LAB 1: ACTION POTENTIAL

OBJECTIVES

- To understand the ionic basis of the action potential including the conductances, currents, and channels that underlie it
- To simulate electrophysiological experiments to explore the factors that affect action potential shape, duration and firing rate

INTRODUCTION

Action potentials are reversible changes in the membrane potential of excitable cells during which the inside of the cell becomes temporarily positive relative to the outside. Information is transmitted along axons both to and from the brain and spinal cord in the form of action potentials. This information coding is essential to maintain life-sustaining properties.

The electrical properties of cells result from 1) the ionic concentration gradients across the cell membrane and 2) the permeability characteristics of the cell membrane. These two factors give rise to the cellular membrane potential. Normally, the concentrations of Na^+ and Cl^- are higher in the extracellular relative to the intracellular fluid while that of K^+ is higher inside the cell. Na^+ is constantly pumped out of and K^+ into the cell by the Na^+/K^+ ATPase. Membrane permeability influences the degree to which these ions can travel across the cell membrane down their concentration gradients. At the resting membrane potential (RMP), the cell membrane is much more permeable to K^+ than to Na^+ or Cl^-. Consequently, the RMP (-70 mV) is very close to the equilibrium potential of K^+ (-90 mV).

In this laboratory exercise, you will perform a computer simulation to understand the basis of action potential with reference to ion channel gating and conductance. You will also investigate the factors affecting the shape and propagation of action potential. It is important for you to have a clear understanding and strong foundation on membrane potentials, and the influence of ionic concentration difference in the extra and intracellular environment on the relay of information in excitable cells.

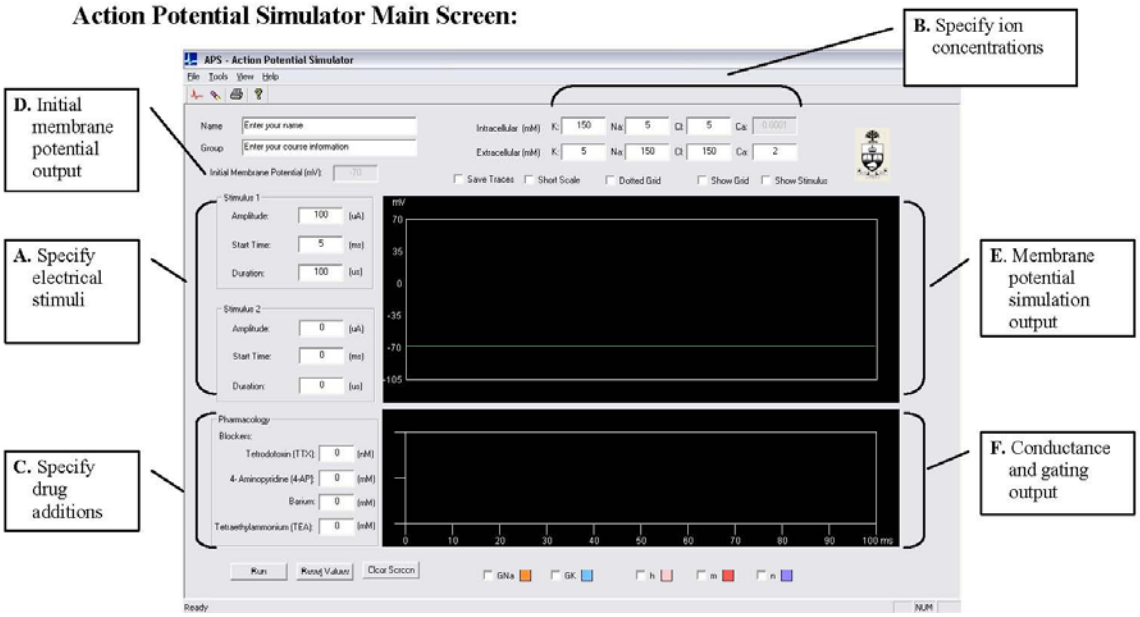
The computer model used in this laboratory is based on that developed by Hodgkin & Huxley for the giant axon of the squid. However, this simulation has been adapted to model mammalian cells. You will observe both the action potential itself, as well as the activity of the Na^+ and K^+ channels that generate it.

This simulation allows you to explore several principals:

- Action potential generation and refractory period
- Nature of sodium and potassium currents/channels during action potentials
- Effect of ion concentrations on resting membrane potentials and action potentials
- Effect of various pharmacological agents on membrane potentials and action potentials

Please do not feel limited by the specific protocols suggested below. ***Experiment!***

Action Potential Simulator Main Screen:



The main APS screen contains several components:

A. Specify electrical stimuli – Here you can specify first and second (optional) electrical stimuli. For each, specify the amplitude of the stimulus (in µA), the start time of the stimulus (in ms from the beginning of the simulation) and the duration of the stimulus (in µs).

B. Specify ion concentrations – Enter the desired intracellular and extracellular ion concentrations (in mM).

C. Specify drug additions – Enter the concentrations of drugs to be added to the simulated cell. TTX (0-15 nM), 4-AP (0-15 mM), Ba^{2+}(unlimited), and TEA (0-25 mM).

D. Initial membrane potential output – Displays the initial resting (unstimulated) membrane potential under the conditions that you specify in B and C.

E. Membrane potential simulation output – Displays tracings of membrane potentials resulting from stimulation. The y axis is displayed in mV and the x axis is displayed as either 100 ms (default) or 20 ms (when 'short scale' is selected). You will be able to display successive runs by selecting 'Save Traces'. For analysis purposes, displaying a grid may be useful. You may also choose to display the electrical stimuli.

F. Conductance and channel output – You may choose to display conductance and channel information for the simulation. Sodium conductance (G_{Na}), potassium conductance (G_K), and open status of the sodium channel gates (h and m) and potassium channel gates (n) can be displayed.

Traces can be 'saved' and superimposed on each other by clicking 'save traces'. You can also show the stimulus pulse responsible for generating the action potential by clicking 'show stimulus'.

For all activities, you may print out your results for your records (and for studying!). For those of you who don't want to print out results, recall that images of the active window can be copied to the clipboard using the ALT+PrtScr function.

A: ACTION POTENTIAL

The action potential results from a **positive feedback** cycle. Depolarization of the cell membrane activates Na^+ channels, causing an influx of Na^+ and depolarizing the membrane further. This opens more Na^+ channels, which results in further depolarization. The cycle continues until the Na^+ channel inactivation gates close the channels and the K^+ channel gates open. At this point, a **negative feedback** loop begins in which K^+ exits the cell, thereby repolarizing the membrane.

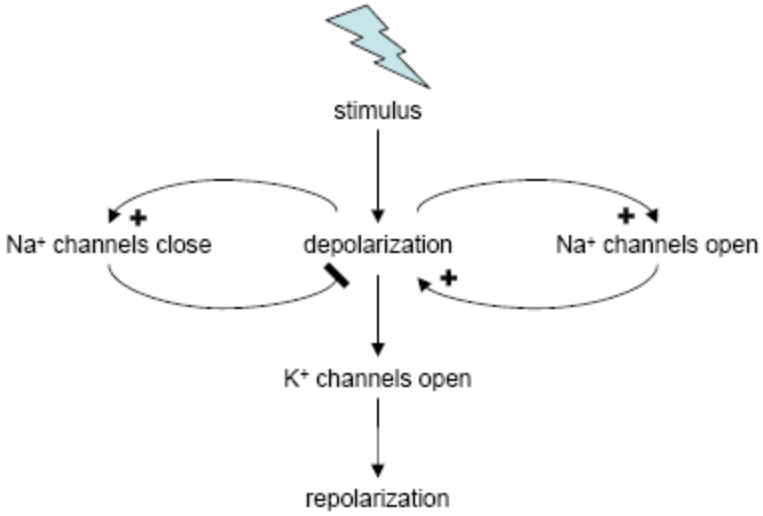


Figure 1.1: Illustration of the feedback loops governing action potential initiation.

A cell will produce an action potential once the sodium conductance exceeds the potassium conductance – this minimum membrane potential that can generate an action potential is known as the **threshold voltage (V_T)**. The **threshold stimulus** is therefore the stimulus required to depolarize a cell to the threshold potential.

The opening/closing of Na^+ and K^+ channels is dictated by the conformation of their gates. Hodgkin and Huxley modeled the Na^+ conductance using three m gates that control activation of the channel and one h gate that controls its inactivation. While originally the number of gates was inferred from parameters in their mathematical model, there is now experimental evidence for the existence of these gates. The m gates are closed at rest; depolarization causes them to open rapidly. The h gate is open at rest; depolarization causes it to close slowly. In other words, they are reciprocally activated/open. Thus, at resting membrane potential, the m gates are closed, while the h gate is open. When the channel is depolarized to threshold value, the m gates open. At this point, the h gate is still open, allowing the entrance of Na^+ into the cell; however, after a time delay of about 0.5 ms, the h gate closes and causes inactivation of the Na^+ channel. The flow of Na^+ ions depends on ion concentration and on the number of open channels (or, on m and h conformation).

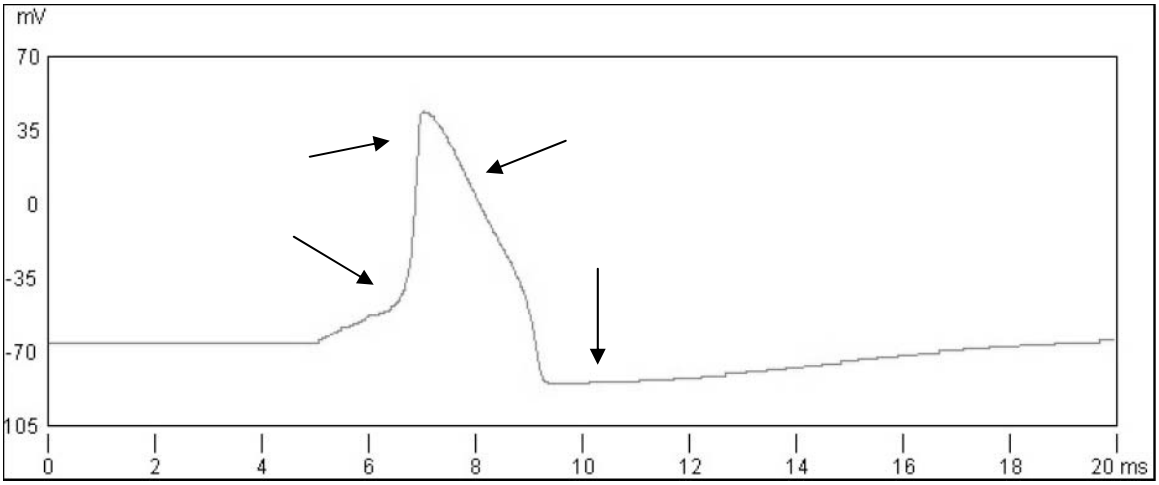
Similarly, Hodgkin and Huxley modeled the K^+ conductance using four n gates, which are slower to open and slower to close than the m gates of Na^+ channels; this leads to repolarization and subsequently hyperpolarization. Channels, which control the flux of ions into or out of the neuron, do not open and close instantaneously. Gates provide this time dimension, controlling the rate at which channels are permitted to open and close. An opened K^+ channel requires that all 4 n gates are activated/open, allowing for the K^+ efflux. Both Na^+ conductance and K^+ conductance are *sigmoidal functions of membrane potential. What does this mean?*

The Experiment

In this first exercise, you will generate action potentials by giving either 1 or 2 electrical stimuli. The computer will plot the membrane potential (voltage) as well as the underlying Na^+ and K^+ conductance (select G_{Na} and G_K options). Use both long and short scales to observe your results. (Remember that **conductance** is proportional to the number of **channels** open which is proportional to the probability that the associated gates are open).

Stimulate an action potential with the default stimulus (amplitude 100 μA, start time 5 ms and duration 100 μs). Using the short (20 ms) scale, observe the shape of the action potential, and correlate it with the rapid increase and decrease in Na^+ conductance (G_{Na}), and slower increase and decrease in K conductance (G_K).

Label the following with the ion conductances and gating kinetics responsible for the shape of the action potential:



*Why is the membrane potential following the action potential more negative than the **resting potential**?* (-70mV is the resting potential of the cell: if you gave no stimulus, the membrane potential should stay at -70 mV indefinitely) *What is the term used to describe this? What is the physiological importance of this? Describe the mechanism of its occurrence.*

Display the status of the Na^+ (h and m) and K^+ (n) gates (note that the vertical scale here is 100% probability of all gates being open (top) and 0% of gates open (bottom)). *Observe the open status of the gates and correlate these with membrane potential, G_{Na} and G_K throughout the action potential. How do the h and m gates control G_{Na} during the action potential? Which part of the action potential do they play a role in? How does the n gate control G_K and how does it affect the action potential?*

What would happen if the m gates opened quicker than normal? What if the h gates opened quicker than normal? What if the n gates opened slower than normal?

Set the stimulus duration to increasing values according to the table. For each stimulus duration, determine the stimulus amplitude required to just initiate an action potential. Select the 'grid' option to read the threshold voltage off the trace. *Determine how this threshold stimulus is related to duration by completing the following table:*

Duration (μs)	**Threshold stimulus (μA)**	**Threshold voltage (mV)**
50		-55
100	60 μA	-55
200		-45
300		-42

What is the relationship between duration and threshold stimulus? Plot a strength duration curve to visualize the relationship.

How does the threshold voltage change with varying stimuli? Explain your results with relation to ion channels and gating. What are the physiological implications of these findings?

B: REFRACTORY PERIOD

The refractory period is the time in which the membrane is unable to fire another action potential due to the inactivation of the Na^+ channel (as a result of the m and h gate configuration as previously described). During the ***absolute* refractory period**, none of the Na^+ channels have been reconfigured, thus no matter how large the stimulus is, an action potential cannot be generated. During the ***relative* refractory period**, however, not all of the Na^+ channels are inactivated, thus a higher-than-normal stimulus (suprathreshold) is able to fire a second action potential.

The Experiment – NOTE: UNCHECK "SHORT SCALE"

Observe the absolute and relative refractory period by first obtaining a control action potential with a large, brief stimulus (stimulus 100 μA, start time 5 ms, duration 100 μs – default value). Next, give a second stimulus starting at 25 ms with the same amplitude and duration as the first.

Does a second action potential form? How does its shape and size compare to that of the first action potential? Describe the physiological mechanism behind your observations.

Decrease the start time of the second stimulus between 25 and 10 ms in one ms intervals (i.e. 24, 23, 22, 21, etc). *What happens? Can you explain the changes in the second action potential shape and in Na^+ conductance?*

Set the delay back to 25 ms and repeat the experiment using an amplitude of 70 μA (near threshold) for the second stimulus. Repeat using 1000 μA. *Are the results the same? When was the axon in its **absolute** refractory period? When was it in its **relative** refractory period? What is the physiological significance of the refractory period?*

Finally, give a single prolonged stimulus to the cell with a start time of 5 ms, and duration of 100 ms (100,000 μs). First, use an amplitude of 10 μA, then try 70, 80, 100, 1000, 2000, 4000 and 8000 μA. *What happens and why? Why is it important in neuronal communication? What is this phenomenon called and in what type of cells is it present? What happens if a negative stimulus is given (i.e. –100 μA)?*

C: ION SUBSTITUTION

Here, you can vary the concentrations of intracellular and extracellular K^+, Na^+, Cl^- and Ca^{2+}. (NOTE: Assume that the extracellular concentration of an ion can be varied without affecting its intracellular concentration and vice versa.)

Membrane potential is dependent on several factors: intracellular and extracellular ion concentrations, ion valance, permeability of the membrane to each ion and temperature. Potential across a cell membrane is described by the expanded Goldman-Hodgkin-Katz Equation, which quantifies the battle between the various permeable ions and predicts how the cell would respond to a perturbation in ion concentration:

$$E_m = \frac{RT}{F} \ln \frac{P_K[K^+]_{out} + P_{Na}[Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{out}} \quad (units = J/C = V)$$

where P_x = *permeability of ion x*
$R = 8.31\ J \cdot mol^{-1} \cdot K^{-1}$ *(Universal Gas constant)*
T = *temperature in K, often 293 K (20°C) for these recordings*
$F = 9.6 \times 10^4\ C \cdot mol^{-1}$ *(Faraday's constant – charge per mole)*

NOTE: A cell is never actually at equilibrium; during an action potential, permeability of the various ions change as a function of membrane potential and the specific dynamics of each channel, and even under steady state conditions, there is flux through various channels because no channels are 100% selective in reality. (Also flux of the dominant permeant ion)

Each of the permeable ions has an equilibrium potential (E_{ion}), a theoretical point at which outward flux of the ion equals the inward. This equilibrium potential is calculated using the Nernst equation: $E_{ion} = (RT/ZF) \ln ([ion]_o/[ion]_i)$ where Z=charge of the ion. The **driving force** on each ion is in the direction towards its equilibrium potential.

Table 1.1: Typical ion concentrations in selected cells of various species. Note the similarities and differences between each. *Default values used in this simulation are approximations of mammalian values.*

	mammalian neuron		frog muscle		squid axon	
ion	[intracellular] (mM)	[extracellular] (mM)	[intracellular] (mM)	[extracellular] (mM)	[intracellular] (mM)	[extracellular] (mM)
K^+	140	5	124	2.25	400	20
Na^+	5-15	145	10.4	109	50	440
Cl^-	4	110	1.5	77.5	40-150	560
Ca^{2+}	0.0001	2.5-5	0.0001	2.1	0.4	10

The Experiment

Try altering intracellular and extracellular [K^+], [Na^+], [Cl^-] and [Ca^{2+}]. (Note that large increases in extracellular electrolytes are not physiological, as that would make the solution hypertonic).

Intracellular [Ca^{2+}] is fixed at 0.1 μM; *why would the simulation be designed this way? What is the primary role of Ca^{2+} in neurons?*

Alter the concentrations of each ion and record the resulting resting (unstimulated) potential (in grey box in the upper-left – "Initial Membrane Potential").
*What happens to the **resting membrane potential** as ion concentrations are altered? Which ion has the greatest effect on resting membrane potential? Which has the least? Explain why this occurs.*

Calculate the equilibrium potentials for Na^+ and K^+ under default conditions. Compare your values to the resting membrane potential. Which value is closer to the resting membrane potential and why? What does this say about the permeability of that ion at steady state compared to the other?

*What happens to the **action potential** as ion concentrations are altered? Summarize the effect of each of the ions on action potential size and shape.*

Do intracellular or extracellular ion concentrations change during or as a result of an action potential? Why?

Experiment with the effect of ion concentrations on action potential thresholds and refractory periods.

D: PHARMACOLOGY

Drugs and toxins are invaluable tools to characterize the kinetics and structures of ion channels. Researchers routinely use a variety of drugs to selectively block specific ion channels or mimic the actions of specific ions. This simulation allows you to use four different drugs as summarized in the following table:

Table 1.2: Pharmacological agents available in the simulation, their dissociation constants, and mechanism of action.

Drug	Available concentrations	Kd	Action
Tetrodotoxin (**TTX**)	0 – 15 nM	1 nM	blocks voltage-gated Na^+ channels
4-Aminopyridine (**4-AP**)	0 – 15 mM	1 mM	blocks voltage-gated K^+ channels
Barium (**Ba^{2+}**)	0 – unlimited	n/a	Ca^{2+} mimetic blocks inwardly rectifying K^+ current
Tetraethylammonium (**TEA**)	0 – 25 mM	2.5 mM	blocks voltage-gated K^+ channels AND K^+ leak channels

The Experiment

On the 20 ms time scale, give a single stimulus (e.g. 100 μA for 100 μs) with no drugs. Then add increasing concentrations of TTX or 4-AP. Remember that 1/2 of the channels will be blocked when the concentration = the dissociation constant, and about 90% will be blocked at 10x higher concentration. *What happens to the shape of the action potential and ion conductances when half the voltage gated Na^+ channels are blocked? When 90% are blocked? What happens when half the voltage gated K^+ channels are blocked? 90%?*

Look at what happens with 4 mM TEA on the long (100 ms) scale. *What is the effect on the initial membrane potential and action potential generation? Explain the mechanism of occurrence in a model neuron with reference to ion conductances. Do any cells do things like this normally?*

Try adding both TEA and 4-AP in various combinations. *What does this tell you about the roles of various K+ channels? Indicate the primary role of each type of K^+ channel with reference to phases of the action potential.*

Add Ba^{2+} in increasing concentrations. *How does this effect the action potential shape produced? Explain your results.*

Experiment with the effect of drugs on refractory periods, thresholds and the interaction with varying ion concentrations.