Lab 2, Ion Channel Simulations

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Introduction

While a larger goal of projects such as the Pysiome Project and the Virtual Physiological Human project are to develop tissue, organ, and system models for human Physiology, smaller scale models such as ion channel models are a vital part of these projects.[1] Mathematical and computational models of Ion channels provide a framework for highly controlled investigation of the properties of these channels as well as the combination of vast amounts of experimental data into a single comprehensive model. The modeling of ion channels contributes greatly to an effort to integrate and interpret experimental data as well as provide a highly detailed way to perform precise and otherwise technically impractical hypothesis testing on such a fine scale.

While there are a vast variety of modeling techniques that range from basic current modeling with Hodgkin and Huxley to computationally intensive atomic scale molecular models, more frequently single ion channels are modeled using a Markovian chain of states model.[1][2] A Markovian model is one in which the next state of the model only depends on the current state, irrespective of the previous states. In the case of ion channels, Markovian models are typically structured into different closed and open states with rate constants that determine the probability of transitioning from one state to another. Each of these different states define different properties about the ion channel in the simulation, such as its permeability to ions, thus the effect on the conductance of those ions.[1] In the most simple case of a two state Markovian ion channel model there is an open state and a closed state. At any given time the model may transition from closed to open or open to closed based on two rate constants, an open to closed rate, and a closed to open rate. If the closed to open rate constant were higher, this would describe a channel has a higher probability of being in the open state as opposed to the closed state. By increasing the number of states these models can be used to describe more complex behaviors such as channels with inactive states or drug/ligand binding that modulate activity. Each of these different state transitions have add rate coefficients describing the transitions between those states and other states, and thus a complex network can be built up.

Each of the parameters that comprise the rate coefficients must be determined. Typically the values used come from direct experimental data from the channels of interest. This data includes structural information gained from crystallography, imaging data, genomic analysis, and perhaps most frequently from patch clamp electrical recordings. A patch clamp is a technique for assaying the electrical behavior of cells down to the single ion channel level. Either a specific cell expressing the channels of interest, such as a neuron or a cardiomyocyte, or a cell that has been made to express the channel of interest is isolated. A glass needle is used to either perforate or isolate a small section or patch of a cell membrane. In the case of perforation this allows access to the intracellular environment whereas in the case of isolation this allows access to the extracellular or intracellular membrane surface, depending on the preparation. In either case the concentrations of ions and other materials can be readily changed in the bath and within the needle. The voltage, injected current, or ion concentrations are then varied, and the resulting response of the ion channels can be measured via electrodes in the needle and extracellular space. Other parameters about the channels are derived from the structural information gained by crystallography and computational analysis given the amino acid sequence of the channels. These analyses are limited by the ability to isolate and crystallize the channels and the computation power and algorithms available to computer the atomic and amino acid scale interactions and models.

As a result of years of work in this field on many different ion channels, vast amounts of data has been generated and funneled into constructing mathematical and computational models. The implementation of these models can often be difficult, however there are a variety of packages designed to incorporate the many different ion channel models into an centralized interface to allow for fast and robust testing of the models. Such libraries as CellML and applications like Jsim utilize these ionic models to allow researchers to perform robust simulations and tests with these models.

In order to efficiently implement these various complex ion channel models, languages such as CellML have been developed. CellML is a markdown language built on top of XML as a way to standardize the development and sharing of models such as ion channel models. CellML allows researchers to easily share their models and parameters between different modeling environments, facilitating collaboration

and distribution of developed models and findings. CellML itself is under active development and new improvements and tools are routinely implemented. Utilizing CellML as a standard language for writing models allows them to be easily shared and implemented in other settings. This significantly cuts down the time it takes to implement new models or test changes and additions to existing ones. Without a standard language like CellML the progress in developing and improving ion channel models would be hindered. For this lab we utilized Jsim as a simulation environment. Jsim is a java based simulation software developed by the Pysiome project for use in computational models and simulation based on experimental data.[1] Jsim can read in CellML files and run the appropriate simulations, then display results in an interactive interface. Through Jsim, model parameters can be exposed for manipulation and testing, allowing for rapid and robust exploration of the model and effects of different parameters. Jsim allows for the testing of hypotheses and exploration of models in a lightweight, computationally inexpensive format that does not require significant experience or expertise with regards to developing CellML models.

During this lab assignment we were required to simulate and manipulate membrane potential and ionic currents as well as a Markovian potassium channel model developed for a cardiac fibroblast.[3] Utilizing Jsim and the CellML cardiac fibroblast model developed by Dr. Frank Sachse we were able to explore the model and investigate the results from changing the different parameters such as ion conductances, stimulation protocols, and rate constants for the different Markovian states. Additionally the robust visualizations available through Jsim allowed for rapid visualization and interpretation of the model outputs and the effects of our changes and tests.

Methods

1:Electrical Modeling of Membranes

During this section we explored the relationship between the voltage at the membrane out our cardiac fibroblast and different stimulation protocols. Additionally we investigated the effects of ion currents on these membrane voltages.

1.1

For the first investigation we loaded the fibroblast CellML model described in [3] and configured it to be a purely capacitive system. To do so we set all of the ionic and background conductances to zero. Specifically those parameters were the background conductance (Gb) the potassium conductance (Gkir) and the potassium shaker conductance (Pshkr). Next we set the stimulation to begin at 0.1 seconds with a duration of 1 second, an amplitude of 0.2 nA and a frequency of 1 Hz. The simulation was then ran for 6 seconds and the membrane voltage was plotted as a function of time. The system we have made here is a capacitor-current source system.

1.2

For the next section we took our purely capacitive cell from 1.1 and added a background (Gb) conductance of $1 \times 10^{-3} \mu S$. We also changed the reversal potential (Eb) to -84 mV. We then ran the same stimulation protocol as before with stimulation to begin at 0.1 seconds with a duration of 1 second, an amplitude of 0.2 nA and a frequency of 1 Hz. After 6 seconds of simulation we plotted membrane voltage, and membrane currents as a function of time. The system we created here is a resistor-capacitor system with a current source.

2: Markovian Models of Ion Channels

During these next sections we manipulated the parameters of the potassium shaker channel Markov model present in the fibroblast model.

2.1

First we adjusted the base fibroblast model to be capacitive and only have the potassium shaker current (Pshkr). The potassium (Gkir) conductance was set to 0, the background (Gb) conductance was set to zero, and the potassium shaker conductance was set to the default value of $5.4 \times 10^{-9} \mu S$. The reversal potential was set to -85 mV and the stimulus was set to begin at 0.1 seconds with a duration of 1 second, and a frequency of 1 Hz. The amplitude of the stimulation was adjusted to cause a membrane voltage peak of $+50$ mV during the simulation. This value was found to be 0.55 nA. The simulation was run for 0.5 seconds, and the membrane voltage, as well as the Markovian states of all of the Pshkr ion channel model were plotted.

2.2

We then took the model generated in 2.1 and modified it to delay the peak of the Pshkr O Markovian state by roughly 20 mS while maintaining a peak above 40%. To do so we modified the values of the Markovian rate constants that dictated the transitions between the states. Specifically by lower the rate constants that dictated the transition between all of the closed states, as well as the closed to open states we could slow the progression through the states thereby delaying the opening peak. However, in order to make sure that the opening still had a significant peak value we needed to make sure to closed to open rate was sufficiently high to allow a quick state change once the model had reached that open to close transition, thereby allowing for a high peak for the open state.

Results and Discussion

1.1

In figure 1 we see the results of the purely capacitive fibroblast with periodic stimulation. On the left in the figure we see the parameters used for this simulation and on the right we see the membrane voltage plotted as a function of time. As a result of setting all conductances to zero, thereby inhibiting any ionic or other current flow, we see that the membrane does not repolarize back to the starting or resting potentials as we might expect from a typical cell model. This demonstrates the necessity of some sort of conductance to be present in order for typical membrane repolarization to occur after a stimulus. Over the course of this simulation the membrane voltage steps incrementally from roughly -58 mV, (Which was set as the initial membrane potential) to roughly 200 mV in steps of roughly 50 mV.

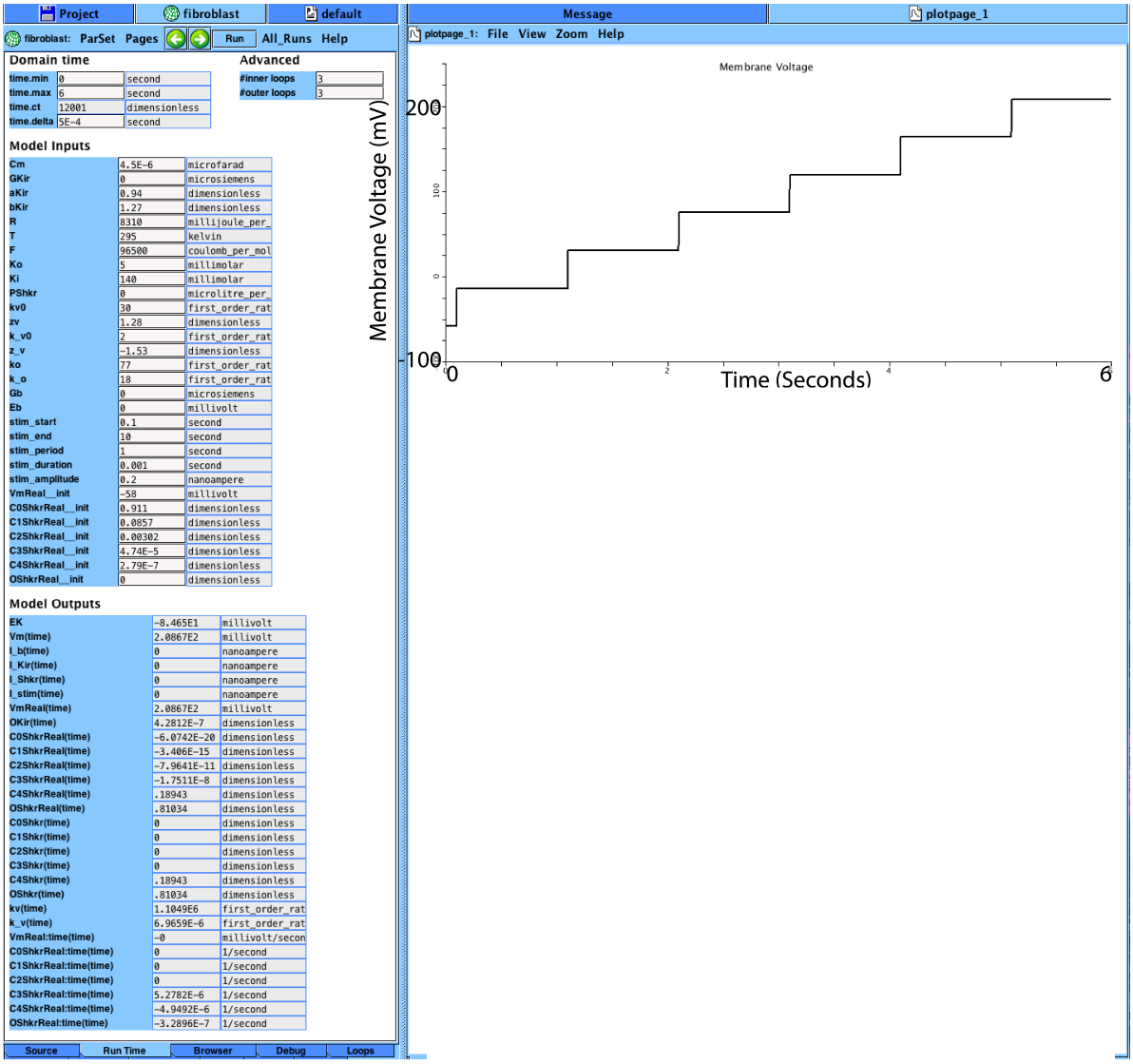

Figure 1: A screen capture of the parameters and output from Jsim for the first section. All conductances have been set to 0 μ S and the stimulation protocol has been set according tot he methods. As can be seen the voltage incrementally steps from roughly -58 mV to roughly 200 mV.

During this simulation we see that the membrane voltage (Vm) increases stepwise with each stimulus of injected current. This is because there are no ion channels present to allow for the current injected to flow across the membrane. By creating this purely capacitive system we are essentially only charging the capacitor with each stimulus, but not allowing any path for the accumulated charge to flow. Thus the membrane (or capacitor) voltage simply increases a fixed amount with each stimulation. Thus we can see from this experiment that the absence of any ion conductances, and therefor the absence of any functional ion channels in this membrane blocks the ability of the membrane to repolarize. The system we have created can be thought of schematically as described in figure 2 wherein our current source charges a capacitor in short bursts but does not afford any path of discharge from the capacitor. In other words, when the current source is turned on (during our stimulu, according to the protocol) the capacitor charges. When the current source is off the capacitor does not charge, but because there is no path for the capacitor to discharged it sayes at the charge voltage from the previous stimulus. Now when the current source turns on it can charge the capacitor further.

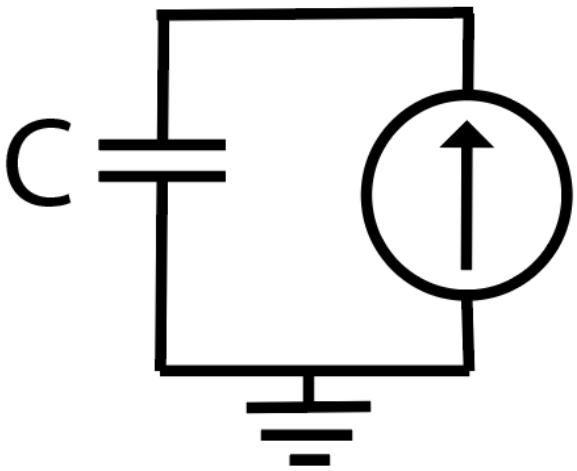

Figure 2: A circuit model of the simulation from 1.1. A current source provides energy to charge the capacitor C at a regular interval, but there is not path for the capacitor to discharge, thus the capacitor voltage will only increase with each successive injection of current from the current source.

1.2

In figure 3 we see the input and output for 1.2. By taking the previously capacitive model from 1.1 and adding a background conductance of $1 \times 10^{-3} \mu S$ we now see that the membrane voltage does not simply accumulate. Instead the membrane voltage jumps during the stimulation, but due to the background conductance, a current is allowed to pass through the membrane, allowing the membrane potential to repolarize back to the resting potential. The depolarization begins at 0.1 seconds (the onset of the first stimulus) and the repolarization completes by about 0.103 seconds. The peak voltage is roughly -74 mV, after which it returns to -84 mV.

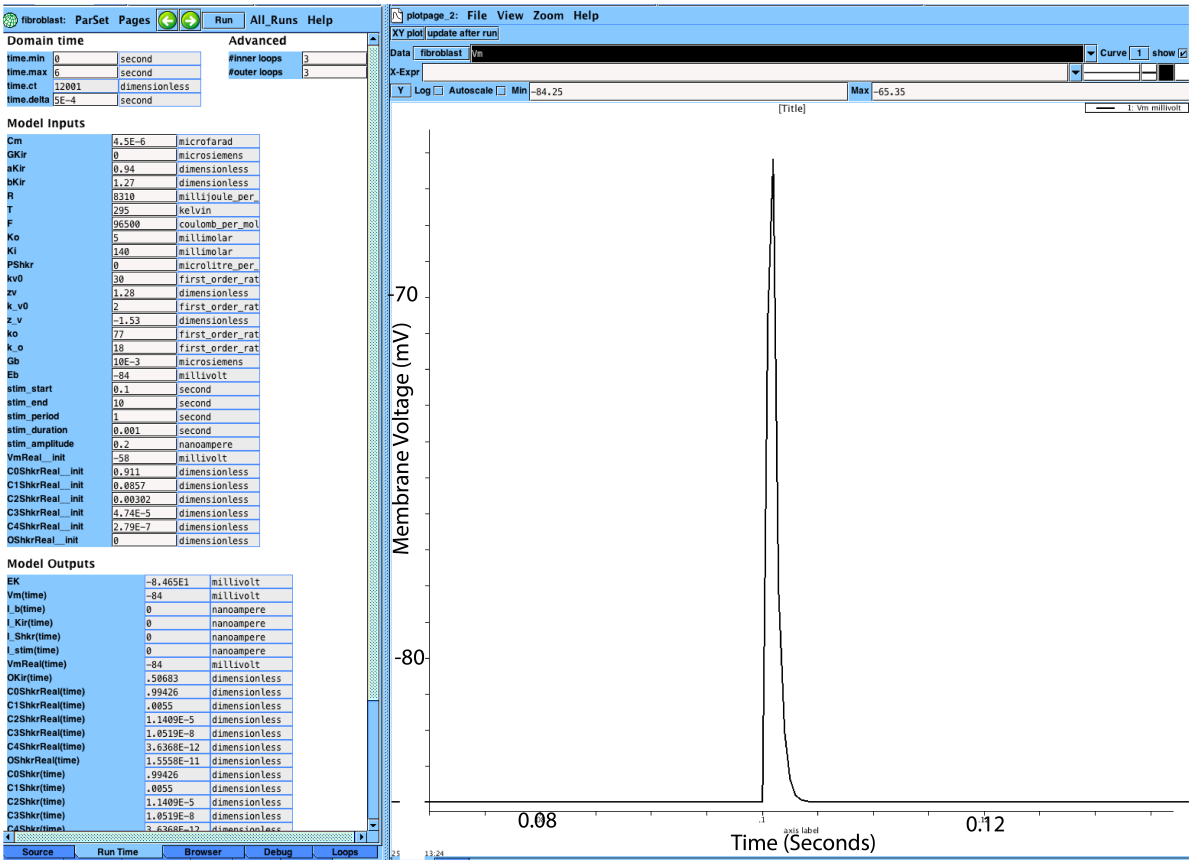

Figure 3: This figure shows the screen capture of the Jsim parameters and plotted membrane voltage for 1.2. Note that the Gkir and Pshkr conductances are set to 0 μS, the reversal potential (Eb) is set to -84 mV, and the background conductance (Gb) is set to $1 \times 10^{-3} \mu S$

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The addition of the background current prevents the star stepping voltage we saw in 1.1. This is becuase we have given the voltage generated by the stimulus a path to discharge and repolarize via the background conductance. Thus our capcitor in our circuit model now has a method of discharge. This can be through of as adding a resistor in parallel with the capacitor, as seen in figure 4. The current source charges the capacitor when it is on (additionally while the current source is on, during stimulus, some current flows through the resistor, additionally the voltage on the capacitor during each stimulus is partly deterined by the resitance of the resistor, which is why we see a lesser voltage jump as compared to that seen in figure 1). When the current source is off we now have a capacitor charged to some voltage in parallel with a resistor. This provides a path of discharge for the capacitor through the resistor. The rate of discharge can be seen in the sloped discharge in figure 3 and can be calculated via equation 1. The value of that resistor is $1/\text{Gb}\ \omega$, thus in this case 1 KΩ.

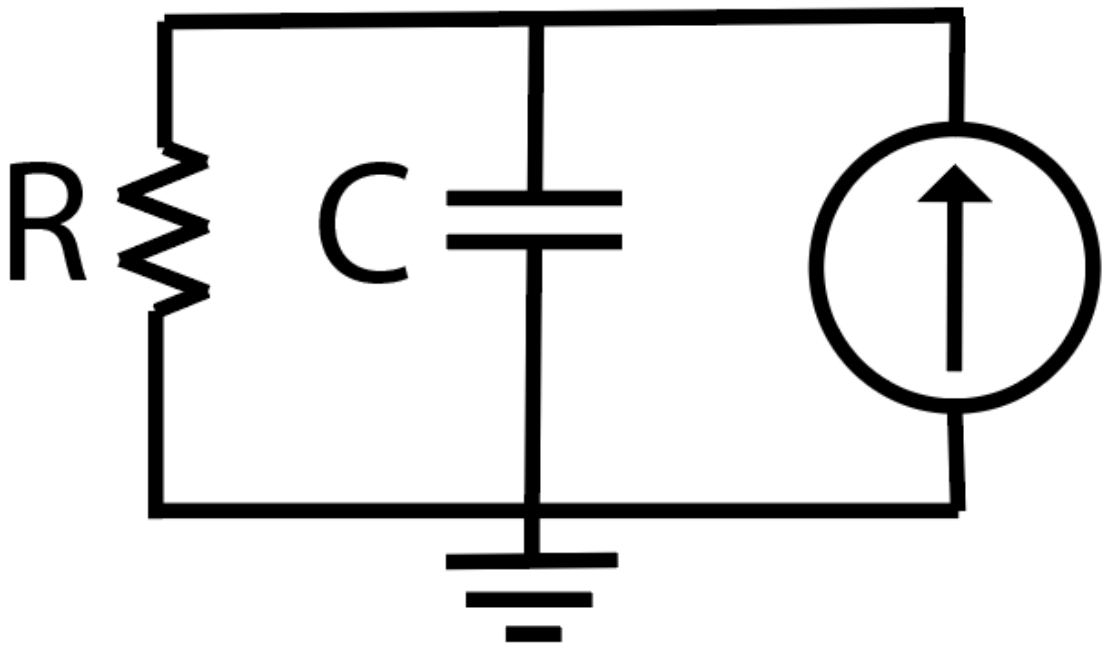

Figure 4

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$$V(t) = V_0 e^{t/RC} \tag{1}$$

2.1

2.2

Conclusions

References

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[2] K. Kojima, H. C. Watanabe, S. Doi, N. Miyoshi, M. Kato, H. Ishikita, and Y. Sudo, "Mutational analysis of the conserved carboxylates of anion channelrhodopsin-2 (ACR2) expressed in Escherichia coli and their roles in anion transport," *Biophysics and Physicobiology*, vol. 15, pp. 179–188, sep 2018. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6194965/ https://www.ncbi.nlm.nih.gov/pmc/PMC6194965/ https://www.ncbi.nlm.nih.gov/pubmed/30349802

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