In 1963 Alan Hodgkin and Andrew Huxley received the Nobel Prize in Physiology and Medicine for their work on the mechanism of the nerve action potential. Their work provided a sound quantitative basis for the understanding of this phenomenon and included a mathematical description of the changes which occur during the action potential. This exercise makes use of their work to provide a computer simulation of the nerve action potential. Three basic aspects of nerve cell physiology will be studied: the threshold voltage and the effects of changes in external sodium and potassium concentrations on the nerve action potential.

In order to study the nerve action potential it is necessary to measure the trans-membrane electrical changes. This is most frequently done with intracellular micro-electrodes (Figure 1.). Usually, it takes about a year of intensive work to master the techniques of pulling micro-electrodes and recording action potentials. In addition, the equipment is costly and is rarely available in undergraduate physiology laboratories. Consequently, only a very few students have the opportunity to perform the experiments that we will simulate.

In Part A the threshold voltage will be measured and the effects of sub-threshold and supra-threshold stimuli on the electrical potentials across the membrane will be determined. The concept of a threshold voltage is one of fundamental importance to the understanding of the all-or-none principle.

In part B the effect of changes in the external sodium concentration ([Na]_{out}) on the resting potential and the peak voltage (see Fig 2) will be determined. Since the action potential is sodium-dependent, changes
in the external sodium concentration should bring about changes in the action potential. The measured values of the resting potential and the peak voltages will be compared with values that are calculated from the Nernst equation (Equation 1). These data should shed some light on the permeability of the nerve cell membrane to sodium during the resting state and at the peak voltage. The data will be analyzed by graphing the electrical potential versus the $\log_{10}$ of the external sodium concentration as shown in Figure 3.

$$E_{in} - E_{out} = \frac{RT}{zF} \ln \frac{C_{out}}{C_{in}}$$

or, after solving at 37 °C, converting to millivolts, and converting to $\log_{10}$

$$E_{in} - E_{out} = \frac{61.5 \text{ mV}}{z} \log_{10} \frac{C_{out}}{C_{in}}$$

Where $E_{in} - E_{out}$ = Electrical Potential Difference (volts)
T = Temperature (Kelvin)
R = Gas Constant (8.314 coulomb volt deg$^{-1}$ mole$^{-1}$)
$z$ = Charge of the ion species
F = Faraday Constant (96500 coulomb mole$^{-1}$)
$C_{in}$ and $C_{out}$ = Ion concentrations on the inside and the outside of the membrane (moles/Liter)

![Figure 3. Membrane Peak Voltage as a Function of External Sodium Concentration.](image)

In part C the effect of changes in the external potassium concentration ($[K]_{out}$) on the resting potential and the peak voltage will be explored. Since the resting potential is potassium-dependent, changes in the external potassium concentration should cause changes in the resting potential. These data should shed some light on the permeability of the nerve cell membrane to potassium during the resting state and at the peak voltage. The data will be analyzed graphically.
The Goldman Constant Field Equation or Goldman-Hodgkin-Katz Equation

The Goldman Equation Constant Field Equation resembles the Nernst Equation, but it describes the voltage generated by a system in a steady state. The Nernst Equation, on the other hand, describes a system at equilibrium. One difficulty in applying the Goldman Equation is that it was derived for the case where the absolute charges on all the relevant ions are the same. Accounting for negative and positive ions is taken care of by placing the outside cations and inside anions in the numerator and outside anions and inside cations in the denominator. When attempts are made to include both divalent and monovalent ions in the same equation the results may not be reliable. This problem becomes important when attempting to account for the effects of Ca^{2+} on the electrical potential in a system where Na^+, K^+, and Cl^- are also present. In the work of Hodgkin and Huxley, Goldman’s work was used as a starting point to derive an equation with a form similar to the equation below. This has become to be known as the Goldman-Hodgkin-Katz equation. Since the major features of the action potential in the giant axon of the squid may be well described by this equation, the problem of mixing monovalent and divalent ions in the equation does not arise.

\[
E_{in} - E_{out} = \frac{RT}{F} \ln \left( \frac{P_{K}[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_{K}[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o} \right)
\]

Where:
- \(E_{in} - E_{out}\) = electrical potential difference (volts)
- \(T\) = Temperature (Kelvin)
- \(R\) = Gas Constant (8.314 coulomb volt deg\(^{-1}\) mole\(^{-1}\))
- \(F\) = Faraday Constant (96500 coulomb mole\(^{-1}\))
- \([K]_o\) = Concentration of potassium ions on the outside (moles/Liter)
- \([K]_i\) = Concentration of potassium ions on the inside (moles/Liter)
- \([Na]_o\) = Concentration of sodium ions on the outside (moles/Liter)
- \([Na]_i\) = Concentration of sodium ions on the inside (moles/Liter)
- \([Cl]_o\) = Concentration of chloride ions on the outside (moles/Liter)
- \([Cl]_i\) = Concentration of chloride ions on the inside (moles/Liter)
- \(P_{K}\) = relative permeability of potassium ions (often set to 1.0)
- \(P_{Na}\) = relative permeability of sodium ions (often set to 0.01)
- \(P_{Cl}\) = relative permeability of chloride ions (often set to 0.45)
STARTING SPIKE

SPIKE is designed to run on an IBM™ PC computer, or compatible computer. To start SPIKE, follow the instructions given to you by your instructor. Absent other instructions, the following notes may be useful.

If SPIKE is on a disk, place the disk in drive A. Go to the Start Menu, click on run, type a:spike and hit <Enter>. Here and elsewhere, when the <> symbols surround a character, this refers to a key on the computer keyboard.

If SPIKE is on a CD, place the CD in the CD ROM drive. Go to the Start Menu, click on run, type d:spike (where d is the name of the CD ROM drive) and hit <Enter>. The drive letter may differ from d, and you may have to determine the drive letter of the CD ROM drive by at "My Computer".

If SPIKE is on the hard drive, double click on the program file (SPIKE.exe). If SPIKE is contained in a ZIP file you must first extract the ZIP file to a directory. In windows XP you can perform this operation by right clicking on the ZIP file and selecting the Extract All option. Extract the zip file to a directory named SPIKE. Open the SPIKE directory and double click on SPIKE.exe. With Windows 7 or Vista you will need to run SPIKE using DOSBox.

PROGRAM OPERATION

1. When SPIKE signs on it will prompt for your ID Code as shown below. When SPIKE recognizes your ID Code it will greet you and assign a nerve cell simulation to you. If SPIKE does not recognize your ID Code, it will greet you as "Unknown Person" and assign a different nerve cell simulation. If you enter your ID code incorrectly, you will be able to enter it again at the MAIN MENU.

2. If you do not have an ID Code then type 'new'. SPIKE will prompt you for your first and last names and then assign a new ID Code.

Write your new ID Code here _____________________________

Use this ID Code if you use SPIKE again. This will ensure that you keep the same simulation.

3. SPIKE will now display the MAIN MENU which appears below. To select an option on the menu, use the <Up Arrow> and <Down Arrow> keys. When your selection is made press <Enter>.
4. If you incorrectly entered your ID code then go back and change this now.

5. Note the selections on the MAIN MENU. At the top of the menu is the name of the person who is currently using SPIKE. Below the menu are recorded the temperature, the internal sodium and potassium concentrations, and the sodium activation constant for the neuron assigned to the user. The parameters for your neuron may differ.

6. Note that the option to quit appears on the MAIN MENU.

7. Select Experiment and press <Enter>. In the upper portion of the screen SPIKE plots two sets of axes: Voltage (mV) versus time (msec) and Stimulus Strength (µA/cm²) versus time (msec). SPIKE uses the bottom lines of the screen to take input and display messages. Before the experiment begins, SPIKE displays a help screen which explains how to provide input for the simulation. When an experiment begins the lower screen will appear as follows:

<table>
<thead>
<tr>
<th>Stimulus µA/cm²</th>
<th>Duration msec</th>
<th>Delay msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.0</td>
<td>0.12</td>
<td>0.0</td>
</tr>
</tbody>
</table>

   [K] out mM 3.0  [Na] out mM 150.0

8. SPIKE allows input for stimulus strength, duration, delay, [K] out, and [Na] out. Use the <Tab> key to move from one input to the next. When the input values have been set, the simulation may be started by pressing <Enter>.

**Stimulus**

The stimulus strength, expressed as a stimulus current, is initially set to 60 µA/cm². Editing with the <backspace> or <left arrow> keys may change this value. If <Enter> is pressed the value is retained and SPIKE will start plotting. Useful values for stimulus strength are -50 to 200 µA/cm². If you wish to change another variable, use the <Tab> key to move to another input.
Duration

The stimulus duration is initially set to 0.12 msec. Useful values generally fall within the range of zero to 20 msec. SPIKE will not accept negative values. SPIKE measures time in intervals of 0.04 msec which is the interval of integration. SPIKE rounds the duration to the nearest 0.04 msec.

Delay

The delay determines how long SPIKE will plot before returning for input. When the value is set to zero, a special case, SPIKE will plot until it is interrupted by a keystroke. A positive non-zero value will cause SPIKE to stop plotting after the specified time. Durations between 0 and 100 msec are accepted.

\([\text{Na}]_{\text{out}}\)

The \([\text{Na}]_{\text{out}}\) input allows the external concentration of sodium to be changed between 1 and 200 mM.

\([\text{K}]_{\text{out}}\)

The \([\text{K}]_{\text{out}}\) input allows the external concentration of potassium to be changed between 1 and 200 mM.

9. Special Keys

Six special keys operate while SPIKE is taking input for the stimulus parameters. They serve the following functions:

\(<\text{Tab}>\) The \(<\text{Tab}>\) key is used to move to the next input.

\(<\text{Esc}>\) The \(<\text{Esc}>\) key causes SPIKE to return to the MAIN MENU.

\(<\text{C}>\) The \(<\text{C}>\) key clears the screen, resets the time to zero, and initializes the simulation. After the \(<\text{C}>\) key is pressed, the nerve cell behaves as if it had not been previously stimulated.

\(<\text{R}>\) The \(<\text{R}>\) key resets the time to zero.

\(<\text{t}>\) The \(<\text{t}>\) key gives access to the Plot menu which allows the user to change some plotting variables and to plot conductances, the Hodgkin Huxley variables, and the sodium and potassium currents.

\(<\text{Enter}>\) The \(<\text{Enter}>\) key starts the simulation.
EXPERIMENTAL PROCEDURE

Following the first part of the exercise is a section called SPIKE DATA ANALYSIS where you may analyze your data and answer the questions, which are posed in this part of the exercise. Record below the internal sodium concentration and the temperature reported at the MAIN MENU. Set $[Na]_{out}$ to 150 mM and begin part A of the experiment.

Temperature = _______ ° C.

Internal sodium ion concentration = _______ mM
Internal potassium ion concentration = _______ mM
Sodium Activation ($\alpha M$) = _______

PART A. DETERMINATION OF THE STIMULUS THRESHOLD VOLTAGE

1. With the outside sodium ion concentration at 150 mM, set the stimulus current to 60 $\mu$A/cm$^2$ and the stimulus duration to 0.12 msec. Allow the experiment to continue until you have determined if an action potential will occur (about 7 msec). Record the value of the Resting Potential.

2. Reset the neuron by pressing the <C> key.

3. If an action potential occurred in step 1, reduce the stimulus current to 30 $\mu$A/cm$^2$. Retain the value of 0.12 msec for the stimulus duration. Continue your experiments until you find the minimum stimulus strength necessary to produce an action potential to within 1 $\mu$A/cm$^2$ of the true value. That is, until you have bracketed the true value within an interval of 1 $\mu$A/cm$^2$.

4. If an action potential did not occur in step 1, increase the stimulus voltage to 90 $\mu$A/cm$^2$ and continue to experiment until you have found the minimum stimulus strength necessary to produce an action potential.

5. Record in Table 1 the resting potential, threshold voltage, and the minimum stimulus current necessary to produce the action potential.

Table 1. Minimum Stimulus Strength. The minimum stimulus strength necessary to produce an action potential at a duration of 0.12 msec.

| External Sodium Concentration ($[Na]_{out}$) | 150 mM (Set) |
| External Potassium Concentration ($[K]_{out}$) | 3.0 mM (Set) |
| Stimulus Duration | 0.12 msec (Set) |
| Resting Potential | _______mV (measured) |
| Threshold voltage | _______mV (measured) |
| Minimum Stimulus Current Necessary to Produce the Action Potential | _______µA/cm$^2$ (measured) |
6. Press <C> to reset the neuron. Increase the stimulus current by 50 µA/cm² while keeping the stimulus duration at 0.12 msec. Allow the experiment to continue for about 7 msec. What is the effect of increasing the stimulus current?

PART B. THE EFFECT OF CHANGES IN EXTERNAL SODIUM ION CONCENTRATIONS

In this part you will design an experiment to determine the effects of changes in external sodium concentration ([Na]_out) on the peak voltage and the resting potential. Choose at least seven different external sodium concentrations between 1 and 200 mM. Before you have settled on the sodium concentrations, however, think about how your results will be displayed. Examine the graph in Figure 3 and note that the abscissa is logarithmic. How would your choices for external sodium appear on a similar graph? In order to produce a graph with equal intervals after the data are log transformed the original data must follow a series in which each concentration increases by a constant factor. For example 1, 2, 4, 8 is a sequence where each successive term increases by a factor of 2. (Note that this differs from the case where each successive term increases by a constant amount such as the sequence 10, 20, 30, 40 …. Record your results in Table 2. Step by step instructions are given below

Table 2. The effect of changes in external sodium ion concentrations.

| External [Na]_out (mM) | | | | | | |
|------------------------|--------|--------|--------|--------|--------|
| Stimulus Strength (µA/cm²) | | | | | | |
| Resting Potential (mV) | | | | | | |
| Peak Voltage (mV) | | | | | | |

1. Set the stimulus current to the minimum current necessary to produce an action potential and set the stimulus duration to 0.12 msec.

2. In a series of at least seven experiments, measure the resting potential and the peak voltage for external sodium ion concentrations between 200 and 1 millimoles/Liter. Use the <Tab> key to move to the input where external sodium concentration is changed.

3. If an action potential does not occur, try increasing the stimulus strength. Why might you have to increase the stimulus strength?

4. For each experiment record the voltage at the peak of the action potential, the external sodium concentration, and the stimulus strength (=stimulus current).
PART C. THE EFFECT OF CHANGES IN EXTERNAL POTASSIUM ION CONCENTRATIONS

In this part you will design an experiment to determine the effects of changes in external potassium concentration ([K]_{out}) on the peak voltage and the resting potential. Choose at least seven different external potassium concentrations between 1 and 200 mM. Record your results in Table 3. Step by step instructions are given below.

Table 3. The effect of changes in external potassium ion concentration.

<table>
<thead>
<tr>
<th>External [K]_{out} (mM)</th>
<th>Stimulus Strength (µA/cm²)</th>
<th>Resting Potential (mV)</th>
<th>Peak Voltage (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Reset the external sodium concentration (which you changed in the previous experiment) to its initial value of 150 mM. Set the stimulus current to the minimum current necessary to produce an action potential and set the stimulus duration to 0.12 msec.

1. In a series of at least seven experiments, measure the resting potential and the peak voltage for external potassium ion concentrations which vary between about 200 and 1 millimoles/Liter. Use the <Tab> key to select the input where external potassium concentration is changed.

2. **If an action potential does not occur, try increasing the stimulus strength.** Why might you have to increase the stimulus strength? **Make sure that the lack of an action potential is not caused by subthreshold stimulation.**

3. For each experiment record the voltage at the peak of the action potential, the external potassium concentration, and the stimulus strength (=stimulus current).

4. When you have finished, press <Esc> to return to the MAIN MENU.

5. If you are a new user make sure that you double check that you have recorded your SPIKE ID Code before you quit.

6. Select the Quit option, press <Enter>, and confirm your selection to quit. You may return to SPIKE to gather more data if you decide that this is necessary. SPIKE will return your nerve cell to you in the same condition that you left it if you have your valid ID Code.

SPIKE ID Code ____________________________
SPIKE DATA ANALYSIS

Place an asterisk next to the name that you used when you logged in to SPIKE and obtained the SPIKE ID code.

Copy your results from the previous section to this section. Use this section to report your results and to answer questions about the analysis of your experiments. Turn in this section.

Report the following information about your neuron.

SPIKE ID Code ____________________________________

Temperature = ______° C.

Internal sodium ion concentration = ______ mM

Internal potassium ion concentration = ______ mM

Sodium Activation (αM) = ______ (points deducted for incomplete information)

Part A. Determination of the Stimulus Threshold Voltage

A1. Report the following values determined in Part A:

Resting Potential = ______ mV

Threshold voltage = ______ mV

Minimum Stimulus Current Necessary to Produce the Threshold Voltage (0.12 msec duration) = ______ μA/cm²

A2. Describe what happens when the stimulus current is increased above the minimum value that is necessary to produce an action potential.
Part B. The effect of Changes in External Sodium Ion Concentration

B1. Report your results from Part B of the exercise in Table B1. Note that you will need to find the log_{10} of the sodium concentrations. If you do not have a scientific calculator, you may use Excel. Enter the Excel formula “=LOG(n)” where n is the number you wish to convert.

**Table B1.** Resting potentials, peak voltages for various external sodium concentrations.

<table>
<thead>
<tr>
<th>External [Na]_{out} (mM)</th>
<th>log_{10} [Na]_{out}</th>
<th>Stimulus Strength (µA/cm²)</th>
<th>Resting Potential (mV)</th>
<th>Peak Voltage (mV)</th>
<th>RT \ ln \ [Na]<em>{out} \over zF \ [Na]</em>{in}</th>
</tr>
</thead>
</table>

B2a. On the same set of axes, plot peak voltage (mV) versus log_{10} [Na]_{out} (mM), the resting potential (mV) versus log_{10} [Na]_{out} (mM) and the Nernst potential versus log_{10} [Na]_{out} (mM). You will have to calculate the Nernst potentials using the external concentrations that you used and the internal concentration that is determined by SPIKE. Attach this graph to the report and label it Figure B1. You may wish to use the graphs on the last page of the exercise.

B2b. Run linear regressions for the three lines and determine the slope and the intercept for each line.

<table>
<thead>
<tr>
<th>Graph</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>peak voltage (mV) versus log_{10} [Na]_{out} (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>resting potential (mV) versus log_{10} [Na]_{out} (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nernst potential (mV) versus log_{10} [Na]_{out} (mM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
B2c. Explain the significance of the slopes for each line.

B3a. How are the peak potentials and the resting potentials affected by the external sodium concentration?

B3b. What does this say about the permeability of the membrane to sodium during the resting potential and during the peak of the action potential?
Part C. The effect of Changes in External Potassium Ion Concentration

C1. Report your results from Part C of the exercise in Table C1.

Table C1. Resting potentials and peak voltages for various external potassium concentrations.

<table>
<thead>
<tr>
<th>External [K]_{out} (mM)</th>
<th>log_{10} [K]_{out}</th>
<th>Stimulus Strength (µA/cm²)</th>
<th>Resting Potential (mV)</th>
<th>Peak Voltage (mV)</th>
<th>RT \ln [K]<em>{out} \ ZF \ln [K]</em>{in}</th>
</tr>
</thead>
</table>

C2a. On the same set of axes, plot peak voltage (mV) versus log_{10} [K]_{out} (mM), the resting potential (mV) versus log_{10} [K]_{out} (mM) and the Nernst potential versus log_{10} [K]_{out} (mM). You will have to calculate the Nernst potentials using the external concentrations that you used and the internal concentration that is determined by SPIKE. Attach this graph to the report and label it Figure C1. You may wish to use the graphs on the last page of the exercise.

C2b. Run linear regressions for the three lines and determine the slope and the intercept for each line.

<table>
<thead>
<tr>
<th>Graph</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>peak voltage (mV) versus log_{10} [K]_{out} (mM)</td>
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<td>resting potential (mV) versus log_{10} [K]_{out} (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nernst potential (mv) versus log_{10} [K]_{out} (mM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C2c. Explain the significance of the slopes for each line.

C3a. How are the peak potentials and the resting potentials affected by the external potassium concentration?

C3b. What does this say about the permeability of the membrane to potassium during the resting potential and during the peak of the action potential?
Part D. The Hodgkin Huxley Model of the Nerve Action Potential

D1. Examine the Hodgkin Huxley variables using the plot control in SPIKE and explain the significance of h n m in the Hodgkin Huxley model of the nerve action potential.

D2. Examine the sodium and potassium currents using the plot control in SPIKE and explain the “notch” in the sodium current.

D3. Examine the conductances using the plot control in SPIKE and explain why nerve cells become hyperpolarized following the spike of the action potential.
E. Fitting Data to the Goldman-Hodgkin-Katz Equation.

Examine the SPIKE data for the effects of external potassium and sodium ion concentrations on the resting potentials and note that the data are not linear over the range of external ion concentrations from 1 to 200 mM. See if you can find values for the GHK Equation that provide a better fit for the data. You might start with the relative permeabilities given below.

\[ P_K = \text{relative permeability of potassium ions (often set to 1.0)} \]
\[ P_{Na} = \text{relative permeability of sodium ions (often set to 0.01)} \]
\[ P_{Cl} = \text{relative permeability of chloride ions (often set to 0.45)} \]

You may use Figures E1 and E2 to plot your results if you wish.
Graphs

Figure B1. Peak voltages, resting potentials, and Nernst potentials for an axon exposed to various concentrations of external sodium ions. (You might wish to choose to graph these data using Excel; however, this may add to the level of difficulty.)

Figure C1. Peak voltages, resting potentials, and Nernst potentials for an axon exposed to various concentrations of external potassium ions. (You might wish to choose to graph these data using Excel; however, this may add to the level of difficulty.)
**Figure E1.** Resting potentials for an axon exposed to various concentrations of external sodium ions. The resting potentials have been fitted with a curve that has been calculated using the Goldman Equation. (You might wish to choose to graph these data using Excel; however, this may add to the level of difficulty.)

**Figure E2.** Resting potentials for an axon exposed to various concentrations of external potassium ions. The resting potentials have been fitted with a curve that has been calculated using the Goldman Equation. (You might wish to choose to graph these data using Excel; however, this may add to the level of difficulty.)