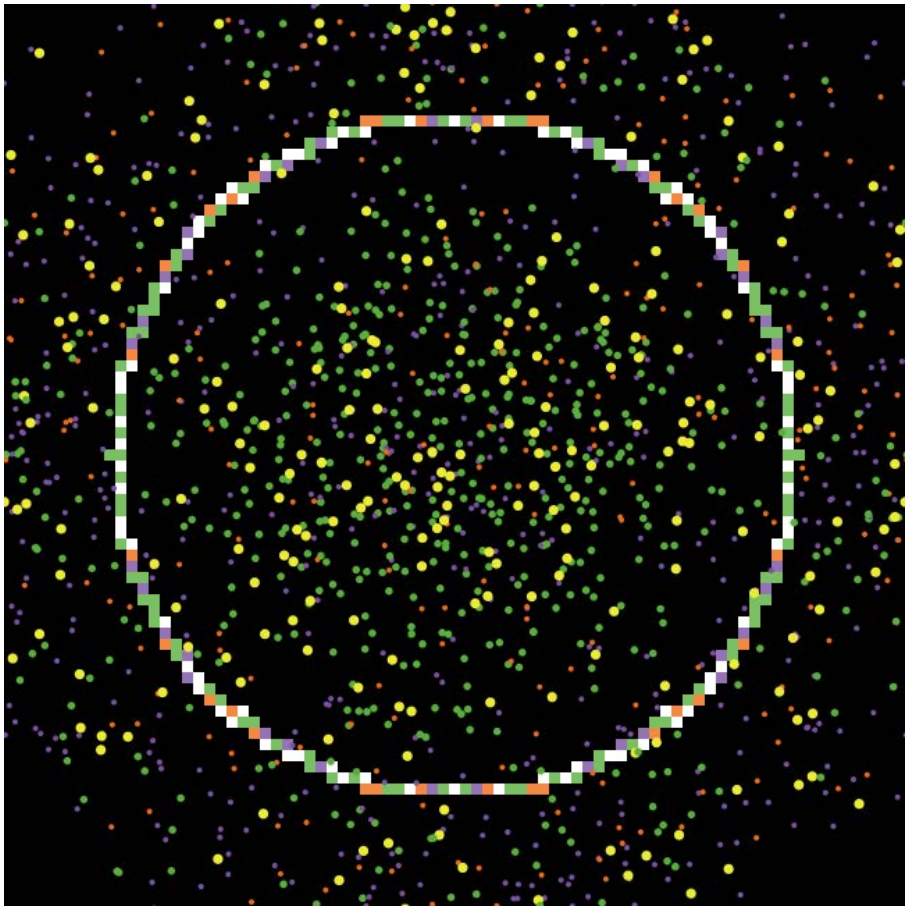
Neuron

SINGLE CELL NEURON ELECTROPHYSIOLOGY AT THE IONIC LEVEL



Brendan Frick
Northwestern University – McCormick School of Engineering
Computer Science, Integrated Science Program and Neuroscience Majors
EECS 372
Professor Uri Wilensky
Spring 2015

An Agent Based Model of a Single Cell Neuron

I designed an agent based model of a neuron at the ionic level. My hypothesis is that it is possible to make several abstractions defining the rules of intracellular neuron behavior and still predict accurate electrophysiological behavior. I plan to validate my model by eliciting an action potential and tail current solely by changing the ionic concentrations and channel densities. All agent rules closely resemble scientifically accepted formulas and definitions. My model will visualize the mechanisms that produce electrophysiological patterns from specific cell environment and membrane compositions. The emergent pattern in this model is the system transitioning through recognizable local equilibria states with kinetics that are replicated in *in vivo* systems.

Background

A single neuron is a complicated system. In this model, I simplified the system so that only the behaviors discussed in this section influence the system. This section is in not a comprehensive description of neuron behavior but serves to provide enough background to understand this model. Fully understanding neural electrophysiology requires a lot of time and appropriate coursework in biology, chemistry, and physics. This section is intended to help the reader better understand **membrane potential**, **voltage gating**, and **action potentials** at the conceptual level as they apply to this model.

Neuron Electrophysiology

Neurons are nervous system cells that process and transmit information through electrical and chemical signals. This model, in its current state, looks solely at electrical signaling. Electrical signals are produced by the flow of charged ions in and out of the cell. The most common ions in a neuron environment are K^+, Na^+, Ca^{2+}, and Cl^-. Cells will transmit information

based on the electrophysiological state at the membrane, which is represented by the membrane potential. The membrane potential is defined by the Nernst Equation below and is important for predicting action potentials and determining channel states of voltage gated ions. The Nernst Equation describes the membrane potential as a logarithmic ratio of net negative charge outside the cell over the net negative charge inside the cell.

$$V_m = \frac{RT}{z_x F} \ln\left(\frac{[X]_O}{[X]_I}\right)$$

Figure 1: Nernst Equation

Voltage Gated Channel

Neurons, like all cells are constrained by an impermeable cell membrane consisting of a lipid bilayer. Within the lipid bilayer exists membrane channels that pass ions either into the cell or out of the cell. Specific channels are ion specific and direction specific (due to ion concentration gradients) in most cases. One class of channels are ligand gated. This means that the channels are opened and closed by signaling molecules (such as neurotransmitters). These not in the model because they are more complicated and may distract from voltage gating affects.

A second class of channels are voltage gated. This means that the channel undergoes conformational changes to an open or closed state at certain membrane potential threshold. In an open state, the channel can pass ions that fit its selectivity parameters, in a closed state, the channel is impassable. An inactive state also exists. The state change to inactive occurs when a cell remains open for long periods of time. Before opening again, the inactive channel must close. The state diagram is shown below, and can be implemented with a Markov Model.

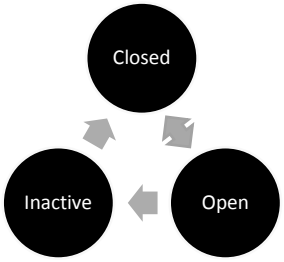


Figure 2: Channel state diagram that can be described with Markov Chain

Action Potential

The electrophysiological behavior of neurons are dictated by two factors: 1) the ionic environment of the cell and 2) the composition of the cell membrane. One such behavior that can be conditionally produced is an action potential. An action potential is a brief electrophysiological event in which the membrane potential rapidly rises (depolarization) and falls (repolarization). This event sends an impulse down the axon of the neuron where it transmits the signal (in terms of bits, high impulse/ low impulse, per time unit) to other neurons.

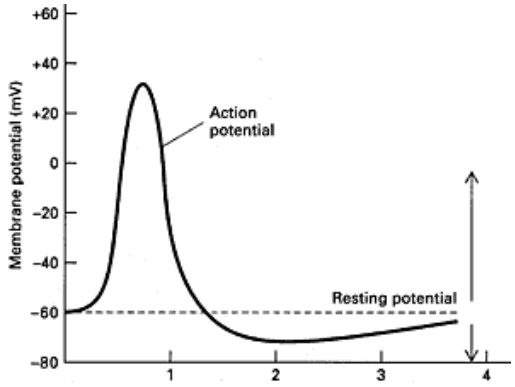


Figure 3: Action potential sketch. Note the peak at + 30 mV and the rectifying current at -70mV (Marban, E.)

Action potentials occur because of the voltage gating properties of Na^+ and K^+ channels. At membrane potentials above -30 mV Na^+ channels will transition from closed to open. At +30mV, Na^+ channels rapidly inactivate. At +30 mV, K^+ channels will open. Somewhere around -60 mV, K^+ channels will close. Because of these properties, neurons are able to reach action potentials to transmit information. However, if the density of a type of channel is too high or too low, the action potential might not occur. A full list of channels, with their voltage gating properties will be included in the *Implementation* section.

Reaching action potential is only possible if the ionic environment is suitable. In an action potential inducing environment, Na^+ is highly concentrated extracellularly and K^+ is highly concentrated intracellularly. So, when Na^+ channels open, the membrane potential rapidly rises until it caps at about +30 mV when Na^+ channels inactivate. The rapid decline starts as K^+

channels open and K^+ flows down the concentration gradient, reducing the membrane potential. If the environment is not suitable, say the Na^+ concentration is too high intracellularly, the concentration gradient will not be steep enough to rapidly pull enough Na^+ into the cell before the channels inactivate.

The conditions for an action potential are highly specific. For example, cells with a lower concentration density of Na^+ channels will be less likely to fire action potentials because the membrane potential will rise less rapidly. There are many more factors and behaviors involving action potentials but most are beyond the scope of this model. For this model, we are only trying to show the rapid rise and decline of membrane potential followed by a rectifying tail current (caused by closure of standard K^+ channels and inward rectifying K^+).

Neuron Summary

In the context of this model, neurons can differ by ionic environment and membrane composition. The combination of these will determine the ability of the cell to reach action potential. If the channels are modelled accurately, and ionic movement through the channels models ionic flow *in vivo* accurately, then manipulating these two factors should determine if the cell's membrane potential over time follows the same pattern as an action potential. If this pattern is replicated it demonstrates that by setting suitable conditions, ionic movement and membrane channel behavior alone can create a reconizable emergent pattern.

Motivation

As described in the *Background* section, single cell neurons are complex systems with many independent variables coming together to form an emergent pattern. There are three thematic reasons for pursuing this model:

1) To help researchers visualize how neuron parameters can effect electrophysiological patterns. This model will help researchers propose and test feasible mechanisms for experimentally observed phenomena
2) To help students learn the fundamentals of single cell electrophysiology. This model will help students learn which channels contribute to electrophysiological events like action potentials and how they contribute.
3) The regression from *in vivo* cell to ABM cell is easy to implement relative to the complex patterns that emerge. This is a system that can easily be defined as a couple of classes and governing rules, making it the perfect system for agent based modelling.

Neuroscience Research Implications

In 1952, Hodgkin and Huxley published groundbreaking research regarding action potentials in neurons (Hodkin and Huxley, 1952). Their hypothesized model explains the ionic mechanisms underlying the generation of action potentials in a squid axon. They derived their model through experimentation by varying extracellular concentrations of K^+ and Na^+ and measuring the net current of the cell over time. Over 60 years later, researchers are using the same scientific method to derive mechanistic models for electrophysiology - scientists propose a mechanism for some phenomenon and then test the electrophysiological response to a set cell environment. Procedures have improved, like the ability to record from single channels, block channels, and genetically manipulate channel concentration, but researchers still build a

hypothesized mechanism by testing and defining constraints. There is visualization of the mechanisms other than what the researchers visualize mentally.

This model can be used to manipulate cell conditions and see what the outcome is. Researchers can set up experimental environments, with specific membrane compositions, to see what *in vivo* experiments are worth pursuing. Genetic manipulation is expensive, time-consuming, and prone to error, so an accurate model would be an asset to researchers. Researchers can go one step further and introduce agents that are incapable of being replicated *in vivo*. Additionally, this model provides perspective to help researchers visualize the mechanisms they are working with and see the potential for new mechanisms. If a researcher can clearly visualize the mechanisms, they are more likely to see potential for new experimentation.

Neuroscience Education Aid

Students in introductory neuroscience classes spend over half of a semester learning about the electrophysiological properties of neurons. A large component of this is learning how different types of channels contribute to electrophysiological events like action potentials. Course instructors teach students the timeline of an action potential, different cell environments, and different cell membrane compositions. By the end of the introductory course, the students should be able to visualize how channels will affect the electrophysiological response of the cell.

If teachers were able to demonstrate how different ion environments and membrane compositions were able to affect membrane potential at specific points in time, students may have an easier time understanding the thermodynamics and kinetics of the system.

In vivo to ABM Regression

A single cell neuron is a perfect candidate for agent based modelling, especially in NetLogo. The electrophysiological system of a neuron is composed of moving particles (ions and

ligands) and stationary particles (membrane segments and channels).These can be represented as turtles and patches respectively. If these agents are defined as the fundamental elements of a neuron, then there are no other elements that affect the electrophysiological system. Even better, all four agents can be described by simple sets of rules.

Ions *in vivo* are nonreactive, have constant charge and size, and follow simple electromagnetic rules of motions. Ligands work the same way, except for that they react with a set of defined proteins. Ligand reactions are relatively simple and predictable. Membrane segments are nonreactive, impassable elements. Finally, channels are the most complex elements. However, the properties can be easily described by computational functions. Voltage gating can be described by a probability function. State transitions can be taught by Markov chains. Channel direction and selectivity can be simple functions or constants. All values have been established experimentally. Implementing values for all channel varieties would be out of the scope of this course, but would be possible. Calibrating the probability function for voltage gating and defining the Markov chains state transitions is also possible with more time.

Implementation

To implement this model in NetLogo, ions are turtles and membrane segments are patches.

Ion Implementation

Classifier, charge, size, and location are attributed to ions when they are created. Each step, ions interact with each other, interact with channels, and move randomly. Ions can interact with other ions by colliding with them or ionically attracting/repulsing one other ion in a nearby radius based on the respective charge. When ions get near channels, they are pulled towards them. If the selectivity parameters fit, the ion is passed through and the location (“int”/”ext”) is

switched appropriately. Random movement leads ions towards the cell membrane, with semi-random headings to prevent overcrowding by the membrane. This is justifiable because ions naturally gravitate towards the membrane because it is polarized. Without this random movement towards the membrane, channel interactions do not occur frequently and the membrane potential is not dynamic. The user can manipulate intracellular and extracellular concentrations of each type of ion.

Channel Implementation

Channels are membrane segments, impassable to ions, that have been converted to let certain classes of ions pass in a certain direction, if the channel is in an open state. Channels are responsible for attracting ions, passing ions, and updating states based on membrane potential. This model only included voltage-gated channels which I have listed below

Channel Name	**Voltage Gate**	**Direction**	**Relevant**
K^+	~ 20-30 mV	Outward	Decline from action potential peak
Leak K^+	None	Outward	Maintaining outward current
Inward Rectifying K^+	~ -60 mV	Inward	Tail current back to equilibrium
Na^+	~ -30 mV	Inward	Incline to action potential peak
L-Type Ca^{2+}	~ 10 mV	Inward	Action potential and signaling
T-Type Ca^{2+}	~ -10 mV	Inward	Action potential and signaling

Figure 4: Table detailing approximate voltage gating properties and relevance to action potential events

In the draw-cell function, I have encoded these voltage gating patch properties for each channel. The user set densities determines the number of each type of channel.

One aspect I had trouble with was the state transitions. I could not find a function that effectively transitioned state from “open” to “inactive.” Effectively in the cell, when the membrane potential is +20 mV, most Na^+ cells are inactive. I could not find a probability

function that did not ruin the kinetics of the system. Instead, I set the to “inactive” transition to occur over a certain threshold. This is justified because this is effective for sodium channels. In extensions, I would develop a sophisticated Markov Chain with real probabilities for state changes based on experimental data. However, I have not seen an accurate source.

Analysis

If the neuron is implemented correctly, I should be able to input realistic ion concentrations (high extracellular Na^+ and intracellular K^+) and manipulate the cell membrane composition and achieve realistic results. If I have mostly Na^+ and K^+ channels, the neuron will be more likely to fire because Na^+ channels will bring the membrane potential up, opening up K^+ channels which will bring the membrane potential back down to rest. If you include a lot of Leaky K^+ channels, which are always open, the cell will never fire because the outward current is too strong. If you include a lot of inward rectifying K^+ channels the cell will rebound quickly to equilibrium if the cell falls below -65 mV. All of these situations were tested with relative success in the model, which lends me to believe that the model is able to accurately represent electrophysiological behavior. The most distinguishable behavior is the action potential.

Due to some limitations with my model, including the lack of ligand gated channels, we are unable to see a full action potential in one trial. Ligand gated channels kick-start the upward climb of membrane potential, opening Na^+ channels that accelerate that climb. Therefore we started the cells at ~ -20 mV (where Na^+ channels are open). Another limitation was the kinetics of repolarization was too slow. After potassium channels opened, the cell was already flooded with Na^+ and it made it hard for K^+ to reach the membrane to pass through channels. However the repolarization was clear, and in a less hindered cell, the repolarization completed. In future

models I will have to come up with a better way for ions to travel in a crowded space, or make it less likely that the space becomes crowded.

Below is a sketch of an *in vivo* action potential and the NetLogo outputs from an environment with 150 mM K^+ intracellular, 150 mM Na^+ extracellular, 15 mM Ca^{2+} extracellular, 25% density K^+ channels, 25% density Na^+ channels, 6% density Leak K^+ channels, 6% density Inward Rectifying K^+ channels and 13% density $Ca2^+$ channels,

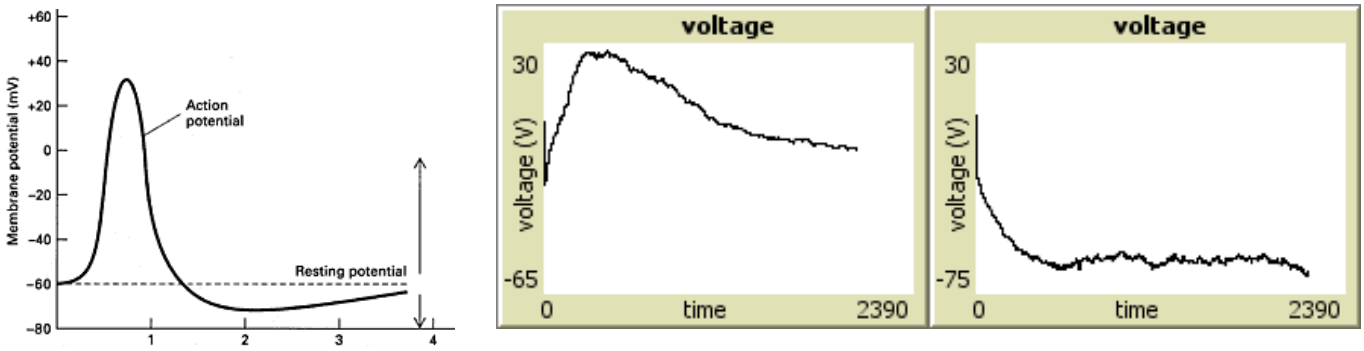


Figure 5: Comparison of action potential sketch to two NetLogo recordings. The left NetLogo image demonstrates the Na^+ inactivation and K^+ opening, while the right demonstrates the ineward rectifying tail current

As you can see the depolarization (upward climb) is replicated well, the inactivation of Na^+ channels coupled with opening of K^+ channels at +30 mV is clear with the local maximum. While the downward repolarization is less steep, it is apparent. This is a matter of intracellular kinetics, not thermodynamics so it is most likely a problem with ion overcrowding. When the membrane potential is allowed to drop it never reaches below -65 mV because the inward rectifying current does its part to rapidly rectify a current that falls below. This is the tail current back to resting potential. The tail current from NetLogo is not as smooth as the sketch. If the Markov Chain is calculated correctly, the probability distribution would smooth the tail current to look more like the sketch.

Conclusion

The model proves the hypothesis that is possible to make several abstractions defining the rules of intracellular neuron behavior and still be able to predict accurate electrophysiological behavior. The model is rough and not entirely accurate in terms of kinetics. In order to make a model that is feasible to use in scientific settings, kinetic and probability factors need to be calibrated so the model can exactly describe an electrophysiological event. However, the purpose of this model is to show that computationally modeling a neuron with ABM is possible. I have shown that by defining rules for ionic movement and parameters for membrane permeability, you can produce complex emergent electrophysiological patterns that model real life phenomena.

Next iterations of this model will include more diverse agent sets to better represent a neuron, and more carefully calibrated calculations and distributions so that the agent rules and behavior is identical to cells *in vivo*. Once ligands and ligand-gated channels are added, the model will be able to fully represent the elements involved in mechanisms that produce electrophysiological events. This will also make the model more relevant to research because most of modern molecular electrophysiology deals with signaling pathways. Ligand gated channels will also allow the model to implement neurotransmitters so that the model can demonstrate post-synaptic neural connections.

In terms of accuracy and precision, the most critical aspect that needs retooling is the states kinetics. The state chain that describes channel state is delicate and highly specific or each channel. It is this precision and specificity that leads to such complex yet predictable electrophysiological behavior. Once these issues are addressed, and more features are added to create a more realistic model, this model may have real implications in electrophysiological research.

Works Cited

"Addiction." *NIH Office of Science Education* N.p., n.d. Web 07 June 2015

Bear, Mark F., Barry W. Conners, and Michael A. Paradiso. *Neuroscience:Exploring the Brain*. 3rd ed. New York: Lippincott Williams and Wilkins, 2007. Print.

Eva Horne et al., *Principles of Biology*. OpenStaxCNX. 07 Junes, 2015.

Hodgkin A.L, Huxley A.F. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J Physiol. 1952

Marbán, E. Cardiac channelopathies. *Nature* 415, 213-218 (2002).

"Neuronal Action Potential." *PhysiologyWeb*. N.p., n.d. Web. 07 June 2015.