expressed in the cell that bind to intracellular Ca^{2+} ions so tht they can't have any action on other things. In fact, about 99% of all intracellular Ca^{2+} ions are bound by buffers, leaving only 1% with the ability to do anything.

One way that Ca^{2+} can do so many things is that it is localized into several distinct parts or compartments of the cell. In neurons, most of the Ca^{2+} enters the cell through Ca^{2+} ion channels. At the inner mouth of an open channel a Ca^{2+} microdomain forms, where the free Ca^{2+} con-

centration can be as high as 100 μM. In comparison, the resting Ca^{2+} level in the cell **cytosol** (i.e., the aqueous component of the cytoplasm) is 0.1 μM. The microdomains of nearby open Ca^{2+} channels can overlap, forming a submembrane compartment in which the Ca^{2+} concentration is significantly elevated above the level in the rest of the cytosol. Ca^{2+} is also pumped into intracellular organelles, such as the cell's nucleus, mitochondria, golgi, and endoplasmic reticulum, where it is maintained at a much higher concentration than in the bulk cytosol. In compartmental models we keep track of the Ca^{2+} concentration in some of these domains or organelles, writing down differential equations that depend on the Ca^{2+} flux into or out of the compartment. This is done because we want to simulate the effect of Ca^{2+} on some downstream target. If the target is ion channels, then we need to know at least the bulk cytosolic Ca^{2+} concentration. We will next construct a model for the handling of Ca^{2+} in just the cytosol, and then couple it to the cell's voltage equation through K(Ca) current.

Cytosolic Ca^{2+} and Activation of K^+ Channels

During an impulse, Ca^{2+} enters the cells through open Ca^{2+} channels in the plasma membrane. It is also pumped out of the cell by Ca^{2+} pumps

(Fig. 1). The fluxes can be described as:

$$J_{in} = -\alpha I_{Ca} \mathbf{V}_c \tag{1}$$

$$J_{out} = k_{pmca} Ca_c \mathbf{V}_c \tag{2}$$

where the units of the fluxes are moles/ms. The negative sign accounts for the convention that inward Ca^{2+} current is negative, α is a conversion factor, and k_{pmca} is a parameter reflecting the strength of the Ca^{2+} pumps (pmca stands for "plasma membrane Ca^{2+} ATPases"). Finally, Ca_c is the concentration of free (not bound to buffers) Ca^{2+} in the cytosol, and $\mathbf{V}_c$ is the volume of the cytolic compartment. The total number of free Ca^{2+} ions in the cytosol is $Ca_c \mathbf{V}_c$ (units of moles) and the rate of change of this number is then

$$\frac{d\left(Ca_c \mathbf{V}_c\right)}{dt} = f_c(J_{in} - J_{out}) \quad . \tag{3}$$

Dividing through by the volume and using Eqs. 1,2,

$$\boxed{\frac{dCa_c}{dt} = -f_c(\alpha I_{Ca} + k_{pmca} Ca_c)} \tag{4}$$

where f_c is the fraction of Ca^{2+} that is free (i.e., not bound by Ca^{2+} buffers in the cytosol).

Now to see how Ca^{2+} activation of K(Ca) currents can be important we modify the Morris-Lecar model discussed earlier in the semester by adding a K(Ca) current, $I_{K(Ca)}$. There are several possible formulations, but we

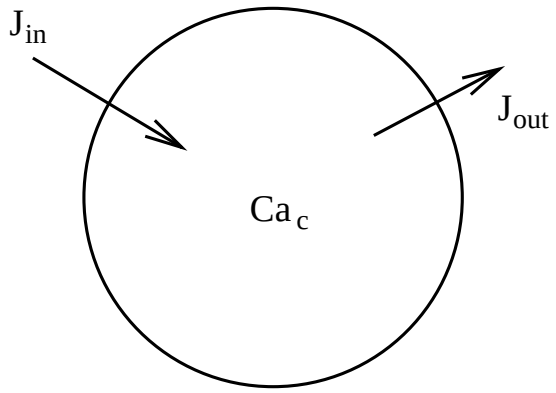

Figure 1: Ca^{2+} fluxes across the plasma membrane in a typical cell.

use:

$$I_{K(Ca)} = \bar{g}_{K(Ca)} \left(\frac{Ca_c^3}{Ca_c^3 + K_D^3} \right) (V - V_K) \tag{5}$$

where a Hill function with exponent 3 is used to describe the Ca^{2+} activation. With this current, the Morris-Lecar model coupled to the Ca^{2+} equation becomes:

$$\frac{dV}{dt} = -[I_{Ca} + I_K + I_L + I_{K(Ca)}]/C \tag{6}$$

$$\frac{dw}{dt} = [w_\infty(V) - w]/\tau(V) \tag{7}$$

$$\frac{dCa_c}{dt} = -f_c[\alpha I_{Ca} + k_{pmca} Ca_c] \ . \tag{8}$$

In the next in-class exercise we will see how this model can produce electrical bursting.

Calcium Handling in the Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is an organelle that processes proteins that are bound for the plasma membrane. It is also a storehouse

for Ca^{2+}. The illustration in Fig. 2 shows some important Ca^{2+} fluxes between compartments.

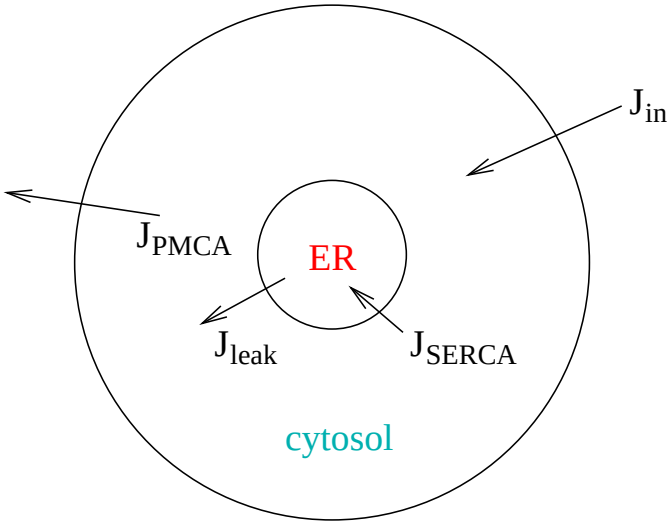

Figure 2: Ca^{2+} fluxes into and out of cytosol and ER.

With this new ER compartment the cytosolic Ca^{2+} equation must be modified, and a new ODE introduced:

$$\frac{dCa_c\mathbf{V_c}}{dt} = f_c(J_{in} - J_{PMCA} - J_{SERCA} + J_{leak}) \quad (9)$$

$$\frac{dCa_{ER}\mathbf{V_{ER}}}{dt} = f_c(J_{SERCA} - J_{leak}) \ . \quad (10)$$

If we define $j_x = J_x/\mathbf{V_c}$, then the equations become

$$\frac{dCa_c}{dt} = f_c(j_{in} - j_{PMCA} - j_{SERCA} + j_{leak}) \quad (11)$$

$$\frac{dCa_{ER}\mathbf{V_{ER}}}{dt} = f_c\mathbf{V_c}(j_{SERCA} - j_{leak}) \ . \quad (12)$$

If we next define $\mu = \frac{\mathbf{V_c}}{\mathbf{V_{ER}}}$, the volume ratio, then the Ca^{2+} equations

become

$$\frac{dCa_c}{dt} = f_c(j_{in} - j_{PMCA} - j_{SERCA} + j_{leak}) \quad (13)$$

$$\frac{dCa_{ER}}{dt} = f_c\mu(j_{SERCA} - j_{leak}) \ . \quad (14)$$

The **Ca^{2+} leak** out of the ER is proportional to the concentration gradient:

$$j_{leak} = k_{leak}(Ca_{ER} - Ca_c)$$

and a reasonable model for flux through **SERCA pumps** (SERCA=Sarco-Endoplasmic Reticulum ATPase) is

$$j_{SERCA} = k_{SERCA}Ca_c \ .$$

The G_q signaling pathway

One reason to include the ER in our Ca^{2+} model is so that we can simulate the activation of the G_q signaling pathway. This pathway is activated when a hormone or neurotransmitter molecule binds to a G-protein coupled receptor. This leads to the splitting of a molecule of phosphatidylinositol 4,5-bisphosphate (PIP_2) into two molecules, one called diacyglycerol and the other is called inositol trisphosphate (IP_3). Although both molecules have downstream targets, we focus here on IP_3. An IP_3 molecule binds to IP_3 receptors in the membrane of the ER, which when activated acts as a Ca^{2+} channel. An open IP_3 receptor/channel leads to efflux of Ca^{2+} from

inside the ER to the cytosol, since the Ca^{2+} concentration is higher in the former than the latter.

To enter an open state, three molecules of IP_3 must bind to the receptor, as well as three cytosolic Ca^{2+} molecules. This reflects the fact that the receptor is a homotrimer, with three identical subunits. The receptor also inactivates, but on a slow time scale. So there is an inactivation variable, h, that represents the probability that the channel is not inativated. This inactivation process is gated by cytosolic Ca^{2+}. The expression for flux through the IP_3 receptor/channel is:

$$j_{\mathrm{IP3}} = P\left(\frac{Ca_c}{Ca_c + k_a}\right)^3 \left(\frac{IP_3}{IP_3 + k_i}\right)^3 h^3(Ca_{ER} - Ca) \tag{15}$$

where P is a parameter representing the flux through an open channel and k_a and k_i are Ca^{2+} affinity parameters. There is also a differential equation for h. With this additional flux term, the Ca^{2+} equations become

$$\frac{dCa_c}{dt} = f_c(j_{in} - j_{PMCA} - j_{SERCA} + j_{leak} + j_{IP3}) \tag{16}$$

$$\frac{dCa_{ER}}{dt} = f_c\mu(j_{SERCA} - j_{leak} - j_{IP3}) \tag{17}$$

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \tag{18}$$

where

$$h_\infty(Ca_c) = \frac{K_d}{K_d + Ca_c} \tag{19}$$

and

$$\tau_h(Ca_c) = \frac{A}{K_d + Ca_c} \tag{20}$$

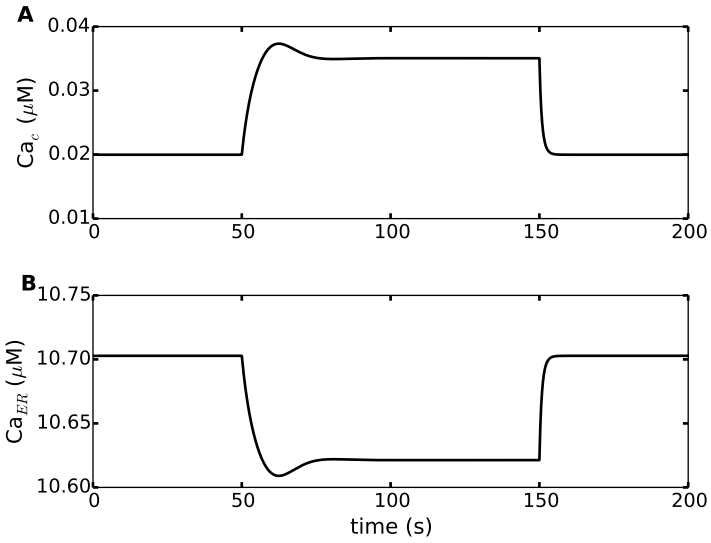

Figure 3: Ca^{2+} responses to a pulse of IP_3 beginning at time 50 s and lasting until 150 s. The IP_3 concentration is 0.6 μM.

is the Ca^{2+}-dependent time constant.

Figure 3 show how the system responds to a 100 s application of IP_3. The Ca^{2+} concentration in the ER drops while that in the cytosol increases. Things look quite different when a somewhat larger IP_3 pulse is applied. Now a train of Ca^{2+} spikes are produced in the cytosol, with corresponding drops in the ER concentration. In fact, the mechnism behind these spikes is very similar to that of action potentials. There is fast positive feedback due to Ca^{2+} activation of IP_3 receptors, assumed in the model to be instantaneous, and slow Ca^{2+}-dependent inactivation of the receptors through the variable h. So the Ca^{2+} model, in the presence of IP_3, is an excitable system!

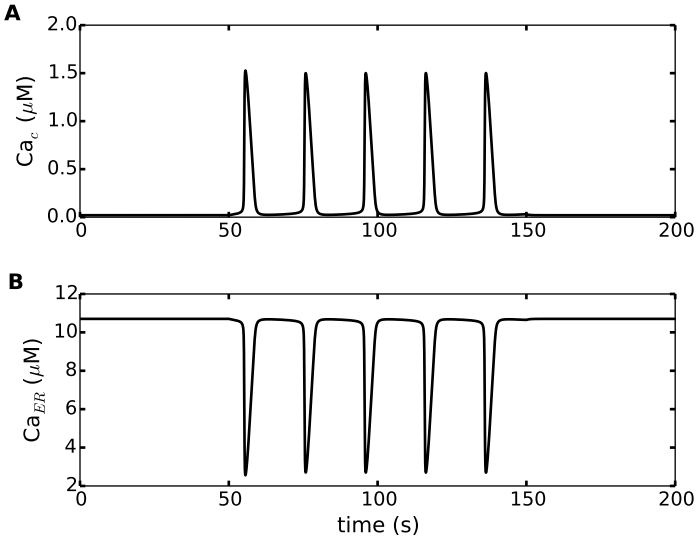

Figure 4: Ca^{2+} responses to a larger pulse of IP_3 beginning at time 50 s and lasting until 150 s. The IP_3 concentration is 0.8 μM.