

Lab 1: Simulation of Resting Membrane Potential and Action Potential

Overview

The aim of the present laboratory exercise is to simulate how changes in the ion concentration or ionic conductance can change the values of the resting membrane potential and equilibrium potential in a model neuron. At rest, the membrane potential is determined by the counterbalance of ion moving in and out of a cell. The Goldman equation can be used to predict the membrane potential (at 25 °C):

$$E_M = 59 \log (P_K[K_{out}] + P_{Na}[Na_{out}] + P_{Cl}[Cl_{in}]) / (P_K[K_{in}] + P_{Na}[Na_{in}] + P_{Cl}[Cl_{out}])$$

According to this equation, the membrane potential depends upon the concentration of the different ions across the membrane and the relative permeability (conductance) of the membrane to these ions. The Goldman equation indicates that the membrane potential is dependent upon the concentration gradients of the different ions. Since the permeability of the resting membrane is highest to potassium, changing the potassium gradient across the membrane might have a great effect of the membrane potential.

The Nernst equation is a special case of the Goldman equation that can be used to predict the equilibrium potential for any particular ion (at 25 °C). For example for Na⁺ ions, the Nernst equation will be: $E_{Na} = 59 \log [Na_{out}] / [Na_{in}]$. At rest, E_{Na} is ~ +60 mV. The Nernst potential is also known as the reverse potential for that particular ion, because at that voltage the net flow of ions across the membrane is 0.

The Na⁺/K⁺ pump plays a very important role in nerve cells. Using ATP as an energy source, 3 positive ions (Na⁺) are pumped out of the cell for every 2 positive ions (K⁺) pumped into the cell. This means that there are more positive charges leaving the cell than entering it when the Na⁺/K⁺ pump is functioning. As a result, positive charge builds up outside the cell compared to inside the cell. The Na⁺/K⁺ pump is also called electrogenic because it creates a potential difference across the cell membrane. In this laboratory exercise you will test how the activity of the Na⁺/K⁺ pump regulate the membrane potential in a model neuron.

The resting membrane potential applies to neuron at rest. An action potential occurs when a neuron sends information down an axon, away from the cell body. An action potential is initiated by a stimulus above a certain intensity or *threshold*. This means that some event (a stimulus) causes the resting potential to move away from rest. When the depolarizing stimuli bring the membrane potential to a specific value (usually around -50 mV), a neuron will fire an action potential. This is the *threshold potential*, which allows a neuron to fire an action potential in an ALL or NONE fashion: if a neuron reaches threshold it will definitively fire an action potential. If the neuron does not reach this critical threshold level, then no action potential will be generated.

Equipment Required

PC computer

NeuroDynamix software

Menu bar contains the following icons: File, Stimulate, Tools, Help

Toolbar contains the following icons: Open File, Save, Reset (■ ◀), Run (▶), Stop (■), Slow advance (◀◀) and Fast advance (▶▶)

Exercise 1. Effect of ion concentration and conductance on the resting membrane potential

Start the Software

1 Click the Windows NeuroDynamix icon

2 When the program opens, select **File > Open Lesson > Soma_resting_potential**. Download the **Soma_resting_potential** lesson.

3 You will see two screens: the first screen represents Scope (Time series) and will show you the changes in resting membrane potential. The second screen represents Scope (Time series) and indicates the changes in conductance. In each screen the **X axis** represents time and the **Y axis** represents voltage (in millivolts, mV)

Make sure that the **X scale** in both screens is the same. This way you can correlate changes in the resting membrane potential with changes in conductance.

4 Click ▶ (run). You will see four (4) lines:

- a) The GREEN line represents the sodium equilibrium potential
- b) The GRAY line represents the chloride equilibrium potential
- c) The BLUE line is the resting membrane potential which is determined by the overall conductance of the membrane to Na^+ , K^+ and Cl^-
- d) The RED line represents the potassium equilibrium potential

5 Click ▶ (run). Let the exercise run for 10 sec.

According to the graph on the screen, determine the values of

- 1) **Resting membrane potential (blue line),**
- 2) **Sodium equilibrium potential (green line)**
- 3) **Chloride equilibrium potential (gray line) and**
- 4) **Potassium equilibrium potential (red line)**

6 Click ■ (Stop). Reset the screen by pressing ■ ◀

7 Click **Tools > New parameters**.

You will see a **new screen** showing the parameters with the initial values used to simulate the resting membrane potential (blue line), sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)

Some of these values are:

Na⁺ conductance (g_{Na})=2	K⁺ conductance (g_K)=16	Cl⁻ conductance (g_{Cl})=5
Na⁺ concentration outside=460	K⁺ concentration outside=20	Cl⁻ concentration outside=560
Na⁺ concentration inside=50	K⁺ concentration inside=400	Cl⁻ concentration inside=60

8 Change **Na⁺ concentration outside** from 460 to 260.

What happen to the resting membrane potential (blue line)?

Did you notice any change in the sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)?

Explain your results.

9 Change the **Na⁺ concentration outside** back to its original value (460).

10 Change the **Na⁺ conductance (g_{Na})** from 2 to 20 (a ten-fold change).

What happen to the resting membrane potential (blue line)?

Did you notice any change in the sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)?

Explain your results.

11 Change the **Na⁺ conductance (g_{Na})** back to its original value (2).

12 Change the **K⁺ concentration outside** from 20 to 200.

What happen to the resting membrane potential (blue line)?

Did you notice any change in the sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)?

Explain your results.

Change the **K⁺ concentration outside** back to its original value (20).

13 Change the **K⁺ conductance (g_K)** from 16 to 1.6 (a ten-fold change).

What happen to the resting membrane potential (blue line)?

Did you notice any change in the sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)?

Explain your results.

14 Change the **K⁺ conductance (g_K)** back to its original value (16).

15 Change Cl^- conductance (g_{Cl}) from 5 to 50 (a ten-fold change).

What happen to the resting membrane potential (blue line)?

Did you notice any change in the sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)?

Explain your results.

Data Analysis

1 Present the data for exercise 1 in the following format:

Parameter Change	Values (in mV)			
	Resting membrane potential	Na^+ equilibrium potential	K^+ equilibrium potential	Cl^- equilibrium potential
Initial values (cell is at rest): Na^+ concentration outside=460 Na^+ conductance (g_{Na})=2 K^+ concentration outside=20 K^+ conductance (g_{K})=16 Cl^- conductance (g_{Cl})=5				
Na^+ concentration outside from 460 to 260				
Na^+ conductance (g_{Na}) from 2 to 20				
K^+ concentration outside from 20 to 200				
K^+ conductance (g_{K}) from 16 to 1.6				
Cl^- conductance (g_{Cl}) from 5 to 50				

Questions

1 What factors determine the resting membrane potential of an excitable cell?

2 Does changes in the extracellular solution change the ion channel permeability?

3 Explain the changes in each of the parameters represented in the table shown above

4 Please compare how a ten-fold change in the Na^+ conductance (g_{Na}), the K^+ conductance (g_{K}) and Cl^- conductance (g_{Cl}) change the resting membrane potential. Which one causes the biggest change in the resting membrane potential?

5 What will you predict will happen to the resting membrane potential if you increase the K^+ conductance (g_{K}) from 16 to 32? Why?

6 Calculate the Na^+ and K^+ Nernst equilibrium potential in a saline solution containing ($[\text{Na}_{\text{in}}] = 17 \text{ mM}$, $[\text{Na}_{\text{ou}}] = 162 \text{ mM}$, $[\text{K}_{\text{in}}] = 17 \text{ mM}$, $[\text{K}_{\text{ou}}] = 207 \text{ mM}$).

Exercise 2. Effect of the Na^+/K^+ pump on the resting membrane potential
Start the Software

1 Select **File > Open Lesson > Soma_electrogenic_Na_pump**. Download the **Soma_electrogenic_Na_pump** lesson.

2 You will see two screens: the first screen represents Scope (Time series) and will show you the changes in resting membrane potential. The second screen represents Scope (Time series) and indicates the changes in the activity of the electrogenic Na^+/K^+ pump. In each screen the **X axis** represents time and the **Y axis** represents voltage (in millivolts, mV)

Make sure that the **X scale** in both screens is the same. This way you can correlate changes in the resting membrane potential with changes in conductance.

3 Click ► (run). You will see one red line representing the resting membrane potential.

4 Click ► (run). Let the exercise run for 10 sec.

According to the graph on the screen, determine the values of the resting membrane potential (red line).

5 Click ■ (Stop). Reset the screen by pressing ◀

6 Click **Tools > New parameters**.

You will see a **new screen** showing the parameters with the initial values used to simulate the resting membrane potential (blue line), sodium equilibrium potential (green line), chloride equilibrium potential (gray line) and potassium equilibrium potential (red line)

Some of these values are:

Na^+ conductance (g_{Na})=20	K^+ conductance (g_{K})=100	Cl^- conductance (g_{Cl})=40
Na^+ concentration outside=145	K^+ concentration outside=5	Cl^- concentration outside=120
Na^+ concentration inside=15	K^+ concentration inside=140	Cl^- concentration inside=7
$\text{JNa}_{\text{pumpmax}}=0$		
$\text{NaPump}_{\text{CnMax}}=14$		
$\text{NaPump}_{\text{sensitivity}}=2$		

7 Change the Na^+/K^+ pump activity (**$\text{JNa}_{\text{pumpmax}}$**) from 0 to 10. Record the membrane potential response for approximately 20 sec.

What happen to the resting membrane potential (red line)? Explain your results.

8 Change the $JNa_{pumpmax}$ back to its original value (0).

9 Change the Na^+ conductance (g_{Na}) from 20 to 100. This is equivalent to a membrane depolarization caused by an action potential.

What happen to the membrane potential (red line)? Explain your results.

10 Now change $JNa_{pumpmax}$ from 0 to 10.

What happen to the resting membrane potential (red line)?

Will this change allow the resting membrane potential to return to its original value? Explain your results.

11 Change back the $JNa_{pumpmax}$ to its original value (0) and the Na^+ conductance (g_{Na}) to 20.

12 Change the Na^+ conductance (g_{Na}) from 20 to 2. This is equivalent to a membrane hyperpolarization (membrane potential become more negative).

What happen to the membrane potential (red line)? Explain your results.

13 Now change $JNa_{pumpmax}$ from 0 to 10.

What happen to the resting membrane potential (red line)?

Will this change allow the resting membrane potential to return to its original value? Explain your results.

Data Analysis

1 Present the data for exercise 2 in the following format:

Parameter Change	Resting membrane potential (in mV)
Initial values (cell is at rest): Na^+ concentration outside=145 Na^+ conductance (g_{Na})=100 K^+ concentration outside=5 K^+ conductance (g_K)=100 Cl^- conductance (g_{Cl})=40 $JNa_{pumpmax} = 0$	
$JNa_{pumpmax}$ from 0 to 10	
Na^+ conductance (g_{Na}) from 20 to 100	
Na^+ conductance (g_{Na}) from 20 to 100 $JNa_{pumpmax}$ from 0 to 10	

Na⁺ conductance (g_{Na}) from 20 to 2	
Na⁺ conductance (g_{Na}) from 20 to 2 JNa_{pumpmax} from 0 to 10	

Questions

- 1 What is the role of the electrogenic Na⁺/K⁺ pump in regulating the membrane potential?
- 2 Explain the changes in each of the parameters represented in the table above
- 3 Which one of the following causes the biggest change in membrane potential: a ten-fold change in **JNa_{pumpmax}** (from 0 to 10) at rest (when the membrane potential is ~-60 mV), or a ten-fold change in **JNa_{pumpmax}** (from 0 to 10) when the cell is depolarized (following a change in the **Na⁺ conductance (g_{Na})** from 20 to 100)? Why?
- 4 Why during a membrane hyperpolarization caused by a reduction in the sodium ion permeability, activation of the Na⁺/K⁺ pump does not have any significant role?

Exercise 3. Generation of an action potential

Start the Software

- 1 Select **File > Open Lesson > Axon_impulse_threshold**. Download the **Axon_impulse_threshold** lesson.
 - 2 You will see two screens: the first screen represents Scope (Time series) and will show you the changes in membrane potential. The second screen represents Scope (Time series) and indicates the strength of the stimulation pulses (needed to generate an action potential). In each screen the **X axis** represents time and the **Y axis** represents voltage (in milivolts, mV)
- Make sure that the **X scale** in both screens is the same. This way you can correlate changes in the resting membrane potential with the strength of the pulse.
- 3 Click ► (run). You will see one red line representing the resting membrane potential. Notice also a green line (showing the Na⁺ equilibrium potential) and an orange line (representing the K⁺ equilibrium potential).
 - 4 Click ► (run). Let the exercise run for 20 sec.

According to the graph on the screen, you will see an action potential (red line). Draw an action potential and show its main components.

Notice: you can export the recorded values of the action potential (voltage as a function of time) as an Excel file and plot the data using Excel.

- 5 Click ■ (Stop). Reset the screen by pressing **◀**
- 6 Click ► (run). Let the exercise run continuously. Click **Tools > New parameters**.

You will see a new screen showing the parameters with the initial values used to simulate the action potential.

Some of these values are:

		Resting membrane potential ($V_{m_{hold}}$)=-65
Na^+ equilibrium potential (E_{Na})=50	K^+ equilibrium potential (E_K)=-77	Cl^- equilibrium potential (E_{Cl})=-55

7 Click **Tools > New parameters > Istim**

Change the **amplitude** to 0 and press Fire.

If the stimulation amplitude is switched to 0, will you see an action potential? Explain your results.

8 Now start increasing the amplitude of the stimulation by 0.01 and press Fire (you will see that the light will flash every time you press Fire).

With which stimulation do you see an action potential? This is the threshold for the generation of an action potential.

9 Once you have determined that certain stimulation causes an action potential, perform the following exercise: apply a few stimuli immediately after the termination of the action potential

What will happen if you apply a stimulus immediately or shortly after the termination of the action potential? Why no action potential can be generated immediately after another action potential?

10 Click **■** (Stop). Reset the screen by pressing **◀**

11 Click **▶** (run). Let the exercise run continuously.

You will see a new screen showing the parameters with the initial values used to simulate the action potential.

12 Click **Tools > New parameters**. Change the holding potential ($V_{m_{hold}}$) from -65 to -50 mV. Now the membrane potential should be reset to -50 mV

13 Click **Tools > New parameters > Istim**

14 Now start increasing the amplitude of the stimulation by 0.01 and press Fire. At which stimulation do you see an action potential? Why a stronger stimulus is needed in order to generate an action potential?

Questions

1 Determine what is the threshold for the generation of an action potential when the membrane potential is $V_{m_{hold}} = -65$ and $V_{m_{hold}} = -50$ mV.

2 Explain how the absolute and relative refractory period regulate the ability of a neuron to generate action potentials.

3 Explain why changing the holding potential ($V_{m_{hold}}$) from -65 to -50 mV will affect the stimulation threshold. Why?

5 What do you predict will happen to the threshold for an action potential if you decrease the membrane potential to $V_{m_{hold}} = -80$ mV? Will you require stronger or weaker stimuli in order to generate an action potential? Why?