NeuroSim

Simulation of membrane potentials, action potentials, and synaptic potentials.

This simulation exercise is designed to increase your understanding of the factors affecting the membrane potential, the local responses, the action potentials, and the synaptic potentials. The simulation program has been created by Professor Nanna MacAulay and Stud. BioMed. Eng. Jonas Uno Kristian Mortensen, partly based on the equations published by Hodgkin and Huxley in 1952.

In the action potential simulations, we use the Hodgkin Huxley framework and treat a patch of neuronal membrane as a simple electrical circuit consisting of a capacitor in parallel with three resistive branches that represent a fast voltage-gated Na^+ channel, a voltage-gated, delayed rectifier K^+ channel, and a constant K^+ leak pathway. By sketching this circuit, we can apply basic electrical theory, specifically Kirchhoff's current law, to derive a single differential equation for the membrane voltage. The resistive branches are then replaced by conductances that vary with voltage and time according to the Hodgkin Huxley rate equations. Together these equations describe how the membrane charges, generates an action potential, and returns to rest while leaving out unnecessary molecular detail.

Access to the simulation program can be found here: https://neurosim.ku.dk/

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OPERATING INSTRUCTIONS

Introduction

NeuroSim is a computer program that models the basic electrical properties of neurons, axons, and dendrites.

Different aspects of neuronal behavior are highlighted in the five lessons presented in NeuroSim:

1. Equilibrium potentials and the resting membrane potential
2. Membrane length constant
3. The action potential
4. The action potential refractory period
5. Summation of synaptic potentials

The lessons in NeuroSim do not attempt to model the full complexity of neuronal behavior. The simulations simplify neuronal properties, highlighting the basic principles of neuronal function. The lessons on *action potentials* are based on the Hodgkin-Huxley equations while the lessons on *passive membranes* are based on the cable equation.

Operation of NeuroSim

NeuroSim runs automatically when the program is opened. A lesson is selected from the menu in the top left corner. The five lessons in NeuroSim run independently of each other. If the parameters in one lesson are changed, the changes will not affect the operation of the other lessons.

Changing parameter values

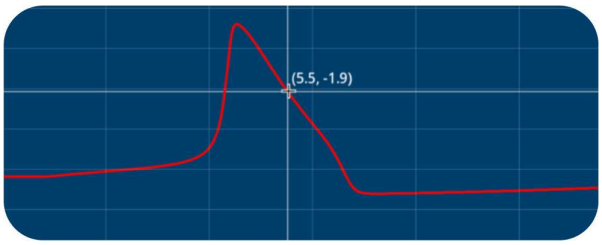
Experiments are run in NeuroSim by changing the values of the parameters displayed in the left portion of the screen. Parameter values can be changed by moving the slider. All parameters can be reset to their default values by selecting "Reset".

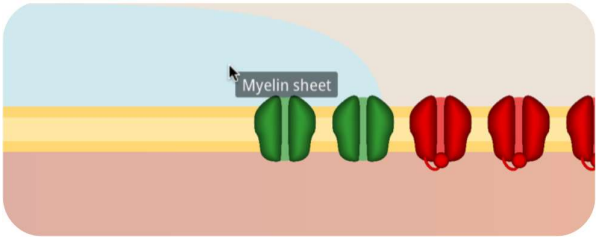
Measuring traces with the cursor

The X and Y values of any point on a graph can be determined by moving the mouse over the graph while pressing left mouse button.

What is does the drawing show?

Hover over items in the simulator to see what they represent.





LESSON 1, EQUILIBRIUM POTENTIALS AND THE RESTING MEMBRANE POTENTIAL

Lesson 1 illustrates how Na^+, K^+, and Cl^- channels contribute to the generation of the resting membrane potential. The neuron in this lesson is modeled by passive conductances to the ions, i.e., these conductances are voltage-independent and this 'neuron' therefore does not generate action potentials (in this lesson). The concentrations of Na^+, K^+, and Cl^-, both outside and inside the cell, can be varied and so can the membrane conductance (see box 1) for each ion.

Box 1. Membrane conductance (g) - the reciprocal of the membrane resistance (R) – represents how easily an ion can permeate the membrane: The more open ion channels of a give type, the higher the conductance (and lower resistance) for that given ion. Conductance thus represents the same as a membrane permeability (P), but is measured in a different manner (conductance measures the charge that crosses the membrane, permeability measures the molecule that crosses the membrane)

Equilibrium potential

The program calculates the electrochemical equilibrium potential (V_{eq}) for each ion (see box 2), based on the ion concentration gradient across the membrane, using the Nernst equation:

$$V_{eq} = \frac{58}{z} \times log \frac{Co}{Ci}$$

Where z is the valence (charge) of the ion, C_o = ion concentration on the outside of the cell and C_i = ion concentration on the inside of the cell. This is the 'short version' of the Nernst equation in which the effect of temperature on the equilibrium potential is included in the number '58', which is based upon temperature (room temperature here), the gas constant, the valence of the ion, and Faraday's constant (see the textbook).

Box 2. Each ion has an equilibrium potential, which is the membrane potential where the ion is in electrochemical equilibrium, meaning that the chemical and electrical forces are of equal magnitude but oppositely directed. It follows that with a larger ionic gradient across the membrane (larger chemical forces), it takes a larger membrane potential (electrical force) to prevent ion flux. The numerical size of the equilibrium potential thus depends directly on the ion gradient across the membrane.

Student exercises

1.1 Calculate the equilibrium potentials using Nernst equation for the ion concentrations in bold in Table 1 and note.

[Ion]	Inside (mM)	Outside (mM)	Calculated V_{eq} (mV)	Measured V_{eq} (mV)
Na^+	**10**	**145**		
Na^+	10	110		
Na^+	10	70		
K^+	**140**	**5**		
K^+	140	10		
K^+	140	15		
Cl^-	**5**	**110**		
Cl^-	5	60		
Cl^-	15	110		

Table 1. Numbers marked in bold are the normal values

To measure an equilibrium potential for a given ion, no other ions can be permeable at the same time. Therefore, choose one ion at a time, and **set the conductance of the other two ions to zero** to read the value off the large center display. Alternatively find the values for each equilibrium potential in the colored boxes.

1.2 Vary the concentrations of Na^+, K^+, and Cl^- in the simulation program as indicated in the table and note all equilibrium potentials.

1.3 Explain the correlation between the ion gradients and the obtained equilibrium potentials.

1.4 Explain why the equilibrium potential of each of the three ions is positive/negative. We measure the inside minus the outside (outside always set to '0').

1.5 Describe what determines the numeric size of the equilibrium potential?

Resting membrane potential

The membrane potential (V_m, see box 3) of the neuron is the weighted average of the permeable ions' equilibrium potentials, which is here calculated with the Millman equation:

$$V_m = \frac{V_{Na} \times g_{Na} + V_K \times g_K + V_{Cl} \times g_{Cl}}{g_{Na} + g_K + g_{Cl}}$$

Where V_x is the equilibrium potential for a given ions and g_x is the conductance for a given ion.

Box 3. The membrane potential of a given cell is the weighted average of the permeable ions' equilibrium potential: The more permeable (P) a given ion is (= the higher conductance (g) it has), the heavier that ion's equilibrium potential will weigh in the combined membrane potential. We usually employ the relative conductances.

Student exercises

1.6 Employ the equilibrium potentials obtained in Table 1 (those arising from the normal concentrations marked in bold) to calculate the membrane potentials in the below scenarios (Table 2).

Measurement	g_{Na} (relative)	g_{K} (relative)	g_{Cl} (relative)	Calculated V_m (mV)	Measured V_m (mV)
1	0.1	0.8	0.1		
2	0.8	0.1	0.1		
3	0.1	0.1	0.8		
Table 2. g = conductance (relative)					

1.7 Adjust the concentrations of Na^+, K^+, and Cl^- in the simulation program as indicated in bold in Table 1 and adjust the conductances according to Table 2 to obtain the measured membrane potentials. Add to table 2.

1.8 Explain (and test in the simulation program) what happens to the resting membrane potential with exposure to an inhibitor of the leak K^+ channels (the ones that are open at rest)?

1.9 Explain (and test in the simulation program) what happens to the resting membrane potential if one eats poorly-prepared FUGU (sushi made on the puffer fish), in which there is too much of the neurotoxin TTX (which inhibits the voltage-gated Na^+ channels)?

1.10 Explain (and test in the simulation program) what effect an increase in extracellular K^+ concentration (to 10 mM) will have on neuronal excitability?

1.11 Explain how ion permeability/conductance affect an ion's equilibrium potential?

1.12 Explain how ion permeabilities/conductances affect the membrane potential?

LESSON 2, MEMBRANE LENGTH CONSTANT

Lesson 2 illustrates the effect of dendritic properties on passive spread of voltage, as quantified by the length constant (λ, see box 4). The longer the λ, the higher possibility of eliciting an action potential either on its own or via spatial summation with other synaptic potentials). When a dendrite is depolarized at a point (X = 0), the potential will decay with distance as it is passively conducted down the dendrite (in both directions).

Box 4. A local depolarization (for example in a dendritic synapse) will spread down the length of the dendrite but diminishes in amplitude as positive charges are lost through open K^+ channels. It spreads further (larger λ) with a smaller inner resistance (larger dendritic diameter) and with fewer open K^+ channels (larger resistance/smaller conductance). The length constant expresses how far down a dendrite a given depolarization has travelled at the point where there is 37% (1/e) left of the original depolarization.

Length constant - exponential decay of voltage with distance
Student exercises

In lesson 2 of the stimulus program, keep "stimulus amplitude" at 1.0 nA/mm^2, adjust the "dendrite radius" to 0.5 µm, and set the "membrane conductance" to 1.0 µS/mm^2 – and press "inject current" to see the depolarization spread down the dendrite.

Note that the basal membrane potential is -65 mV, so the depolarization amplitude is the difference between that and the obtained membrane potential at length = 0.

2.1 Calculate the length constant (the length down the dendrite at which the membrane depolarization is reduced to 37% of its original (maximal) value).

2.2 Decrease the "membrane conductance" (equaling an increase in membrane resistance, R_m), mimicking partial closing of leak K^+ channels to 0.6 µS/mm^2 and calculate the new length constant.

2.3 Increase the "membrane conductance" (mimicking opening of other dendritic channels, i.e. a GABA receptor) to 1.4 µS/mm^2 and calculate the new length constant.

2.4 Explain the relation between R_m and λ?

2.5 Discuss the physiological implications of decreasing/increasing λ.

2.6 Return the "membrane conductance" to the default 1.0 µS/mm^2 and now increase the "dendrite radius" to 1 µm and calculate the new length constant.

2.7 Explain the relation between dendrite diameter and the length constant.

LESSON 3, THE ACTION POTENTIAL

Lesson 3 illustrates how voltage- and time-dependent Na^+ and K^+ conductances generate the action potential (see box 5).

Box 5. The action potential is generated when the membrane potential reaches the threshold (-50 mV) for the voltage-sensitive Na^+ channels. These swiftly open and shifts the membrane potential closer to the equilibrium potential for Na^+, which represents the peak of the action potential. The Na^+ channels then inactivate and the voltage-sensitive K^+ channels open, which together brings the membrane potential to the undershoot representing the afterhyperpolarization, which – in turn – permits closing of the voltage-sensitive K^+ channels.

All-or-none response
Student exercises

An action potential is said to display all-or-nothing responses. That is; there is no 'information' in the amplitude of the action potential, it is the frequency of action potentials that holds the 'information'. So either there is a full-scale action potential or there is none (all-or-none response).

3.1 When the simulation 'injects current' and thus creates a stimulus (the square form on the red line on the screen), what is the equivalent in a real neuron (i.e., what brings the membrane potential to the threshold potential)?

3.2 Set the "stimulus amplitude to 10 nA/mm^2 and the stimulus duration to 2 ms and record an action potential (press "inject current"). Note the amplitude potential.

3.3 Decrease the "stimulus amplitude" from the 10 nA/mm^2 to 4 nA/mm^2 and perform a recording.

a. What happens? Why?
b. Increase the stimulus duration to 3 ms. What happens? Why?

3.4 Return the stimulus duration to 2 ms and increase the "stimulus amplitude" to 20 nA/mm^2 and record an action potential. Note the amplitude potential.

a. What effect does this larger stimulus have on the amplitude of the action potential? Why?
b. What effect does it have on other parameters of the action potential? Why?

Action potentials and the Na^+ equilibrium potential
Student exercises

3.5 Return the "stimulus amplitude" to 10 nA/mm^2 and the stimulus duration to 2 ms and record an action potential. Reduce the Na^+ concentration on the outside to 70 mM and on the inside to 30 mM and record a new action potential. Explain the effect on the action potential amplitude? Why?

LESSON 4, THE ACTION POTENTIAL REFRACTORY PERIOD

Lesson 4 illustrates the action potential refractory period (see Box 6), which indicates the time between action potentials where it is either impossible (the absolute refractory period) to elicit a new action potential or possible, but with a larger stimulus (the relative refractory period).

Box 6. The 'information' in a train of action potentials lies in the frequency of action potentials and not in the amplitude of each action potential. Action potential separation is therefore ensured with the refractory period, which consists of i) an *absolute* refractory period, where NO action potentials can be elicited because of Na^+ channel inactivation and ii) a *relative* refractory period, where a subsequent action potential CAN BE elicited, although requiring a larger stimulus due to the membrane hyperpolarization and increased conductance in the afterhyperpolarization phase (which equals the relative refractory period), due to voltage-gated K^+ channels opening.

Refractory period
Student exercises

In lesson 4, one can elicit two stimuli. The first one is automatically assigned an amplitude of 20 nA/mm^2 and a duration of 2 ms. The second stimulus can be adjusted regarding amplitude ("2nd stimulus amplitude" and delay after the first stimulus ("2nd stimulus delay").

Initiate the simulation by adjusting "2nd stimulus amplitude" to 20 nA/mm^2 and the "2nd stimulus delay" to 10 ms. With these settings, one should observe two identical action potentials.

4.1 Reduce the "2nd stimulus delay" by 1 ms at a time until there is no longer elicited an action potential (note the stimulus delay). With this setting, increase the "2nd stimulus amplitude" until an action potential is elicited (note the stimulus amplitude and also the amplitude of the 2nd action potential. Continue to reduce the 2nd stimulus delay" by 1 ms and find the "2nd stimulus amplitude" required to elicit an action potential at the given stimulus delay.

Stimulus delay (ms)	1st stimulus amplitude (nA/mm^2)	2nd stimulus amplitude (nA/mm^2)	2nd action potential peak (mV)
10	20	20	
9	20		
8	20		
7	20		
6	20		
5	20		
4	20		
3	20		

Table 3

4.2 Indicate the approximate time interval for the absolute refractory period (from the peak of the 1st action potential).

4.3 Describe the molecular mechanism underlying the absolute refractory period.

4.4 Explain what happens to the amplitude of the stimulus 2 required to elicit an action potential as a function of the reduced interval between the two stimuli? Why?

4.5 Indicate the approximate time interval for the relative refractory period.

4.6 Describe the molecular mechanism underlying the relative refractory period.

4.5 Explain why the amplitude of the 2nd action potential diminishes with reduced stimulus intervals.

LESSON 5, SUMMATION OF SYNAPTIC POTENTIALS

Lesson 5 illustrates spatial summation of synaptic potentials (see box 7) in the dendritic compartment. In this lesson, there are two excitatory synapses (in red) and one inhibitory synapse (in blue). Each can be moved along the dendrite and activated alone and together.

Box 7. Synaptic potentials arise with opening of the ligand-gated neurotransmitter receptors, with excitatory receptors promoting a local depolarization and inhibitory receptors promoting a local hyperpolarization. These synaptic potentials travel along the dendrite and reach the soma/axon initial segment, where an action potential will be generated when the membrane potential exceeds the threshold of the voltage-sensitive Na^+ channels. The synaptic potentials can be summed so that two excitatory postsynaptic potentials can add up and depolarize the membrane potential sufficiently to reach the threshold. Alternatively, an inhibitory postsynaptic potential arriving in the axon initial segment at the same time as an excitatory postsynaptic potential may prevent action potential generation.

Summation of synaptic potentials

Student exercises

In lesson 5 one can adjust the location of each synapse on the dendrite and the stimulus amplitude of each synapse (equaling mimicking of neurotransmitter release). The blue synapse is an inhibitory synapse (e.g. GABA) and the red synapses (I and II) are excitatory synapses (e.g. glutamate).

Ensure that the stimulus amplitude is at '0' for the inhibitory synapse and the excitatory synapse II.

5.1 Place the excitatory synapse closest to the synapse at a distance of 0.1 mm from the soma and increase the stimulus amplitude (in intervals of 0.1 nA/mm^2) until an action potential is generated in the axon initial segment. Note the stimulus amplitude in Table 4. Move the synapse as indicated in Table 4 and find the new stimulus threshold required to elicit an action potential.

Excitatory synapse I		Excitatory synapse II		Inhibitory synapse		AIS
Distance from soma (mm)	Stimulus amplitude (nA/mm^2)	Distance from soma (mm)	Stimulus amplitude (nA/mm^2)	Distance from soma (mm)	Stimulus amplitude (nA/mm^2)	Action potential (yes/no)
0.10			0		0	Yes
0.30			0		0	
0.40			0		0	
0.50			0		0	
0.60			0		0	
0.60	1.0	1.50	0		0	
0.60	0	1.50	0.8		0	
0.60	1.0	1.50	0.8		0	
0.60	1.0	1.50	0.8	1.0	-1.0	

Table 4. AIS = axon initial segment (where action potentials are initiated)

5.2 Explain why a larger stimulus amplitude is required to elicit an action potential as the synapse moves down the dendrite away from the soma.

5.3 Keep the excitatory synapse I at 0.60 mm and stimulate with 1.0 nA/mm^2. Note in Table 4 whether an action potential is generated? Why?

5.4 Turn the stimulus amplitude to '0' for Excitatory synapse I and place Excitatory synapse II at 1.50 mm with a stimulus amplitude of 0.8 nA/mm^2. Note in Table 4 whether an action potential is generated? Why?

5.5 Combine the settings from 5.3 and 5.4 (Excitatory synapse I: 0.60 mm and 1.0 nA/mm^2, Excitatory synapse II: 1.50 mm and 0.8 nA/mm^2). Note in Table 4 whether an action potential is generated? Why?

5.6 Retain the values from 5.5 (Excitatory synapse I: 0.60 mm and 1.0 nA/mm^2, Excitatory synapse II: 1.50 mm and 0.8 nA/mm^2) and place the inhibitory synapse at 1 mm with a stimulus amplitude of -1.0 nA/mm^2. Note in Table 4 whether an action potential is generated? Why?