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A Quantitative Study of Neuronal Calcium Signaling

by

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Abstract

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Neurons have both a fast and slow mode of signaling. Fast signals are communicated by transmembrane voltage changes, while calcium levels within the cell communicate information on a much slower time scale. Calcium acts as a second messenger responsible for modulating neuronal excitability in many ways including the mediation of gene transcription in the cell and the sensitivity of the cell to further stimulus. I develop a voltage model of the neuron’s electrical signal with ion diffusion and drift which includes voltage-gated calcium currents and calcium-dependent potassium currents. The influx of calcium resulting from the voltage model will prime the endoplasmic reticulum with calcium. A model of the dynamics of calcium induced calcium release from the endoplasmic reticulum via IP3 receptors which includes diffusion of calcium and IP3 as well as calcium buffering by the mitochondria results in a calcium wave similar to what has been observed experimentally.
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# Contents

List of Figures

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2 Background</td>
<td>4</td>
</tr>
<tr>
<td>3 Generation of Calcium Waves</td>
<td>19</td>
</tr>
<tr>
<td>4 Model</td>
<td>27</td>
</tr>
<tr>
<td>4.1 Derivation of Equations</td>
<td>30</td>
</tr>
<tr>
<td>4.1.1 Voltage Equation</td>
<td>30</td>
</tr>
<tr>
<td>4.1.2 Calcium Wave Equations</td>
<td>35</td>
</tr>
<tr>
<td>4.2 Voltage Model Results</td>
<td>38</td>
</tr>
<tr>
<td>4.3 Calcium Wave Model Results</td>
<td>42</td>
</tr>
<tr>
<td>5 Future Work</td>
<td>49</td>
</tr>
<tr>
<td>Appendix A. Gating Variable Equations</td>
<td>55</td>
</tr>
<tr>
<td>Appendix B. Voltage Equation Implementation</td>
<td>57</td>
</tr>
<tr>
<td>Appendix C. Calcium Wave Implementation</td>
<td>66</td>
</tr>
<tr>
<td>Appendix D. IP3 Generation Process</td>
<td>72</td>
</tr>
<tr>
<td>Bibliography</td>
<td>76</td>
</tr>
</tbody>
</table>
List of Figures

2.1 Ion channel in the membrane .................................................. 6
2.2 Action Potential ................................................................. 7
2.3 Parts of a neuron .................................................................... 8
2.4 Neuron showing soma, dendrites and spines ......................... 9
2.5 Depiction of a synapse ............................................................ 10
2.6 Post-synaptic calcium cascade of interactions ...................... 11
2.7 Calcium wave ...................................................................... 13
2.8 Endoplasmic reticulum in the neuron ................................... 16

3.1 Ionotropic receptor ............................................................... 20
3.2 Metabotropic receptor .......................................................... 21
3.3 IP$_3$ production ................................................................... 23
3.4 Components of localized calcium flow ................................... 26

4.1 Sodium Channel Density for Voltage Model ......................... 38
4.2 Back-propagating Action Potential ........................................ 39
4.3 Potassium, Sodium, Leak, and Calcium-dependent Potassium currents .......................................................... 40
4.4 Calcium, AMPA, NMDA, and Total currents ...................... 41
4.5 Sodium, Potassium, and Calcium Concentrations ................ 42
4.6 Receptor-Dependent Activation Levels for Calcium Wave Model .................. 43
4.7 Cytosolic Calcium Waves ...................................................... 44
4.8 Cytosolic Calcium Concentration - another view ................ 45
4.9 IP$_3$ Waves ......................................................................... 46
4.10 IP$_3$ waves - another view .................................................... 47
4.11 Calcium in the ER ................................................................. 48
Chapter 1

Introduction

Calcium plays a critical role as a second messenger in the neuron to modulate the excitability of the cell. Calcium signals must reach the nucleus to enable gene transcription necessary in the formation of long-term memories. Although many models are available for the voltage signaling in neurons, no model has been developed that incorporates the interplay of voltage signaling with the calcium dynamics in the cell.

Hodgkin and Huxley developed a model for voltage signaling in the neuron in 1952 [15] using a resistor-capacitor circuit model for the neuron. They used gating variables to model the various channel states, but assumed a concentration of ions within the cell that remained essentially constant and, thus, neglected diffusion of ions longitudinally within the neuron. Qian and Sejnowski [32] developed an electrodiffusion model of the neuron beginning with first principles. Their model includes diffusion
and drift of ions, but does not include the gating variables which are necessary to predict the action potentials seen in neurons. In 1996 a model was developed by Rapp, et al. [33] to incorporate specific receptors on the spines. Others have included calcium currents and calcium-dependent currents into their voltage models [16],[38]. Meyer and Stryer [26] constructed a model for calcium release from internal cellular stores that resulted in spiking when interplay with the mitochondria was included. Their model included no diffusion of ions or coupling with the previously developed voltage models.

I implement a voltage model incorporating the elements most crucial to calcium signaling. I begin with a resistor-capcitator model that includes gating variables, the diffusion and drift of ions, and currents present on the spines. I add transient calcium currents and calcium-dependent potassium currents and track the resulting calcium levels in the cell. I add a spatial component with diffusion terms to the equations of the Meyer and Stryer model to generate calcium waves.

The next chapter provides an introduction to terms and processes from neuroscience. Chapter 3 describes in detail how calcium enters the cell, what happens to it after it enters, and the key elements responsible for calcium wave generation. I then describe mathematical models for both fast and slow neuronal signaling and show the resulting graphs. The fast signaling model shows the voltage change in the cell as well as the corresponding currents and concentrations. The slow signaling model results in the generation of calcium waves. Finally, I discuss ways in which the model
could be improved and pose other questions requiring further investigation.
Chapter 2

Background

Our ability to make and recall memories is critical to our ability to function. Calcium levels inside our nerve cells are a vital part of the memory mechanism. This paper will explore how calcium waves are generated and their effect. Nerve cells or neurons function as a result of both electrical and chemical processes which must be understood in order to understand the effects of calcium.

Our brains control our bodily functions, process input from the world around us, make decisions about how we will respond to the world, determine our emotional state, and hold our memories of past events. Right now, your eye is transmitting information about the input of colors and shapes contained in these words to your brain. Your brain compares these patterns to the patterns it knows (letters and words) and determines a meaning from the input it has received. Your brain also determines the level of attention you are giving to this reading and what your emotional
state is while reading it. It will call upon memories of past information stored to put
the information here in a more understandable context. At the same time that all
this is occurring, your brain is also controlling your respiration and other autonomic
functions. How is it able to do such complicated tasks?

Your brain is composed of over 100 billion individuals neurons that must com-
municate through complex interactions. In fact, a neuron on average has about
10,000 input sites where it receives information from other neurons. In addition,
there are neurons that are specialized to receive inputs from our environment. These
specialized neurons contain receptors that are sensitive to either light rays (optical
nerves), sound waves (auditory nerves), odors (olfactory nerves), tastes, touch, pain,
and temperature (somatic sensory nerves). When stimulated sufficiently by an input
from either other nerves or the environment, the neuron will produce an electrical
signal called an action potential. This action potential is a change in transmembrane
voltage which passes along the neuron. In this sense the cell is excitable. Calcium
waves regulate and modulate this neuronal excitability.

The action potential is possible because the concentration of ions within the cell is
different from the concentration outside the cell. The cell membrane will not allow the
passage of ions through the membrane itself. Instead, the ions must pass through
channels which are embedded in the wall of the membrane of the cell. Figure 2.1
shows a 2-D graphical representation of an ion channel in the cell membrane.
Figure 2.1: Ion channel in the membrane adapted from Travis ([37]). The ion channel is a transmembrane protein which opens under certain conditions, allowing ions to flow through.

There are many different types of channels in the membranes of neurons and various types of neurons have differing distributions of the available channel types. Although normally closed, these ion channels will open under the appropriate conditions and allow the ions to flow across until the channel is again closed. The concentration of sodium is higher outside the cell than it is inside the cell. Thus, the tendency is for sodium to flow into the cell. Similarly, potassium concentration is higher inside the cell, and tends to flow out. The action potential results from the movement of sodium and potassium ions through their respective channels. Initially, the sodium channels open resulting in an inflow of sodium ions. The potassium channels then open, allowing potassium ions to begin flowing out of the cell. After a short time, the sodium channels inactivate, causing them to close. The result is a change in voltage over time at a given place in the cell as depicted in figure 2.2.
Figure 2.2: Action Potential adapted from Cannon [4]. When a neuron depolarizes above a certain threshold, a large percentage of sodium ($Na^+$) channels open. Potassium ($K^+$) channels open a little more slowly. The result is a spike in the membrane potential called an action potential.

Because this paper will be referring to the parts of the nerve cell, a drawing depicting the parts of the cell is shown in figure 2.3 from EnchantedLearning.com [12].
In general, the dendrites receive inputs; the soma or cell body processes the inputs; the axon transmits information from the cell. The dendrites frequently contain structures called spines which is where the inputs occur. Like the name infers, these are spiny looking structures that protrude out of the sides of the dendrites. Figure 2.4 from Chicurel [5] is a photograph of a neuron - the spines are the red dots, the yellow lines are the dendrites and the round part in the middle is the soma. The axon is not clearly visible with this staining of the cell.
Figure 2.4: Neuron showing soma, dendrites and spines from Chicurel [5]

Nerve cells communicate with each other through connections called synapses, most of which are electrochemical. One nerve cell fires an action potential (electrical signal) which results in the release of neurotransmitters (chemicals) from its axon terminals. These neurotransmitters diffuse across a narrow synaptic cleft and bind to receptors on the dendrite (input portion) of the receiving neuron. The binding of the receptor causes an electrical signal in the receiving neuron. Figure 2.5 is a drawing of a synapse from Purves, *et al.* [31], p. 142.
Figure 2.5: Depiction of a synapse from Purves, et al. [31], p. 142. Neurotransmitter is released from the pre-synaptic neuron and diffuses across the synaptic cleft. The neurotransmitter binds to receptors on the post-synaptic neuron which allows ions to enter and initiate an electrical signal in the post-synaptic neuron.

Memories are stored as a combination of synaptic strengths. Synapses are strengthened when used through various means including increasing the probability of release on the sending, or pre-synaptic, side; increasing the amount of neurotransmitter released each time; adding receptors on the receiving, or post-synaptic, side;
and adding post-synaptic receiving sites. The long-term strengthening of a synapse is called LTP (long-term potentiation). The early phase of LTP is caused by a calcium influx into the cell leading to insertion of additional receptors. With repeated stimulation, the calcium levels increase, resulting in a set of chemical interactions involving cyclicAMP protein kinase (PKA), adenylyl cyclase and cyclicAMP response element binding protein (CREB) that eventually lead to structural changes in the synapse [35], p. 150-151. Some of the elements involved in the process are shown in figure 2.6 from Sheng [40].

Figure 2.6: Post-synaptic calcium cascade of interactions from Sheng [40]. Important players in the increase of cytosolic calcium include the NMDAR receptor, the AMPAR receptor, and the mGluR. Calcium interacts with many other chemicals. However, the Ras / Raf / MEK / ERK cascade is important in regulating nuclear gene expression.
These structural changes are set in motion by gene transcription in the nucleus. In order to induce gene transcription, a calcium signal must enter the nucleus [3]. This paper explores how the calcium signal gets to the nucleus of the post-synaptic neuron and touches on questions regarding other ways in which calcium modulates the excitability of the neuron.

The part of the brain which consolidates declarative memories is the hippocampus; other regions of the brain are responsible for emotional and motor memories. The hippocampus turns short-term memories of facts, images, and thoughts into long-term memories. One of the main neurons involved in the memory consolidation in the hippocampus is called the CA1 pyramidal neuron. The model will use the CA1 pyramidal neuron parameters where possible.

Jaffe and Brown observed and measured calcium waves in neurons in 1994. These waves spread from the site of synaptic input down to the cell body. Figure 2.7 is a picture taken from Jaffe and Brown [17] showing the calcium increases over time at three different points in the dendrites and the soma of a hippocampal CA1 pyramidal neuron.
Panel A is a picture of the neuron with areas numbered 1 to 4. Area 1 is a basal dendrite; area 2 is the soma; area 3 is the portion of an apical dendrite close to the soma; area 4 is a more distant portion of the apical dendrite. Panel B1 shows the change in calcium concentration over time for each of the 4 regions resulting from application of ACPD to region 1 for 250 milliseconds. Notice that the calcium increase starts at region 1 which is where the input occurred. The calcium increase spreads through the soma and down another dendrite. Panel B2 shows the outward current at the site of the input. Applying a depolarizing stimulus for 1 second produced smaller rapid increases in calcium in both the soma and dendrites (shown
in C1). These small increases did not propagate as a wave. C2 shows the train of action potentials produced by the depolarizing stimulus.

In the experiment, Jaffe and Brown activated the metabotropic glutamate receptor (mGluR, described in section 3) by applying a substance called ACPD to the receptor. ACPD is an agonist for mGluR; it binds to the receptor and activates it in the same way glutamate would activate it. Using an agonist instead of glutamate allows the effect of the mGluR to be isolated. If glutamate were used, it would also activate the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors in the area. Jaffe and Brown applied ACPD in a local area for 250 milliseconds and measured the increase in calcium. The calcium level was determined using the $Ca^{2+}$-sensitive dye Flouro-3. The dye levels were measured and compared to the dye levels at rest. This normalized dye level ($\Delta F/F_0$) was then plotted over time. Taking these measurements it was found that the ACPD application produced a localized increase in $Ca^{2+}$ near the source of the stimulus. The increase in $Ca^{2+}$ propagated from the point of ACPD application through the soma and into the apical dendrite at a velocity of about 40 $\mu m/s$. A slow outward current (panel B2 of the figure) was also measured in response to the calcium wave, presumably as a result of a $Ca^{2+}$-dependent $K$ current.

After the initial application, repeated applications no longer produce calcium waves unless preceded by several trains of voltage stimuli to the cell. Thus, the voltage stimulus is necessary to open voltage-gated calcium channels, allowing cal-
cium to flow into the cell and be buffered in the endoplasmic reticulum. Once the amount of calcium in the endoplasmic reticulum (ER) exceeds a threshold, the storage is primed so that stimulation of the mGluR will result in a calcium wave.

More recently, Zhou and Ross [42] perfected and streamlined the experiments performed earlier by Jaffe and Brown. Zhou and Ross measured regenerative calcium waves in the apical dendrites of hippocampal CA1 pyramidal neurons resulting from repetitive synaptic activation. The $[Ca^{2+}]_i$ increases associated with these waves were on the scale of several micromolar, much larger than the $Ca^{2+}$ entry through NMDA receptor channels or $Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels opened by backpropagating action potentials.

What causes a calcium wave and where does the calcium come from to make the wave? Most of the calcium that enters a cell is quickly stored in the ER. As Berridge suggested [3], the ER can be thought of as a neuron within a neuron. It is a contiguous structure that stores calcium inside the neuron and extends even into the spines on the dendrites.
Figure 2.8: Endoplasmic reticulum in the neuron from Berridge [3]. The ER is a contiguous structure throughout the neuron, protruding even into the spines.

Figure 2.8, borrowed from Berridge's paper, shows the ER in red and the membrane of the neuron in black. Under appropriate conditions, the interplay between the ER and the synaptic information received by the cell will result in a calcium wave that travels from the input site to the soma.

Calcium plays an important role in modulating neuronal excitability. Neuronal $Ca^{2+}$ signaling depends on a binary membrane system. The cell membrane integrates excitatory and inhibitory inputs from other neurons and generates a relatively fast voltage change mainly using voltage-dependent $Na^+$ and $K^+$ channels. The
ER monitors internal signals like $Ca^{2+}$, $IP_3$, cyclic ADP ribose, and cyclic AMP and produces a slower signal using calcium released through the $IP_3$ and ryanodine receptors located on the ER membrane. The two membrane systems are linked because the levels of $Ca^{2+}$, $IP_3$, cyclic ADP ribose, and cyclic AMP are affected by inputs to the cell membrane. Similarly, the $Ca^{2+}$ released by the ER modulates what occurs at the cell membrane. "These two membranes are intimately tied together through a variety of reciprocal interactions to regulate specific neuronal processes such as excitability, associativity, transmitter release, synaptic plasticity, and gene transcription" [3], p.17.

$Ca^{2+}$ released from the ER alters the membrane potential, thus modulating neuronal excitability. $Ca^{2+}$ also has an effect on the behavior of other ion channels. There are two types of $K^+$ channels which are dependent on the local level of $Ca^{2+}$, which result in slow and fast after-hyperpolarizing currents. The slow after-hyperpolarizing potential is most likely a result of $Ca^{2+}$ released from the ER and results in a suppressed response to inputs to the cell membrane. Because the ER stores calcium generated by previous activity, it may act as an associative mechanism for integrating synaptic inputs that happen at different times.

Entry of $Ca^{2+}$ through voltage-gated $Ca^{2+}$ channels is necessary for exocytosis, the process of releasing neurotransmitter from the cell. A local release of $Ca^{2+}$ from the ER may increase the probability of release of small synaptic vesicles and may be required to raise the concentration of $Ca^{2+}$ above the threshold required for release.
of the larger dense-core vesicles.

A neuron subjected to simulation is capable of either enhancing or reducing synaptic strength based on the timing and strength of the inputs received. One possible means of communicating to the nucleus the timing and strength of inputs is through the amplitude as well as the spatial and temporal nature of the $Ca^{2+}$ signal. In order to make long-term changes affecting synaptic strength, it is necessary for $Ca^{2+}$ to reach the nucleus and induce gene transcription.

Chapter 3 will describe in detail how calcium enters the cell, what happens to it after it enters, and the key elements responsible for calcium wave generation. I will then present a mathematical model that accounts for the interplay between most of those elements. Finally, I will discuss ways in which the model could be improved and discuss other questions requiring further investigation.
Chapter 3

Generation of Calcium Waves

Calculated waves are thought to be generated in the following manner:

1. Synaptic input is received which causes

   (a) the soma of the cell to fire a back propagating action potential (BPAP)
   and

   (b) initiation of a complex process resulting in generation of IP$_3$ at the site of
   the synaptic input.

2. The BPAP causes

   (a) voltage-gated calcium channels to open and calcium to flow into the cell
   and

   (b) unblocking of NMDA channels.
3. The calcium is taken up by the endoplasmic reticulum.

4. Additional synaptic input is received, allowing calcium to flow into the cell.

5. Calcium acts together with IP$_3$ to release calcium from the endoplasmic reticulum.

The dendrites receive information at the synapses through receptors. Receptors can be ionotropic or metabotropic. Both types contain binding sites outside the cell that a particular neurotransmitter binds to. When bound, ionotropic receptors shown in figure 3.1 change shape, opening a channel through the receptor which allows ions to pass through.

![Figure 3.1: Ionotropic receptor from Purves, et al. [31], p. 169. When the neurotransmitter binds to the ionotropic receptor, a pore opens in the receptor allowing ions to flow through.](image)

Bound metabotropic receptors (shown in figure 3.2) change shape to expose G-protein binding sites inside the cell, initiating a complex series of events.

![Diagram of metabotropic receptor]

Figure 3.2: Metabotropic receptor from Purves, et al. [31], p. 169. Binding of the neurotransmitter to the G-protein receptor opens a G-protein binding site in the cell. When the G-protein attaches, it exchanges GDP for GTP, resulting in its activation.

The process begins post-synaptically when the neurotransmitter glutamate is released from the pre-synaptic (sending) neuron and binds to the receptors. Receptors are predominantly located in the cell membrane of spines on the dendrites. Two of the receptors that glutamate binds are ionotropic receptors: AMPA and NMDA. Glutamate also binds to a metabotropic glutamate receptor (mGluR). Each receptor plays a different role in processing the signal. The mGluR begins a relatively slow process of producing inositol 1,4,5-trisphosphate ($IP_3$). The AMPA receptor is an
ionotropic receptor that allows for rapid depolarization of the membrane while the NMDA receptor acts as a coincidence detector.

Binding of glutamate to the mGluRs results in a G-protein cascade that results in the production of $IP_3$. The mGluR is a transmembrane protein with binding sites outside the cell for glutamate and inside the cell for a G-protein. Binding of glutamate to the extracellular site of the receptor results in a conformational change to the receptor, opening up the intracellular binding site for the G-protein, $G_q$. The G-protein contains $\alpha$, $\beta$, and $\gamma$ subunits with the $\alpha$ subunit bound to guanine diphosphate (GDP). When the G-protein binds to the intracellular binding site of the mGluR, the GDP is replaced by guanosine-5'-triphosphate (GTP). The $\alpha$ subunit dissociates from the $\beta\gamma$ complex and in concert with $Ca^{2+}$ activates the effector enzyme phospholipase C (PLC). Phosphatidylinositol 1,4,5-bisphosphate is partially membrane bound. PLC cleaves it into $IP_3$ and diacylglycerol (DAG). DAG remains membrane bound and is used along with cytosolic calcium to activate protein kinase C (PKC). Now that $IP_3$ is free from the membrane, it can diffuse the short distance to the $IP_3$ receptors in the nearby endoplasmic reticulum [20], [31]. The last step in the G-protein cascade is shown in figure 3.3.
Figure 3.3: IP₃ production from Purves, et al. Neuroscience [31], p. 173. Activated PLC cleaves PIP₂ resulting in DAG and IP₃. IP₃ is available to bind to the IP₃ receptor of the ER or to be broken down by phosphatase.

The NMDA receptors are normally blocked by Mg²⁺ and ion flow is restricted even when bound by glutamate. The initial binding of glutamate to the AMPA receptors allows the passage of Na⁺ and K⁺, resulting in a depolarization of the cell. Membrane depolarization opens voltage gated Na⁺ and K⁺ channels, further depolarizing the membrane. Generally, the depolarization is not enough to reach the threshold required to generate an action potential in the dendrite. However, the depolarization spreads with decreasing amplitude until it reaches the soma. Because the distribution of voltage gated channels in the dendrites differs significantly from the distribution in the soma, the depolarization that reaches the soma may be above the threshold there. Another possibility is that other synaptic inputs combine their
depolarization effects to produce an action potential in the soma or the axon hillock. This action potential spreads in both directions. When it reenters the dendrite, it is called a back propagating action potential (BPAP).

The BPAP spreads along the neuron in both directions, causing voltage-gated calcium channels to open. Calcium enters the cell everywhere as a result and is quickly buffered into the endoplasmic reticulum. Now the ER is a contiguous tube inside the neuron that extends throughout the cell, even into the spines [3]. Thus, the ER is uniformly loaded in the cell following a BPAP. The depolarization that occurs during the BPAP also causes the Mg$^{2+}$ plug to be dislodged from the NMDA receptors. Over time the calcium in the ER will escape through a leak channel and either be buffered in the cell by the mitochondria or other compounds binding with it or be pumped out of the cell by the Na$^+$/Ca$^{2+}$ exchanger or the ATP-ase pump in the cell membrane. Also over time, the Mg$^{2+}$ plugs will return to block the NMDA channels again.

If another input is received quickly enough that the ER is still primed and the NMDA channels remain unblocked, the glutamate will bind to the NMDA receptor and both Na$^+$ and Ca$^{2+}$ will be free to flow through the channel. When the calcium enters the cell, some of it will bind to the IP$_3$ receptor on the ER along with some of the IP$_3$ that was generated by the G-protein cascade started during the previous input. The combination of Ca$^{2+}$ and IP$_3$ binding opens the IP$_3$ receptor allowing calcium to flow out of the ER. The diffusion of IP$_3$ along with that of Ca$^{2+}$ allows
for a calcium wave to flow down the dendrite to the soma.

Over time, the calcium is buffered by other compounds or pumped either back into the ER or out of the cell, the $I_P^3$ is destroyed by a phosphatase and the magnesium block returns to the NMDA receptors. An overview of most of the components of the localized calcium flow are shown in figure 3.4.
Figure 3.4: Components of localized calcium flow from Purves et al. [31], p. 173. The red arrows indicate processes resulting in an increase in the level of cytosolic \( Ca^{2+} \) while the green arrows indicate a decrease.
Chapter 4

Model

A model is required that includes the voltage changes of the neuron both spatially and temporally as well as changes in concentrations of calcium and the interplay between calcium, \( IP_3 \) and the endoplasmic reticulum.

The model considers the effects on voltage of the membrane capacitance, longitudinal current flow, and cross-membrane currents generated by leak channels, voltage-gated sodium, potassium, and calcium channels, calcium-dependent potassium channels and ligand-gated AMPA and NMDA channels. Diffusion, mGluR initiated G-protein activity and degradation by phosphotase compose the changes in \( IP_3 \) concentration. The calcium in the ER is assumed to be buffered and not able to diffuse. So, the contributions to the change in the calcium in the ER will be the release through \( IP_3 \) receptors, the \( Ca^{2+} \) ATP-ase pump in the ER membrane, and the ER leak. The change in intracellular calcium will come from diffusion, voltage-
gated calcium channels, the NMDA receptors, the buffering and release of $Ca^{2+}$ by the mitochondria, release from the ER via the $IP_3$ receptors, the ATP-ase pump in the ER membrane, and the ER leak. The model will take the form:

$$C_m V_t = \frac{a}{2r_i} V_{xx} - I_L - I_K - I_{Na} - I_{Ca} - I_{AMPA} - I_{NMDA}$$ (4.1)

$$p_t = D_p p_{xx} + k_y I_{AMPA} \left[ \frac{c}{c + K_3} \right] - k_b p$$ (4.2)

$$s_t = -k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] + k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_s s^2$$ (4.3)

$$c_t = D_c c_{xx} + f_1 I_{Ca} + f_2 I_{NMDA} + k_m \left[ 1 - \left( \frac{c}{K_4} \right)^{3.3} \right]$$ (4.4)

$$+ k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] + k_s s^2$$

$$V(x, 0) = V_{\text{rest}} ; p(x, 0) = p_0$$

$$s(x, 0) = s_0 ; c(x, 0) = c_0$$

$$V_x(0, t) = V_x(l, t) = 0$$

$$p_x(0, t) = p_x(l, t) = 0$$

$$c_x(0, t) = c_x(l, t) = 0$$
for

\[ 0 < x < l ; 0 < t \]

where \( s \) is the concentration of calcium in the ER, \( p \) is the cytosolic concentration of \( IP_3 \), \( c \) is the cytosolic concentration of calcium, \( V \) is the transmembrane potential, \( x \) is the distance from the end of the neuron, and \( t \) is time.

In the expression for \( p \), \( D_p \rho_p x \) measures the diffusion of \( IP_3 \) through the cytosol. The term \( k_\beta I_{AMP\,A} \left[ \frac{c}{c + K_3} \right] \) represents the change in \( IP_3 \) based on activation of the mGluR and the resulting cascade of processes which ultimately result in the production of \( IP_3 \). Since a measure of the glutamate binding to mGluR is provided by the AMPA current, \( k_\beta \) will convert from AMPA current to mGluR activation and account for an overall rate of formation of \( IP_3 \) from mGluR activation. Finally, \( -k_{k,p} \) accounts for the destruction of \( IP_3 \) by phosphatase.

Since \( Ca^{2+} \) does not diffuse in the ER, \( s \) has no diffusion term. The release of \( Ca^{2+} \) from the ER is a function of both the level of \( Ca^{2+} \) in the ER and the level of \( IP_3 \) in the cytosol available to bind to the receptor and is given by \(-k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right]\) where \( K_1 \) is the concentration at which half the \( IP_3 \) binding sites on the \( IP_3 \) receptor are filled and \( k_1 \) is the corresponding release rate. The rate at which cytosolic \( Ca^{2+} \) is pumped into the ER is a function of the concentration of cytosolic \( Ca^{2+} \) with Hill coefficient of 2: \( k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] \). There is a slow leak of \( Ca^{2+} \) out of the ER which is dependent on the square of the concentration of \( Ca^{2+} \) in the ER: \(-k_s s^2\).

When the ER gains \( Ca^{2+} \), it comes from the cytosol; when the ER loses \( Ca^{2+} \),
it goes into the cytosol. Thus, cytosolic $Ca^{2+}$ will change by $-s_t$. $D_{c_cxx}$ measures the diffusion of $Ca^{2+}$ through the cytosol. Cytosolic $Ca^{2+}$ is also gained through the voltage-gated $Ca^{2+}$ channels and the ligand-gated NMDA channels. By using $f_1$ to convert from current to the concentration change, $f_1 I_{Ca}$ measures the concentration change in $Ca^{2+}$ due to the voltage-gated $Ca^{2+}$ channels. Because $Ca^{2+}$ represents only a portion of the current flowing through the NMDA channels, $f_2$ must not only convert from current to the concentration change, but also multiply by the percentage of current owing to $Ca^{2+}$. The mitochondria sequesters $Ca^{2+}$ with the rate of $Ca^{2+}$ uptake dependent on the 3.3 power of cytosolic $Ca^{2+}$, while the rate of efflux is independent of the mitochondrial calcium level. So, for $K_4 = \text{concentration at which mitochondrial influx of } Ca^{2+} \text{ is equal to its efflux}$, the change in cytosolic $Ca^{2+}$ concentration due to the mitochondria is $k_m \left[ 1 - \left( \frac{c}{K_4} \right)^{3.3} \right]$.

The following sections of this chapter give the background of the above equations and show the results of the model. For a description of the model implementation, refer to Appendices B and C.

4.1 Derivation of Equations

4.1.1 Voltage Equation

The cable equation is a resistor-capacitor model of a neuron. It was developed by Hodgkin and Huxley in 1952 [15] and assumes that concentrations within the cytosol
of various ions remain constant. The resulting equation is

\[ C_m V_t = \frac{a}{2r_i} V_{xx} - \sum I_j \]

where \( V \) is the membrane potential (voltage), \( C_m \) is the membrane capacitance, \( a \) is the fiber radius, \( r_i \) is the axial resistivity, \( I_j \) are the cross-membrane ionic currents, and \( A \) is the membrane surface area.

Ions pass through the cell membrane by way of ion channels. Some ion channels are in one of four states at any given point in time: open or closed and either activated or inactivated. In the open and activated state, the channel allows specific ions to flow through. The most common progression is for a channel to start closed, then open, then become inactivated. It then becomes activated and is available to open again. Other ion channels have only a closed and an open state.

Many open ion channels are found to obey Ohm’s Law. When this is confirmed experimentally, the current through the open channel is proportional to the voltage drop across the membrane. So,

\[ I_L = g_L (V - V_L) \]

where \( g_L \) is the leak conductance of the membrane (through the channels) measured in milliSiemens per square centimeter. Since these channels are always open, \( g_L \) = conductance per channel * channel density. The potassium channel is also ohmic.
So

\[ I_K = g_K (V - V_K). \]

Now the potassium channel can be either open or closed. So the conductance \( g_K \) is the product of the conductance per open \( K \) channel times the density of \( K \) channels times the probability that a \( K \) channel is open at time \( t \). So

\[ g_K = G_K n^4(t, V) \]

for

\[ n'(t) = \alpha_n(V)(1 - n(t)) - \beta_n(V)m(t). \]

The functions \( \alpha_n \) and \( \beta_n \) are experimental fits to exponential functions.

The sodium channel can be open, closed or inactivated. To account for this, equations for both opening

\[ m'(t) = \alpha_m(V)(1 - m(t)) - \beta_m(V)m(t) \]

and activation

\[ h'(t) = \alpha_h(V)(1 - h(t)) - \beta_h(V)h(t) \]

are needed. Thus,

\[ I_{Na} = g_{Na}(V - V_{Na}) \]
and

\[ g_{Na} = G_{Na} m^3(t, V) h(t, V) \]

since a channel must be both open and activated to allow ions to pass. As before, \( G_{Na} \) is the conductance per open Na channel times the Na channel density.

Similarly, the transient \( Ca^{2+} \) current is given by

\[ I_{CaT} = G_{CaT} M^2 H(V - V_{Ca}). \]

The \( Ca^{2+} \)-dependent K channel has the same form

\[ I_{KCa} = g_{KCa} (V - V_K) \]

with

\[ g_{KCa} = G_{KCa} C^4(t, V, c). \]

Now, however, \( C(t, V, c) \) is dependent on the intracellular \( Ca^{2+} \) concentration as well as time and voltage.

Equations for \( \alpha_n, \beta_n, \alpha_m, \beta_m, \alpha_h, \beta_h, M, H, \) and \( C \) are given in Appendix A.

I will use the equations for \( I_{ampa} \) and \( I_{nmda} \) given in Rapp, et al. [33]:

\[ I_{ampa} = -g_{peak} \left( \frac{t}{t_{peak}} \right) \exp \left( 1 - \frac{t}{t_{peak}} \right) (V - V_{ampa}) \]
\[ I_{nmda} = -g_{nmda} \frac{\exp\left(-\frac{t}{\tau_1}\right) - \exp\left(-\frac{t}{\tau_2}\right)}{1 + \eta[Ca^{2+}] \exp\left(-\frac{\gamma}{V - V_{nmda}}\right)} (V - V_{nmda}). \]

Qian and Sejnowski [32] developed an electro-diffusion model that included the diffusion and drift of ions in the neuron. The electro-diffusion model does not assume a constant axial resistivity, but rather computes it based on the ion concentration. However, the electro-diffusion model does not account for the gating of the transmembrane currents. I will combine the diffusion and drift terms of the electro-diffusion model along with the variable axial resistivity with the gated transmembrane currents. Thus, the voltage model implemented will take the form:

\[ C_m V_t = \frac{aF10^6}{2} \left( \sum_{ions} Dz\xi n \right) V_{xx} + \sum_{channels} i_k + \frac{aF10^6}{2} \left( \sum_{ions} Dz n_{xx} \right) + \frac{aF10^6}{2} \frac{V_x}{V} \left( \sum_{ions} Dz\xi n_x \right) \]

\[ 0 < x < l ; 0 < t \]

\[ V_x(0, t) = V_x(l, t) = 0 \]

\[ V(x, 0) = V_{rest} \]

Initially the voltage model was implemented with spines at a uniform density. However, the spines provided no significant differences in the levels of \( Ca^{2+} \) and were removed.
For a description of the implementation and parameters used, see Appendix B.

4.1.2 Calcium Wave Equations

I begin with the simplified model of the interplay between calcium, $IP_3$, and the endoplasmic reticulum developed by Meyer and Stryer [26]. Meyer and Stryer developed a model cross-coupling $IP_3$ and cytosolic $Ca^{2+}$ which is strictly local and thus does not include diffusion or drift of ions. Also, the assumptions for binding kinetics lacks a basis in the biophysics of the receptors, relying instead on a fit to experimental data. The change in cytosolic calcium is determined in their model by a release from the ER (a function of the amount of calcium in the ER as well as the amount of $IP_3$ in the cytosol), and a flow back into the ER driven by the ATP-ase pump in the ER membrane (a function of the amount of cytosolic calcium and the amount of calcium in the ER). The change in $IP_3$ concentration is the result of additional $IP_3$ being generated by the receptor less the destruction of $IP_3$ by phosphotase. The equations they give are

$$c_t = k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_m \left( 1 - \left( \frac{c}{K_4} \right)^3 \right) + k_s s^2$$

$$p_t = k_4 R \left[ \frac{c}{c + K_4} \right] - k_h p$$

where $c$ is the cytosolic calcium concentration, $p$ is the $IP_3$ concentration, $s$ is the calcium concentration in the ER, and $R$ is the degree of receptor-dependent activation.
The model combines all the effects of the G-protein cascade on $IP_3$ generation into the $k_4R$ term.

The $IP_3$ induced flux of $Ca^{2+}$ out of the ER is the product of a rate constant, $k_1$, with the concentration of $Ca^{2+}$ in the ER and the fraction of open $IP_3$ channels. The fraction of open channels was approximated based on experimental data in rat basophilic leukemia (RBL) cells as $\frac{p^2}{(K_1 + p)^3}$ where $K_1$ is the $IP_3$ concentration with half the binding sites occupied.

An ATP-ase pump transports two $Ca^{2+}$ ions per reaction cycle into the ER and was thought to be proportional to the square of the cytosolic $Ca^{2+}$ concentration. The amount of $Ca^{2+}$ in the ER is limited, probably through a reversal of the ATP-ase pump. Meyer and Stryer accounted for the reversal with a negative term proportional to the square of the ER $Ca^{2+}$ level. Constants were fit from RBL experimental data.

Calcium is sequestered by the mitochondria when cytosolic $Ca^{2+}$ levels exceed a certain threshold, $K_4$. Experimental data suggests that the rate of entry of $Ca^{2+}$ into the mitochondria is dependent on the 3.3 power of the cytosolic calcium concentration and that the rate of efflux from the mitochondria is independent of the level of $Ca^{2+}$ in the mitochondria. So, the rate of $Ca^{2+}$ into the mitochondria is $k_m\left(\frac{c}{K_4}\right)^{3.3}$ while the efflux rate is $k_m$.

$IP_3$ production depends on activation of a G-protein receptor which leads to PLC activation. Studies show different levels of stimulatory effect of $Ca^{2+}$ on PLC activity. Assuming that PLC activity depends on both stimulation of the receptor and the $Ca^{2+}$
level, the rate of formation of \( IP_3 \) is given by \( k_1 R g \) where \( k_1 \) is the rate constant, \( R \) is the degree of receptor-dependent activation and \( g \) is the modulatory effect of cytosolic \( Ca^{2+} \). Meyer and Stryer assume a simple binding expression for \( g = \frac{c}{c + K_3} \). Constants again are fit from experimental data in RBL cells.

I modified their model to include the diffusion of \( Ca^{2+} \) and \( IP_3 \) within the cytosol and to track the amount of calcium contained in the ER. With \( D_c = \) diffusivity constant of \( Ca^{2+} \) and \( D_p = \) diffusivity constant of \( IP_3 \), the calcium wave model implemented takes the form:

\[
\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_m \left( 1 - \left( \frac{c}{K_4} \right)^{3.3} \right) + k_s s^2
\]

\[
p_t = D_p \frac{\partial^2 p}{\partial x^2} + k_4 R \left[ \frac{c}{c + K_3} \right] - k_h p
\]

\[
s_t = -k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] + k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_s s^2
\]

\[0 < x < l ; 0 < t\]

\[c_x(0, t) = c_x(l, t) = 0\]

\[p_x(0, t) = p_x(l, t) = 0\]

\[c(x, 0) = c_0 ; p(x, 0) = p_0 ; s(x, 0) = s_0.\]

For implementation details, see Appendix C.
4.2 Voltage Model Results

Implementation of the voltage equations with synaptic input location and sodium channel densities, shown in figure 4.1 resulted in a back-propagating action potential shown in figure 4.2.

Figure 4.1: Sodium Channel Density for Voltage Model
Figure 4.2: Back-propagating Action Potential initiated by synaptic input 3/4 of the way down the dendrite; Channel conductances used in \( \text{ms}^2/\text{cm}^2 \): \( g_{\text{L}} = 0.3 \), \( g_{\text{Na}} = 120 \), \( g_{\text{K}} = 36 \), \( g_{\text{Ca}} = 1.3 \), \( g_{\text{KCa}} = 40 \); NMDA channel parameters: \( g_{\text{NMDA}} = 0.05 \text{e} - 6 \text{ mS} \), \( \tau_1 = 80 \text{ ms} \), \( \tau_2 = 0.67 \text{ ms} \), \( \eta = 0.33 \text{ mM}^{-1} \), \( \gamma = 0.06 \text{ mV}^{-1} \); AMPA channel parameters: \( g_{\text{peak}} = 0.1 \text{e} - 6 \text{ mS} \), \( t_{\text{peak}} = 0.3 \text{ ms} \); other parameters in Appendix B.

The sodium, potassium, calcium-dependent potassium and leak currents associated with the synaptic input and the BPAP are depicted in figure 4.3.

The NMDA, AMPA, and voltage-gated \( Ca^{2+} \) currents are shown in figure 4.4.

Note that the calcium current is of the order \( 10^{-6} \mu A \) resulting from the BPAP.
Figure 4.3: Potassium, Sodium, Leak, and Calcium-dependent Potassium currents associated with synaptic input and BPAP
Figure 4.4: Calcium, AMPA, NMDA, and total currents associated with synaptic input and BPAP

The AMPA current occurs only at the time of the initial synaptic input, whereas the NMDA current peaks with the BPAP.

Associated concentration changes of sodium, potassium, and calcium are shown in figure 4.5. Note that the concentration change is highest where the sodium channel density is maximal. Also, note that the calcium concentration rises to about .15 mM as the result of the BPAP’s. Here, however, there is no ER buffering the calcium.
Figure 4.5: Concentrations of Sodium, Potassium, and Calcium associated with BPAPs

4.3 Calcium Wave Model Results

Implementation of the Meyer and Stryer equations produced the $Ca^{2+}$ waves shown below. I used a dendrite of length .1 cm divided into 128 compartments, $dt = .025$ s, degree of receptor-dependent activation of 0.14 everywhere except for 10 contiguous compartments $\frac{3}{4}$ of the way down the dendrite where it was set to 0.4. Initial levels of $IP_3$, $[Ca^{2+}]_{cyt}$, and $[Ca^{2+}]_{ER}$ were set to 0.01 $\mu$M, 0.1 $\mu$M, and 60 $\mu$M respectively. The receptor-dependent activation levels are shown in figure 4.6.
Figure 4.6: Receptor-Dependent Activation Levels for Calcium Wave Model

Figures 4.7 and 4.8 shows the calcium waves generated by the mGluR stimulus. Note that the wave is propagating at about $\frac{0.08 \text{ cm}}{70 \text{ s}} = 11.4 \mu\text{m/s}$ whereas Jaffe and Brown measured the propagation at about $40 \mu\text{m/s}$ ([17]). Also, the calcium concentration peaks at about $2 \mu\text{M} = 0.002 \text{ mM}$ whereas the calcium concentration resulting from the BPAP peaked at 0.15 mM. So the models give about 100 times the concentration from repeated BPAP's than from the $IP_3$ generated calcium wave. The calcium concentration peak of $2 \mu\text{M}$ resulting from the calcium wave model is consistent with the experimental data from Zhou and Ross ([42]) of calcium levels of
Figure 4.7: Cytosolic Calcium waves generated from the interplay of calcium in the cytosol with IP3 levels and calcium in the ER.

several micromolar. However, the calcium level resulting from the BPAP in the voltage model is much higher, while Zhou and Ross found experimentally that it is much smaller than the level resulting from the calcium wave.
Figure 4.8: Cytosolic Calcium Concentration - another view

The $IP_3$ concentration also exhibits waves as demonstrated in figures 4.9 and 4.10.
Figure 4.9: IP3 waves resulting from the generation of IP3 from a G-protein cascade and the interplay of IP3 with calcium
Figure 4.10: IP3 waves - another view

The corresponding change in the level of calcium in the ER, shown in figure 4.11, appears to be driven by the change in the \( IP_3 \) level.
Figure 4.11: Changes in the level of calcium stored in the ER as a result of the interplay with cytosolic calcium and IP3.
Chapter 5

Future Work

I will build a predictive model for understanding modulation of excitability in neurons by \( Ca^{2+} \) at differing time scales. To show the modulation at a fast time scale, the model must include ion channels which depend upon \( Ca^{2+} \) concentrations to show how neuronal excitability is suppressed by after-hyperpolarizing potentials or enhanced by depolarizing after-potentials. At a slower time scale, the timing and amplitude of \( Ca^{2+} \) waves must be related to the pattern of synaptic inputs. On the pre-synaptic side, the model should show how the ER store and release of \( Ca^{2+} \) affects neurotransmitter release rates.

In particular, a future model will include:

1. cell geometry changes

2. inclusion of \( Ca^{2+} \) dependent currents
3. channel and receptor distributions to match experimental data

4. drift terms in concentration equations

5. buffering of calcium by other compounds in the cytosol

6. \( \text{Na}^{+}/\text{Ca}^{2+} \) exchanger and ATP-ase pump in cell membrane

7. channel kinetics for release of \( \text{Ca}^{2+} \) from the ER.

8. G-protein cascade interactions resulting in \( IP_3 \) production

9. Neurotransmitter release

The current model includes only a dendrite of uniform radius attached to a soma. A more realistic model of the geometry including tapered dendrites would result in a more complete picture. Also, the distribution of ion channels within the cell needs to reflect experimental values. I will modify the calcium currents to reflect experimental distribution of different types of calcium channels and other calcium-dependent afterhyperpolarizing potassium currents.

Rather than including only an ohmic transient calcium current, the model should use the different types of calcium currents known to exist in neurons. These currents are not ohmic. Thus rather than using the ohmic equation, \( I_{Ca}(V) = g_{Ca} (V - V_{Ca}) \), a future model should use the Goldman-Hodgkin-Katz current equation:

\[
I_{Ca}(V) = P_{Ca} z F \xi V \left( \frac{[Ca^{2+}]_{in} \exp(\xi V) - [Ca^{2+}]_{out}}{\exp(\xi V) - 1} \right)
\]
Like Na\(^+\) channels, Calcium channels are activated by depolarization, have inward current at normal membrane potentials, and have some degree of inactivation. The model should include an appropriate distribution of the four main types of calcium currents: L, T, N, P. Hopefully, this change will provide calcium concentrations that fit the experimental data more closely.

We will add the contribution to the membrane currents of a calcium-dependent after-hyperpolarizing \(K\) current, \(I_{K(\text{AHP})}\). This channel generates a long, slow hyperpolarization following a single action potential which is even more prominent following a train of APs.

\[
I_{K(\text{AHP})} = m_{Ca} g_{K(\text{AHP})}(V_m - V_K)
\]

where \(m_{Ca}\) is dependent only on \([\text{Ca}^{2+}]_{in}\) [19].

Factors for the buffering of calcium by other chemicals and into structures other than the ER should be incorporated along with the contributions due to the Na\(^+\)/Ca\(^{2+}\) exchanger in the cell membrane.

Rather than using a simplified model of the \(IP_3, \text{Ca}^{2+}\), and ER interactions, a future model should incorporate the work by Goldbeter [13], DeYoung and Keizer [9], and Li and Rinzel [24], combining contributions from these models to make the interplay reflect the biophysics of the channels and receptors.

The Goldbeter model includes the cooperative effect of \(IP_3\) and \(\text{Ca}^{2+}\) on release of \(\text{Ca}^{2+}\) from the endoplasmic reticulum, the endoplasmic reticulum pump, a leak from the endoplasmic reticulum, an input of \(\text{Ca}^{2+}\) into the cell and a linear transport
of $Ca^{2+}$ from the cytosol to the extracellular medium.

Li and Rinzel produce a simplified model based on the kinetics of the $IP_3$ receptor. This model includes an ATP-ase pump that takes calcium up into the ER, a leak of calcium from the ER and release of calcium through the InsP$_3$R channel. Each subunit of InsP$_3$R has 3 binding sites: one for InsP$_3$ and 2 for Ca$^{2+}$ (an activation site and an inactivation site). In order for the calcium to flow out, the InsP$_3$R must be in the 1,1,0 state (InsP$_3$ site and Ca$^{2+}$ activation site bound, Ca$^{2+}$ inactivation site not bound). The Li and Rinzel equations are:

$$c_t = -c_1 v_1 m^3 h^3 (c - s) - c_1 v_2 (c - s) - \frac{v_3 c^2}{k^2_s + c^2}$$

$$h_t = \frac{h_\infty - h}{\tau_h}$$

where

$$m_\infty = \left( \frac{p}{p + d_1} \right) \left( \frac{c}{c + d_5} \right)$$

$$\tau_h = \frac{1}{a_2 (Q_2 + c)}$$

$$h_\infty = \frac{Q_2}{Q_2 + c}$$

$$Q_2 = d_2 \left( \frac{p + d_1}{p + d_3} \right)$$

$c_1$ is the percentage of calcium in the cell that is not free in the cytosol which is stored in the endoplasmic reticulum and $\nu_1, \nu_2, \nu_3, k_3, a_2, d_1, d_2, d_3, d_5$ are various rate
constants, and $h$ is the fraction of channels not yet inactivated by calcium. This model, however, does not include diffusion for the calcium.

DeYoung and Keizer provides the basis for the Li and Rinzel model and contributes the following equation for the change in $IP_3$ concentration in the neuron:

$$p_t = v_4 \left( \frac{c + (1 - \alpha)K_3}{c + K_3} \right) - k_hp$$

where $\alpha$ reflects the strength of the dependence of the $IP_3$ rate on calcium concentration, $v_4$ is the maximum rate of $IP_3$ production, $K_3$ is the dissociation constant for $Ca^{2+}$ stimulation and $k_h$ is the rate constant for degradation of $IP_3$ by phosphotase.

The differential equations representing the series of actions required for the metabotropic glutamate receptor to generate $IP_3$ should replace the simplified term $k_4R \left[ \frac{c}{c + K_3} \right]$ in the current set of equations. My current understanding of this process is documented in Appendix D.

Calcium waves have been successfully generated and the voltage equation produces the expected result. However, there are many unanswered questions. How will the coupling of the two models affect the results? What are the correct distributions and types of calcium channels and how does that effect the priming of the ER? Which calcium channels are most important for generation of the calcium waves and are they essential for the wave or just for the loading of the ER? What types and locations of mGluR participate in generation of the calcium wave? What are the timing
and location requirements of mGluR activation necessary to raise the $IP_3$ levels? What are the local and global levels of cytosolic calcium and $IP_3$ needed for wave propagation? What is the threshold of calcium required in the ER? Is there a role for the ryanodine receptor in the propagation of calcium waves? What are the locations and densities of the $IP_3$ receptor? What is the role of the mitochondria, $Na^+/Ca^{2+}$ exchanger, and the ATP-ase membrane pump in the interplay between fast and slow signaling?
Appendix A. Gating Variable

Equations

The equations used in computing the gating variables are:

\[ \alpha_n(V) = 0.01 \frac{10 - (V - V_{rest})}{\exp\left(1 - \frac{V - V_{rest}}{10}\right) - 1} \]

\[ \beta_n(V) = 0.125 \exp\left(\frac{-(V - V_{rest})}{80}\right) \]

\[ \alpha_m(V) = 0.1 \frac{25 - (V - V_{rest})}{\exp\left(2.5 - \frac{V - V_{rest}}{10}\right) - 1} \]

\[ \beta_m(V) = 4 \exp\left(\frac{-(V - V_{rest})}{18}\right) \]

\[ \alpha_h(V) = 0.07 \exp\left(\frac{-(V - V_{rest})}{20}\right) \]

\[ \beta_h(V) = \frac{1}{\exp\left(3 - \frac{(V - V_{rest})}{10}\right) + 1} \]

\[ M_\infty(V) = \frac{1}{\exp\left(\frac{-(V + 57)}{6.2}\right) + 1} \]
\[ \tau_M(V) = 0.612 + \frac{1}{\exp\left(\frac{-(V+132)}{16.7}\right) + \exp\left(\frac{(V+16.8)}{18.2}\right)} \]

\[ H_\infty(V) = \frac{1}{\exp\left(\frac{V+81}{4}\right) + 1} \]

\[ \tau_M(V) = \begin{cases} 
\exp\left(\frac{V+467}{66.6}\right) & \text{if } V < -80 \\
28 + \exp\left(\frac{-(V+22)}{10.5}\right) & \text{if } V \geq -80 
\end{cases} \]

\[ C_\infty(V, [Ca]) = \left(\frac{\frac{[Ca]}{[Ca] + 3e - 3}}{\exp\left(\frac{-(V+28.3)}{12.6}\right) + 1}\right) \left(\frac{1}{\exp\left(\frac{-(V+28.3)}{12.6}\right) + 1}\right) \]

\[ \tau_C(V) = 90.3 + \frac{75.1}{\exp\left(\frac{-(V+46)}{22.7}\right) + 1} \]

The \( \alpha \) and \( \beta \) functions are taken from Hodgkin and Huxley's work on the squid axon [15]. The equations for \( M \) and \( H \) are taken from Huegenard and McCormick's work in the thalamic relay neuron [16] while the \( C \) equations are taken from the Turrigiano et al. work on stomatogastric ganglion neurons [38].
Appendix B. Voltage Equation

Implementation

I implemented the differential equations for voltage concentrations in Matlab. The geometry modeled is a cylindrical fiber of length $ell$ discretized into $N$ compartments, each of radius $a$. The transmembrane currents used are the gated ohmic currents for sodium, potassium, leak, calcium, and calcium-dependent potassium. Channel densities are assumed to be uniform except for sodium. Currents from AMPA and NMDA receptors are included.

The model uses hybrid Euler finite differences to approximate a solution to the voltage equations:

$$C_m V_t = \frac{aF10^6}{2} \left( \sum_{ions} Dz\xi n \right) V_{xx} + \sum_{channels} i_k$$

$$+ \frac{aF10^6}{2} \left( \sum_{ions} Dz n_{xx} \right) + \frac{aF10^6}{2} V_x \left( \sum_{ions} Dz\xi n_x \right)$$
where $a$ is the fiber radius, $C_m$ is the membrane capacitance, $D$ is the ion-specific diffusivity constant, $z$ is the valence of the ion, $n$ is the ion concentration, and $\xi = \frac{Fz}{RT}$ ($R$ = gas constant, $T$ = absolute temperature).

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Model parameters and constants related to $V$ equation:

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<td>Conc_out(1)</td>
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<tr>
<td>Conc_out(3)</td>
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<td>Purves [31]</td>
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<tr>
<td>z(1)</td>
<td>valence of Na</td>
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<tr>
<td>z(2)</td>
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<tr>
<td>z(3)</td>
<td>valence of Ca</td>
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<td>R</td>
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Other model parameters and constants related NMDA, AMPA currents:

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<td>Cox</td>
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<td>ms</td>
<td>Rapp [33]</td>
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<td>mS</td>
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<td>eta</td>
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<td>(\text{mM}^{-1})</td>
<td>Rapp [33]</td>
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<tr>
<td>gam</td>
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<td>Rapp [33]</td>
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<td>Cox</td>
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<tr>
<td>V_AMPA</td>
<td>AMPA receptor reversal potential</td>
<td>30</td>
<td>mV</td>
<td>Cox</td>
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</tbody>
</table>

**Code Overview**

Set synaptic input at the patch 3/4 of the way down the dendrite

Vary the densities of the Na channels by factor of 1 for patches 1-10; 2.5 for patches 11-30; 1 for patches 31 - 40; .5 for patches 41- 60; and .4 for patches 61-N.

Compute VRest using the Goldman-Hodgkin-Katz voltage equation:

\[
V_{rest} = \frac{RT_{\text{emp}}}{F} \log \left( \frac{\sum (P) (Conc_{out})}{\sum (P) (Conc)} \right).
\]

Compute reversal potentials for Na, K, and Ca channels using

\[
Nernst = \frac{RT_{\text{emp}}}{zF} \log \left( \frac{\text{Conc}_{\text{out}}}{\text{Conc}} \right).
\]
Compute diameter, surface area of fiber compartment and space step length:

\[ d = 2a \]
\[ A = \pi dd x \]
\[ dx = \frac{ell}{N}. \]

Convert Conc, D, Conc_out, Z into arrays.

Initialize V to Vrest.

Initialize gating variables m, h, n:

\[ n_0 = \frac{\alpha_m(V_{rest})}{\alpha_m(V_{rest}) + \beta_m(V_{rest})}. \]

Initialize gating variables M, H, C:

\[ M_0 = M_\infty(V_{rest}). \]

Set up matrices for 2nd order finite difference:
\[
B_2 = \frac{1}{(dx)^2} \begin{bmatrix}
-1 & 1 \\
1 & -2 & 1 \\
1 & -2 & 1 \\
. & . & . \\
1 & -2 & 1 \\
1 & -2 & 1 \\
1 & -1
\end{bmatrix}
\]

and 1st order finite difference:

\[
B_1 = \frac{1}{2dx} \begin{bmatrix}
-2 & 2 \\
-1 & 0 & 1 \\
-1 & 0 & 1 \\
. & . & . \\
-1 & 0 & 1 \\
-1 & 0 & 1 \\
-2 & 2
\end{bmatrix}
\]

Initialize figures.

For each time step:

increment time step

compute R\_i from current concentrations
\[ R_{-i} = \frac{1}{\sum 10^6 F^2 DZ^2 \text{Conc} / \text{RTemp}} \]

compute gating variable values using hybrid Euler

for \( z = m, n, \) and \( h: \)

\[
\frac{dz}{dt} = \alpha_z(v)(1 - z) - \beta_z(v)z
\]
\[
\frac{z_j - z_{j-1}}{dt} = \alpha_z(v_{j-1})(1 - z_j) - \beta_z(v_{j-1})z_j
\]
\[
z_j = z_{j-1} + dt \left[ \alpha_z(v_{j-1}) - \alpha_z(v_{j-1})z_j - \beta_z(v_{j-1})z_j \right]
\]
\[
z_j[1 + dt(\alpha_z(v_{j-1}) + \beta_z(v_{j-1})]] = z_{j-1} + (dt) \alpha_z(v_{j-1})
\]
\[
z_j = \frac{z_{j-1} + (dt) \alpha_z(v_{j-1})}{1 + (dt) (\alpha_z(v_{j-1}) + \beta_z(v_{j-1}))}
\]

for \( M, H, \) and \( C: \)

\[
\frac{dz}{dt} = \frac{z_\infty(v) - z}{\tau_z(v)}
\]
\[
\frac{z_j - z_{j-1}}{dt} = \frac{z_\infty(v_{j-1}) - z_j}{\tau_z(v_{j-1})}
\]
\[
\tau_z(v_{j-1}) (z_j - z_{j-1}) = dt \left[ z_\infty(v_{j-1}) - z_j \right]
\]
\[
z_j (\tau_z(v_{j-1}) + dt) = \tau_z(v_{j-1})z_{j-1} + (dt) z_\infty(v_{j-1})
\]
\[
z_j = \frac{\tau_z(v_{j-1})z_{j-1} + (dt) z_\infty(v_{j-1})}{\tau_z(v_{j-1}) + dt}
\]
compute gated conductances

\[ g_K = n^4 g_{_K} \]
\[ g_{Na} = m^3 h g_{_Na} \]
\[ g_{Ca} = M^2 g_{_Ca} \]
\[ g_{KCa} = c^4 g_{_KCa} \]

compute conductance through AMPA, NMDA channels

\[ g_{AMPA} = g_{peak} \left( \frac{t}{t_{peak}} \right) \exp \left( 1 - \frac{t}{t_{peak}} \right) \]
\[ \exp \left( \frac{-t}{\tau_1} \right) - \exp \left( \frac{-t}{\tau_2} \right) \]
\[ g_{NMDA} = g_{nmda} \frac{1 + \eta [Mg] \exp(-\gamma(V - V_{nmda}))}{1 + \eta [Mg] \exp(-\gamma(V - V_{nmda}))} \]

compute diffusion term:

\[ Diff = \frac{10^6 Fa}{2} \sum Dz(B2) \text{Conc} \]

compute drift term (except for multiplication by \( V_x \)):

\[ Drift = \frac{10^6 F^2 a}{2RT_{\text{emp}}} \sum Dz^2(B1) \text{Conc} \]
solve

\[
\begin{align*}
\left[ \left( \frac{C_m}{dt} + \sum g_k \right) I - \frac{a}{2R_i} B2 - Drift \ast B1 \right] V &= \frac{C_m}{dt} V_{old} + \sum g_k V_k + Diff \\
\end{align*}
\]

find current / cm² for each ion

\[i_k = -g_k(V - V_k) \frac{\mu A}{cm^2}\]

and current per compartment for each ion

\[I_k = i_k A\]

find value for Conc (concentrations) at this time step from:

\[Conc = Conc - \frac{2 \ast 10^{-6} i_k}{a F z} dt\]

compute new Nernst potentials:

\[Nernst = \frac{R Temp}{z F} \log \left( \frac{Conc_{out}}{Conc} \right)\]

plot voltages, concentrations and currents if at multiple of pinc

Label plots
Appendix C. Calcium Wave

Implementation

I implemented the differential equations for \( IP_3 \) and \( Ca^{2+} \) concentrations in Matlab. The geometry modelled is a cylindrical fiber of length \( \ell \) discretized into \( N \) compartments, each of radius \( a \).

The model uses hybrid Euler finite differences to approximate solutions to the following equations:

\[
c_t = D_c c_{xx} + k_{1s} \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_m \left( 1 - \left( \frac{c}{K_4} \right)^{3.3} \right) + k_s s^2
\]

\[
p_t = D_p p_{xx} + k_A R \left[ \frac{c}{c + K_3} \right] - k_h p
\]

\[
s_t = -k_{1s} \left[ \frac{p^3}{(K_1 + p)^3} \right] + k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] - k_s s^2
\]
So, with $B_2$ = matrix for 2nd order difference in spatial direction,

$$
\frac{p_k - p_{k-1}}{dt} = D_p B_2 p_k + k_4 R \left( \frac{c_{k-1}}{c_{k-1} + K_3} \right) - k_h p_k
$$

$$
p_k = p_{k-1} + (dt) \left( D_p B_2 p_k + k_4 R \left( \frac{c_{k-1}}{c_{k-1} + K_3} \right) - k_h p_k \right)
$$

$$
[(1 + (dt)k_h) I - (dt)D_p B_2] p_k = p_{k-1} + (dt)k_4 R \left( \frac{c_{k-1}}{c_{k-1} + K_3} \right)
$$

Similarly,

$$
\frac{s_k - s_{k-1}}{dt} = -k_1 s_k \left( \frac{p_k^3}{(K_1 + p_k)^3} \right) + k_p \left( \frac{c_{k-1}^2}{(K_2 + c_{k-1})^2} \right) - k_s s_{k-1}^2
$$

$$
s_k = s_{k-1} + (dt) \left( -k_1 s_k \left( \frac{p_k^3}{(K_1 + p_k)^3} \right) + k_p \left( \frac{c_{k-1}^2}{(K_2 + c_{k-1})^2} \right) - k_s s_{k-1}^2 \right)
$$

$$
\left( 1 + (dt)k_1 \left( \frac{p_k^3}{(K_1 + p_k)^3} \right) \right) s_k = s_{k-1} + (dt) \left( k_p \left( \frac{c_{k-1}^2}{(K_2 + c_{k-1})^2} \right) - k_s s_{k-1}^2 \right)
$$

Since all the terms involved in the change in $s$ are also included in the change in $c$,
let \( s_{ch} = s_k - s_{k-1} \). Then

\[
\frac{c_k - c_{k-1}}{dt} = D_c B_2 c_k - k_m \left( 1 - \left( \frac{c_{k-1}}{K_4} \right)^{3.3} \right) + k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] + k_s s^2
\]

\[
c_k = c_{k-1} + (dt) D_c B_2 c_k - (dt) k_m + (dt) \left\{ k_m \left( \frac{c_{k-1}}{K_4} \right)^{3.3} + k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] + k_s s^2 \right\}
\]

\[
(I - (dt) D_c B_2) c_k = c_{k-1} - (dt) k_m + (dt) \left\{ k_m \left( \frac{c_{k-1}}{K_4} \right)^{3.3} + k_1 s \left[ \frac{p^3}{(K_1 + p)^3} \right] - k_p \left[ \frac{c^2}{(K_2 + c)^2} \right] + k_s s^2 \right\}
\]

**Inputs to model**

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<th>suggested value</th>
<th>units</th>
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<td>N</td>
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<td>dt</td>
<td>timestep</td>
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<td>s</td>
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<td>T</td>
<td>stopping time of simulation</td>
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<td>s</td>
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Model parameters and constants:

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<td>k_s</td>
<td>rate for ER leak of Ca</td>
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<td>$\mu\text{M}/\text{s}$</td>
<td>Meyer [26]</td>
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<tr>
<td>K1</td>
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<td>$\mu\text{M}$</td>
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<td>Meyer [26]</td>
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<td>initial ER Ca conc.</td>
<td>60</td>
<td>$\mu\text{M}$</td>
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</table>

**Code Overview:**

Set synaptic input beginning at the patch 3/4 of the way down the dendrite and continuing for 10 compartments by setting $R = R\_\text{high}$ for those compartments and $R = R\_\text{low}$ everywhere else.

Compute space step length $dx = \frac{ell}{N}$.

Initialize IP$_3$ and Ca concentrations
Set up matrix for 2nd order finite difference:

\[
B_2 = \frac{1}{(dx)^2} \begin{bmatrix}
-1 & 1 \\
1 & -2 & 1 \\
& 1 & -2 & 1 \\
& & . & . & . \\
1 & -2 & 1 \\
& 1 & -2 & 1 \\
& & 1 & -1 \\
\end{bmatrix}
\]

Compute portions of left hand sides which will not change with each time step:

\[
B_p = (1 + (dt)k_{\text{h}})I - (dt)D_pB_2
\]

\[
B_c = I - (dt)D_cB_2
\]

Initialize figures.

For each time step:

increment time step

compute new IP3 concentration due to diffusion, activation by mGluR, and decay

\[
B_p p = p + (dt)k_4(R) \left( \frac{c}{c + K3} \right)
\]
compute new ERCa concentration due to pump, leak, and IP3 receptors

\[ \left[ 1 + (dt)k_1 \left( \frac{p}{p + K_1} \right)^3 \right] s = s + (dt)k_p \left( \frac{c}{c + K_2} \right)^2 - k_s s^2 \]

compute new CytCa concentration due to diffusion, ER, and mitochondria

\[ B_c c = c + (dt) \left\{ k_1 \left( \frac{p}{p + K_1} \right)^3 - k_p \left( \frac{c}{c + K_2} \right)^2 + k_s s^2 + k_m \left[ 1 - \left( \frac{c}{K_4} \right)^{3.3} \right] \right\} \]

Label plots
Appendix D. IP3 Generation

Process

My current understanding of the cascade resulting in the production of IP3 is diagrammed below. However, the state changes need to be verified and rate constants determined.

Glutamate (G) binds to the metabotropic glutamate receptors (R) at a rate of $k_{ntb}$ (NeuroTransmitterBind) resulting in an activated receptor (AR) with an exposed G-protein binding site. The glutamate can unbinds at a rate $k_{ntu}$ (NeuroTransmitterUnbind) and can either rebind or diffuse in the synaptic cleft at rate $k_d$ (diffuse).

$$
{\begin{array}{c}
 k_{ntb} \\
 \text{R} + \text{G} \xrightarrow{k_{ntb}} \text{AR} \\
 \xleftarrow{k_{ntu}} \\
\end{array}}
$$

The G-protein $G_q$ binds to the exposed G-protein binding site of the activated metabotropic glutamate receptor (AR) at a rate of $k_{Gb}$ (G-protein Bind) and unbinds at a rate $k_{Gu}$ (G-protein Unbind). When the G-protein is bound to the receptor, it
is in the state GBR for G-protein Bound Receptor.

\[
\begin{align*}
\frac{\dot{k}_{GB}}{k_{GB}} \\
AR + Gq & \xrightarrow{k_{GB}} GBR \\
\end{align*}
\]

The alpha subunit of the G-protein which is bound to the receptor exchanges its GDP for GTP, activating the G-protein. When this occurs, the G-protein dissociates from the receptor and splits into an alpha subunit with GTP (\(\alpha\)GTP) and the \(\beta\gamma\) subunit. This leaves the receptor in the activated, but not GTP-bound state. It is ready to now activate another G-protein. The rate in which the GDP is exchanged for GTP and the subunits dissociate is \(k_{Ga}\) for G-protein activation.

\[
\text{GBR} + \text{GTP} \xrightarrow{k_{Ga}} \alpha\text{GTP} + \beta\gamma + \text{GDP} + \text{AR}
\]

The alpha subunit with GTP attached works with calcium (Ca) to activate phospholipase C (PLC) at a rate \(k_{Pa}\) (PLC Activate) without using up the Ca and \(\alpha\)GTP. The activated PLC (APLC) becomes inactivated at rate \(k_{Pi}\) (PLC Inactivate).

\[
\alpha\text{GTP} + \text{Ca} + \text{PLC} \xrightarrow{k_{Pa}} \text{APLC} \xrightarrow{k_{Pi}} \text{PLC}
\]

The activated PLC cleaves PIP into DAG and IP_3 at rate \(k_c\) ( cleave). The activated PLC remains intact to cleave other PIP units. Once cleaved, the IP_3 and DAG are assumed not to rebind.

\[
\text{APLC} + \text{PIP} \xrightarrow{k_c} \text{DAG} + \text{IP}_3 + \text{APLC}
\]
The alpha subunit with GTP attached exchanges the GTP for GDP at rate $k_{\alpha i}$ ($\alpha$ inactivate).

$$\alpha\text{GTP} + \text{GDP} \xrightarrow{k_{\alpha i}} \alpha\text{GDP} + \text{GTP}$$

The alpha subunit with GDP attached is now able to combine with a $\beta\gamma$ subunit to reform the G-protein $G_q$ at the rate $k_{Gc}$ (G-protein combine). This G-protein is now available to attach to an exposed G-protein binding site.

$$\alpha\text{GDP} + \beta\gamma \xrightarrow{k_{Gc}} G_q$$

The above state changes give rise to the following set of differential equations by applying mass action equations:

$$\frac{d(AR)}{dt} = k_{ntb}(R)(G) - k_{ntu}(AR)$$
$$+ k_{Ga}(GBR) - k_{Gb}(AR)(Gq)$$
$$+ k_{Ga}(GBR)(GTP)$$

$$\frac{d(R)}{dt} = k_{ntu}(AR) - k_{ntb}(R)(G)$$

$$\frac{d(G)}{dt} = k_{ntu}(AR) - k_{ntb}(R)(G) - k_d(G)$$

$$\frac{d(Gq)}{dt} = k_{Ga}(GBR) - k_{Gb}(AR)(Gq) + k_{Gc}(\alpha\text{GDP})(\beta\gamma)$$
\[
\frac{d(\text{GBR})}{dt} = k_{Gb}(\text{AR})(\text{Gq}) - k_{Ga}(\text{GBR}) - k_{Ga}(\text{GBR})(\text{GTP})
\]

\[
\frac{d(\text{GTP})}{dt} = k_{ca}(\alpha \text{GTP})(\text{GDP}) - k_{Ga}(\text{GTP})(\text{GBR})
\]

\[
\frac{d(\alpha \text{GTP})}{dt} = k_{Ga}(\text{GTP})(\text{GBR}) - k_{ca}(\alpha \text{GTP})(\text{GDP})
\]

\[
\frac{d(\beta \gamma)}{dt} = k_{Ga}(\text{GTP})(\text{GBR}) - k_{Ge}(\alpha \text{GDP})(\beta \gamma)
\]

\[
\frac{d(\text{GDP})}{dt} = k_{Ga}(\text{GTP})(\text{GBR}) - k_{ca}(\alpha \text{GTP})(\text{GDP})
\]

\[
\frac{d(\text{PLC})}{dt} = k_{P_t}(\text{APLC}) - k_{Pa}(\text{PLC})(\text{Ca})(\alpha \text{GTP})
\]

\[
\frac{d(\text{APLC})}{dt} = -k_{P_t}(\text{APLC}) + k_{Pa}(\text{PLC})(\text{Ca})(\alpha \text{GTP})
\]

\[
\frac{d(\alpha \text{GDP})}{dt} = k_{ca}(\alpha \text{GTP})(\text{GDP}) - k_{Ge}(\alpha \text{GDP})(\beta \gamma)
\]

\[
\frac{d(\text{PIP})}{dt} = -k_{c}(\text{PIP})(\text{APLC})
\]

\[
\frac{d(\text{DAG})}{dt} = k_{c}(\text{PIP})(\text{APLC})
\]

\[
\frac{d(\text{IP3})}{dt} = k_{c}(\text{PIP})(\text{APLC})
\]
Bibliography


[29] Paul Pfaffinger, NEUR 415 Course notes, Fall, 2002.


