University of Toronto

Faculty of Applied Science and Engineering

BME445 Neural Bioelectricity

Lab 3: Generation of Action Potentials

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Purpose

In this lab, we aim to recreate Hodgkin and Huxley's original experiments in a simulation, to enhance our understanding of the model of action potential generation in the giant axon of the squid. Our purpose is to understand the ionic currents and conductances that underlie such phenomena as the firing of the action potential, frequency encoding of stimulus amplitude, accommodation and refractoriness.

Results/Discussion

A. Excitation

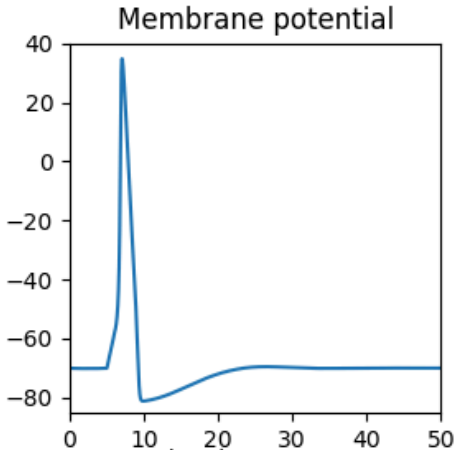

Figure 1: The action potential with default settings and pulse width of 1ms.

Describe the action potential: including characteristics like threshold potential, maximum amplitude, and hyperpolarization.

An action potential is a rapid sequence of changes in the voltage across a membrane as a result of a stimulus. Right before the excitatory stimulus is applied (5ms), we observe the resting membrane potential to be at -70V. The threshold potential is the value of the membrane potential, which when reached, leads to the all-or-non initiation of the action potential. From *Figure 1,* we can observe the threshold potential to be around -55V, creating a depolarization in the membrane when reached that results in the initiation of the action potential. The peak of the action potential is observed to be just below 40V, around 38V. After reaching this value, the membrane goes through a repolarization state in which the cell experiences a decrease of voltage due to the efflux of potassium ions. Due to an excess of open potassium channels and potassium efflux from the cell, the hyperpolarization and undershoot occurs, where the membrane potential briefly dips lower than its resting potential. When the membrane is hyperpolarized, we observe the voltage to be -80V. By looking at the difference between the peak of the action potential and the threshold value, we determine the maximum amplitude of the action potential to be 38V + |-55V| = 93V.

Find the minimum current pulse amplitude (First Pulse Current) which causes the firing of an action potential (excitation threshold) for various pulse widths and plot the results as a Strength-Duration Curve.

Table 1: Corresponding current amplitudes (μA) for varying pulse widths (ms).

Pulse Width (ms)	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	2.0
Current amplitude (μA)	0.258	0.13	0.088	0.067	0.054	0.047	0.041	0.036	0.031

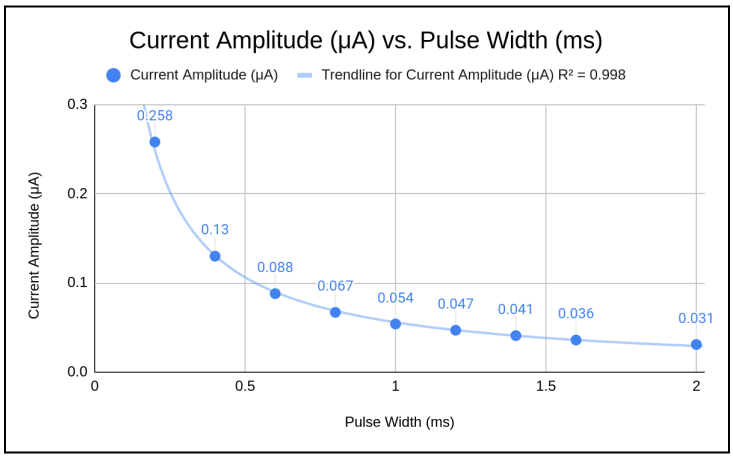

Figure 2: Strength-Duration Curve for the values given in Table 1.

1. **Is there a simple relationship between stimulus duration and strength?**
   With progressively stronger stimulation, more and more nerve fibres are activated. However, the threshold for activation of a fibre depends not only on the stimulus strength but also on the duration of the stimulus. From the plot of the Strength-Duration curve, in *Figure 2*, we observe a decline that is near zero as the stimulus duration is increased. This indicates that the stimulus strength required to reach threshold should decrease during more prolonged stimulation. Therefore, we can conclude that there exists an inverse relationship between the pulse width and the current amplitude. However, due to the resistive and capacitive effects in the membrane, the membrane potential rises exponentially to a plateau during prolonged stimulation, instead of increasing linearly with time. This relationship could be represented by using the power series trendline on *Figure 2,* to be $I = 0.0559D^{-0.928}$ (where the stimulus duration is D and the current required to achieve activation is I).

2. **The rheobase is the maximum amplitude which cannot trigger an action potential regardless of duration. From your graph, what is its magnitude?**
   From *Figure 2,* we observe the rheobase to be 0.031 μA, where the strength-duration curve plateaus.

B. Latency

Produce a point-by-point Latency Curve for the membrane by applying current pulses having a pulse width of 1ms and various pulse amplitudes = [0.06, 0.07, 0.08, 0.1, 0.12, 0.14, 0.16...] µA and measuring the time between the stimulus onset and the time of the peak of the action potential. Plot the results.

Table 2: Corresponding latencies (ms) for varying current amplitudes (µA).

Current Amplitude (µA)	0.06	0.07	0.08	0.1	0.12	0.14	0.16	0.18	0.2
Latency (ms)	3.7	2.87	2.47	2.04	1.79	1.64	1.5	1.43	1.35

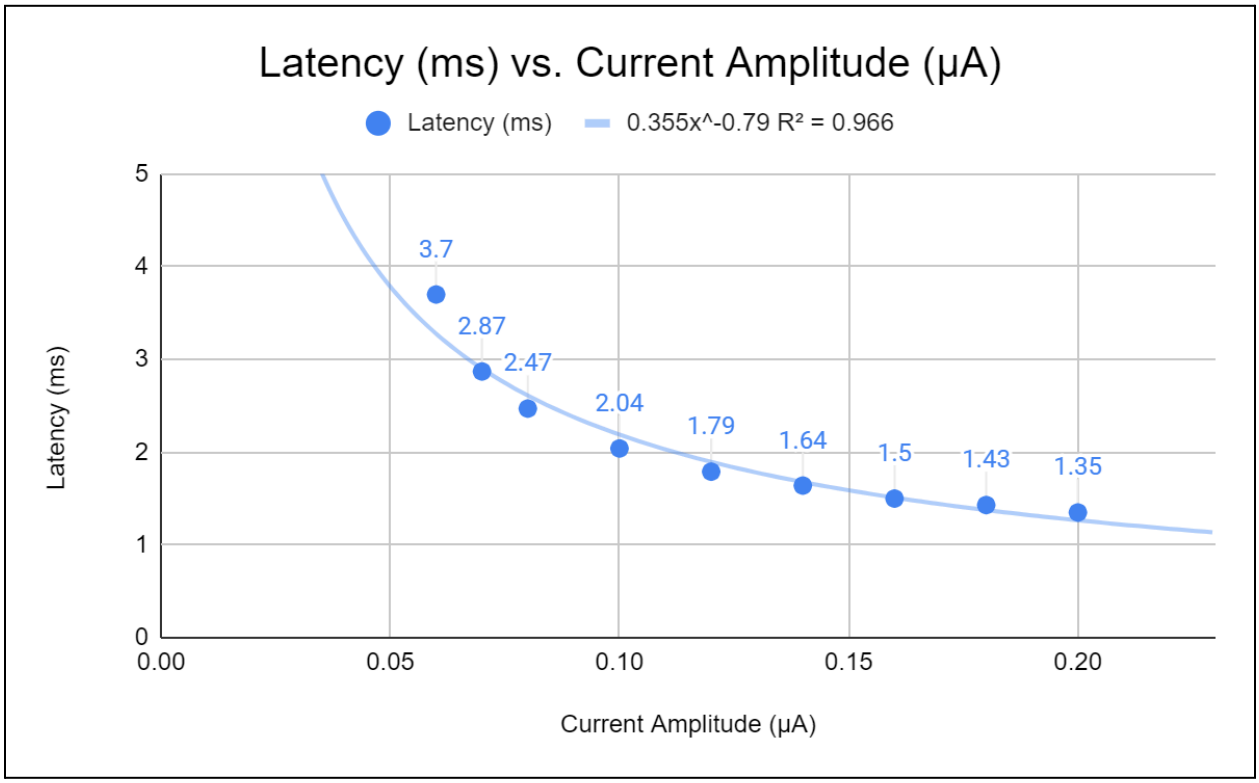

Figure 3: Latency Curve for the values given in Table 2.

From *Figure 3,* we can observe that for increasing values of the current amplitude, the latency time that is required to initiate an action potential decreases.

C. Refractoriness

Plot a graph of the minimum pulse 2 amplitude required to produce a second action potential for various delays.

Table 3: Corresponding second pulse current amplitudes for various second onset delays.

Second Onset Delay (ms)	2	4	6	8	10	12	14	16	18
Second Pulse Current Amplitude (µA)	11	3.14	1.4	0.632	0.392	0.255	0.182	0.15	0.146

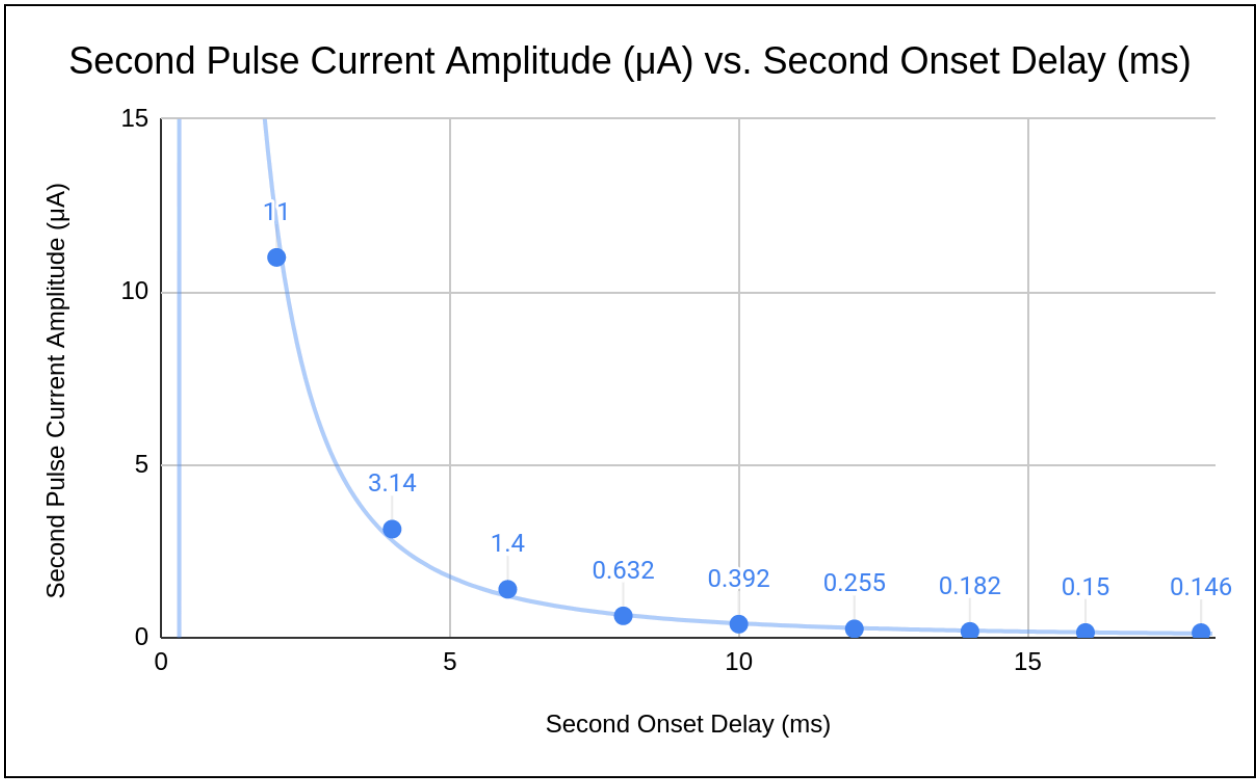

Figure 4: Refractoriness curve for the values given in Table 3.

1. How long is the absolute refractory period?
The absolute refractory period lasts around 2ms, which is from 0-2ms.

2. How long is the relative refractory period
The relative refractory period lasts around 10 ms which is from 2-12ms.

D . Passive Properties

Choose a -0.1µA stimulating step current (i.e. duration 100ms) and observe the plot.

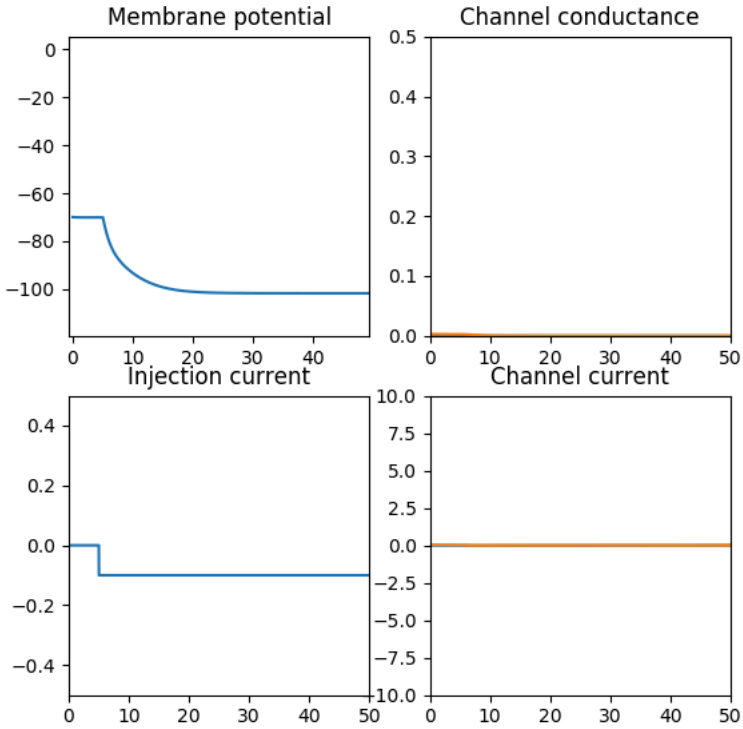

Figure 5: Plots for -0.1µA stimulating step current (i.e. duration 100ms).

The membrane appears to respond in a passive manner. Can this initial hyperpolarizing portion be characterized by a simple RC network? If so, what is the time constant?

Yes, the initial hyperpolarizing portion in the membrane potential plot in *Figure 5,* can be characterized by a simple RC network, since this is a passive property and the time for the membrane to discharge can be represented by a time constant. This initial voltage drop takes place between 5 and 25ms. Since the voltage drop across the capacitor in a discharging RC network is ~37% of its initial value, we can calculate the time constant τ to be:

$$\tau \simeq 37\% \, (25 - 5 \, ms) \simeq 7.4 \text{ ms}$$

E. Na+ and K+ Conductance

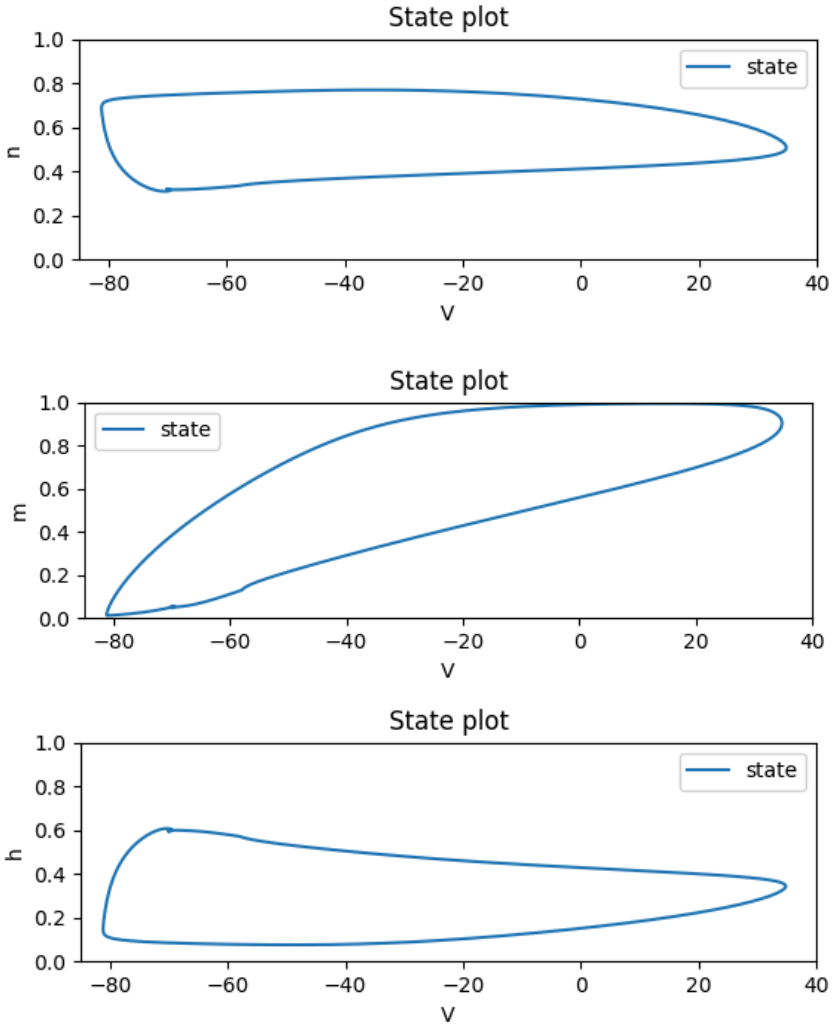

Figure 6: State plots for the values of m, n and h.

Why are the initial values of m, n, and h not zero at the beginning of the trace? (Expose the State Plot to see m, n and h.)

The initial values of m, n and h are non-zero at the beginning of the trace at -70V, because even when the membrane is at rest, leak potassium channels are open ($n \neq 0$) as well as some sodium channels ($m \neq 0$) and a fraction of sodium channels are inactive ($h \neq 0$).

Describe qualitatively the changes in proportion of open Na+ and K+ channels during the course of the action potential.

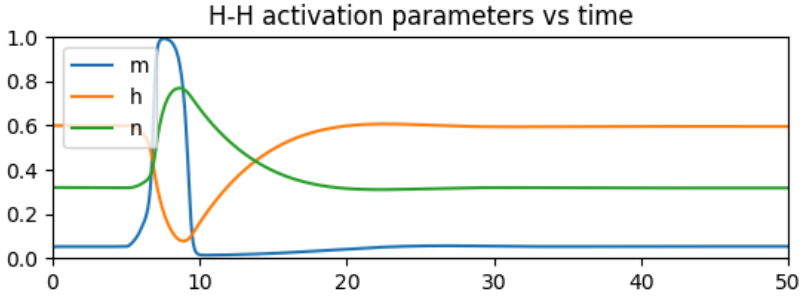

Figure 7: Plot of H-H activation parameters m, n and h vs. time.

At rest, a significant portion of the Na channels are inactive (h) whereas the leak K channels are open (n). When the membrane reaches the threshold (around 5ms) and the action potential is initiated, more and more voltage gated Na channels start to open, causing depolarization. In *Figure 7*, we can observe this as an increase in the activation of Na channels, m, and a decrease in the inactive Na channels, h. As the action potential reaches its peak (around 8ms in *Figure 1*), the Na channels start to close (around 7.5ms in *Figure 7*) and the fraction of active K channels, n, start to increase. This causes the membrane to go under repolarization as the K ions leave the cell. As we can observe in *Figure 7*, K channels cannot deactivate as fast as they are activating. This causes a fraction of K channels to remain open even after the membrane reaches the resting potential. The open fraction of the voltage gated K channels cause the hyperpolarization of the cell. As K channels close, the cell returns to its natural permeability to Na and K during this recovery period, coming back to the resting potential.

What is the time difference between:
1. Peak g Na and the peak of the action potential?

Peak of the action potential: 7.04ms
Peak Na conductance: 7.146ms

The time difference between peak Na conductance and the peak of the action potential is 0.106ms.

2. Peak g K and the peak of the action potential?

Peak of the action potential: 7.04ms
Peak K conductance: 8.616ms

The time difference between peak K conductance and the peak of the action potential is 1.576ms.

F. Voltage Clamp

For different clamping voltages = [-65, -60, -50, -40, -30, -0, 50, 100, 200 ... 500] mV, determine the maximum Na+ conductance. Plot gNa as a function of clamp voltage.

Table 4: Maximum Na+ conductance for various clamping voltages

Clamp Voltage (mV)	-65	-60	-50	-40	-30	0	50	100	200	300	400	500
gNa (mS)	0.0003	0.02	0.016	0.057	0.111	0.24	0.343	0.39	0.435	0.46	0.472	0.48

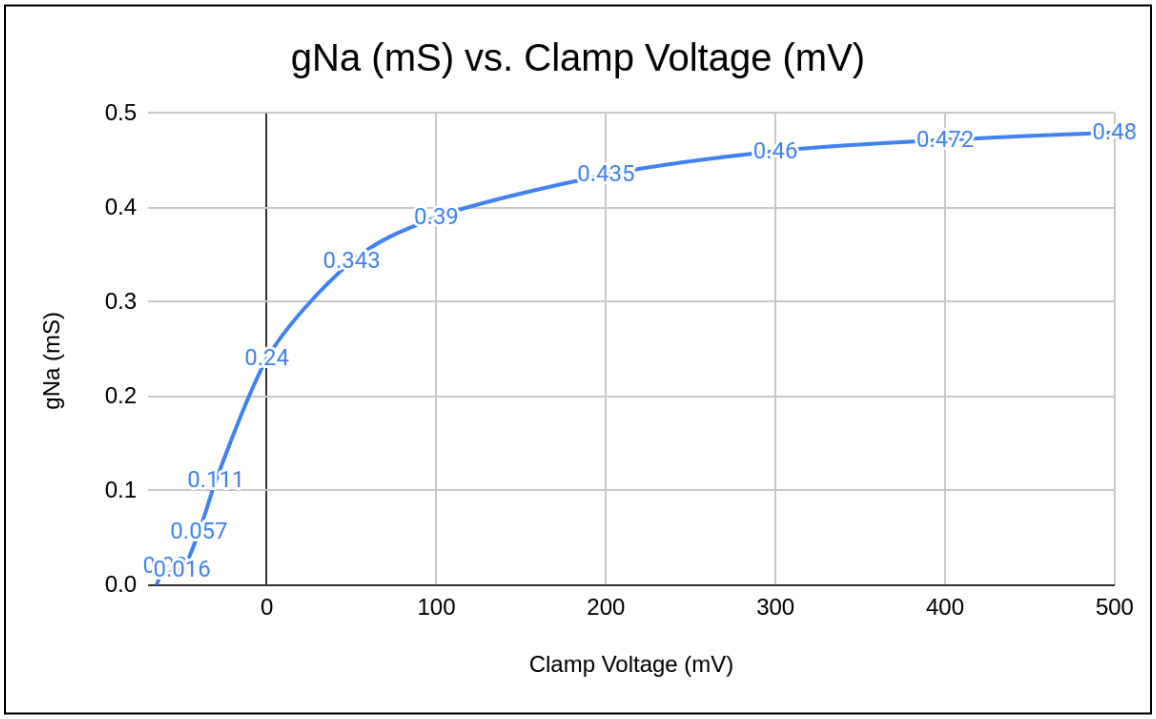

Figure 8: Maximum Na+ conductance across various voltages given in Table 4.

Repeat for K+ , i.e. plot gK vs clamp voltage = [-65, -60, -50, -40, -30, -0, 50, 100, 200 ... 500] mV.

Table 5: Maximum K+ conductance for various clamping voltages

Clamp Voltage (mV)	-65	-60	-50	-40	-30	0	50	100	200	300	400	500
gK (mS)	0.006	0.011	0.039	0.08	0.115	0.198	0.256	0.267	0.276	0.278	0.278	0.28

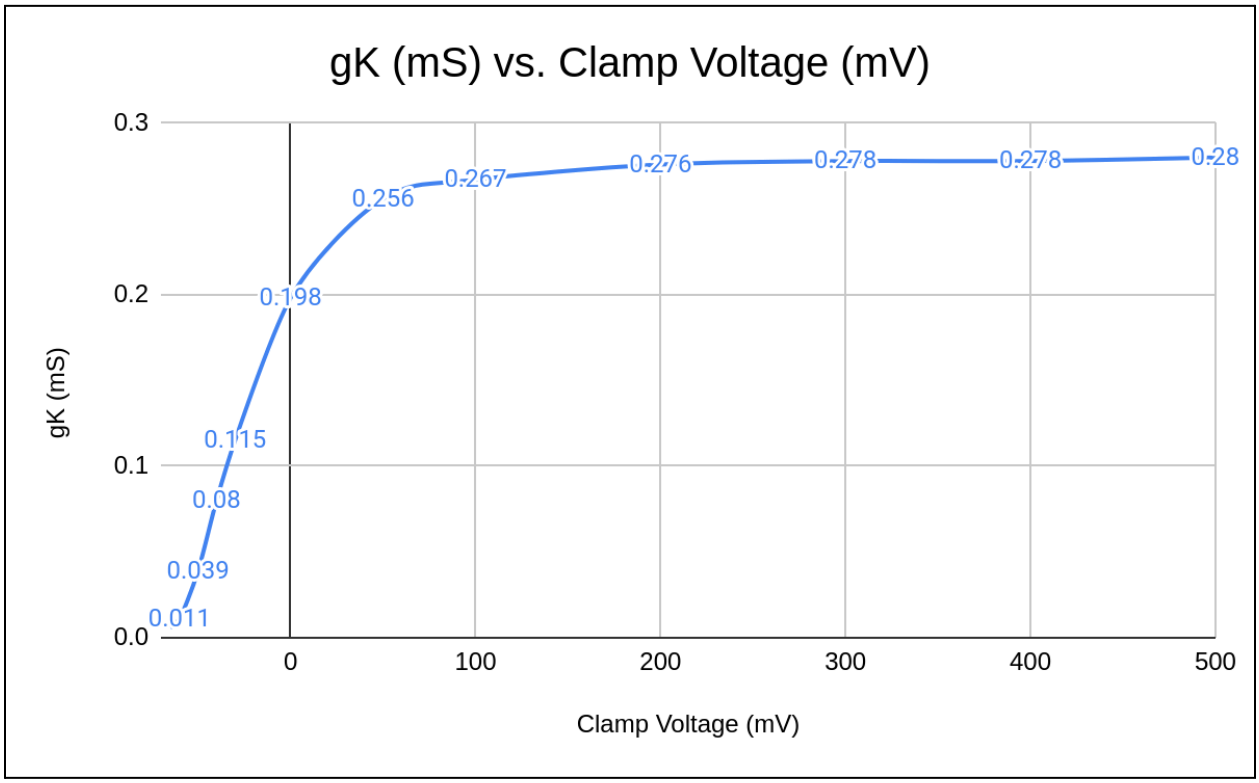

Figure 9: Maximum K+ conductance across various voltages given in Table 5.

At what voltage do the channels start to activate strongly?

Na+ channels start to activate strongly around the threshold value of -55V, initiating the depolarization of the cell.

K+ channels start to activate around the -45V, however this is not significant since the K channels are slow to open. K+ channels start to activate strongly around +30V, where the peak of the action potential occurs.

Here we follow the same calculation steps as H-H performed to determine the rate constants for the channels. For a clamp potential of -50 mV, graphically determine (using the STATE MODE PLOT) α and β values for m, n and h:

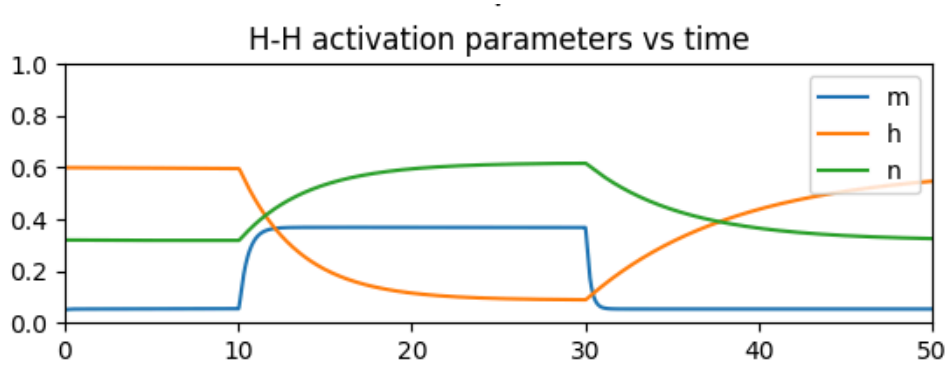

Figure 10: H-H activation parameters vs. time for a clamp potential of -50mV.

At 30 ms from *Figure 10* we observe:

$m_\infty = 0.34$

$n_\infty = 0.6$

$h_\infty = 0.08$

And from Part D: $\tau \simeq 7.4$ ms

αm $= m_\infty / \tau = 0.34 / 7.4 = 0.046\ ms^{-1}$ **βm** $= (1 - m_\infty) / \tau = (1 - 0.34) / 7.4 = 0.089\ ms^{-1}$

αn $= n_\infty / \tau = 0.60 / 7.4 = 0.081\ ms^{-1}$ **βn** $= (1 - n_\infty) / \tau = (1 - 0.60) / 7.4 = 0.054\ ms^{-1}$

αh $= h_\infty / \tau = 0.08 / 7.4 = 0.01\ ms^{-1}$ **βh** $= (1 - h_\infty) / \tau = (1 - 0.08) / 7.4 = 0.12\ ms^{-1}$

What causes the large injected current spikes?

The voltage clamp detects the actual membrane potential, and then very rapidly injects current into the neuron, causing a large injected current spike, to correct any deviation of the actual potential from the desired potentials.

G. Current Clamp and Conductances

Return to current clamp mode and observe an action potential (pulse width 1ms, amplitude 0.1µA).

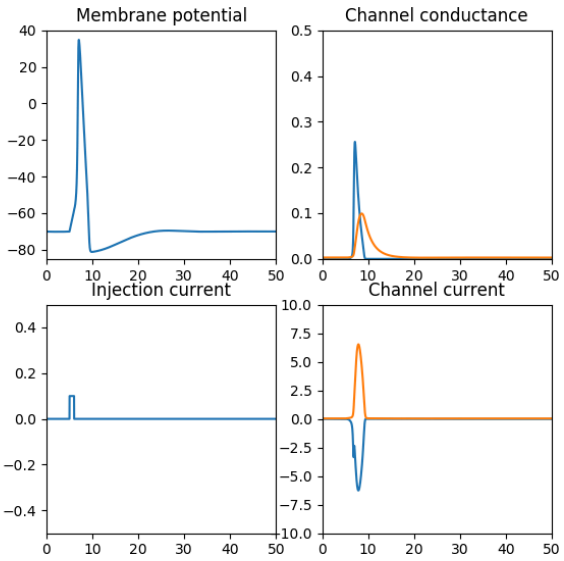

Figure 11: Action potential with pulse width of 1ms and amplitude of 0.1µA.

1. Why does the action potential stop rising and start to decrease?

The action potential stops rising as the Na+ channels start to close and it starts decreasing as the K+ channels are opening and there is a net efflux of K+ ions.

2. Describe the time course of the total ionic current during an AP. Why is there an initial sharp peak in the sodium current? What happens to the instantaneous total of ionic currents during an action potential? (Hint: Think back to part E)

As the membrane voltage reaches the threshold value, Na+ channels start to activate and the total ionic current is dominated by the Na+ influx. The initial sharp peak in the Na+ current results from this rapid inflow of Na+ and results in the depolarization of the membrane. As the action potential reaches its peak, the Na+ channels start to close, and as a result we observe a drop in the Na+ current. Simultaneously, the opening of the K+ channels results in an efflux of K+ ions and balances out the total ionic current. As the total ionic current is dominated by the efflux of the K+ ions, the membrane repolarizes. Due to the slow deactivation of K+ channels, K+ current slowly decreases while still dominating the total ionic current, causing the action potential to undershoot and hyperpolarize. After K+ channels are closed, the total ionic current recovers its initial balance as the cell returns to its natural permeability to Na+ and K+ ions.

3. By blocking the Na+ and K + channels, determine the leakage voltage. By unblocking the channels and balancing the currents without stimulus determine the leakage resistance. What are the specific values of Eleak , gleak ? (Hint: Think back to membrane current equation)

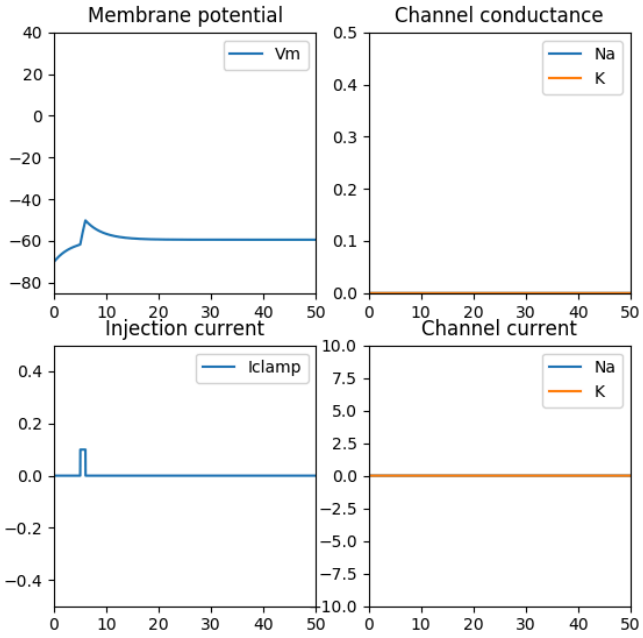

Figure 12: Both Na+ and K+ channels are blocked.

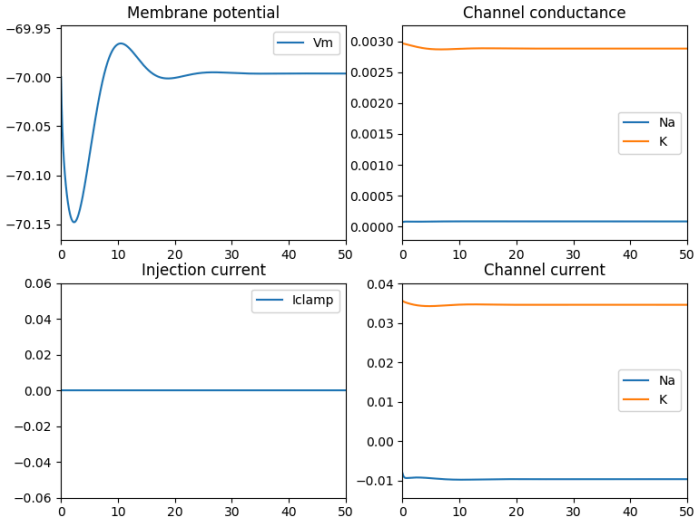

Figure 13: Both Na+ and K+ channels are unblocked, without stimulus applied.

From the Membrane Potential graph in *Figure 12* we can determine the leakage voltage to be:

$$E_{leak} = -60\ mV$$

By unblocking the channels and balancing the currents without stimulus, from the Channel Current Graph in *Figure 13,* we determine the maximum Na+ and K+ values:

$$I_{Na} = -0.009\ \mu A \text{ and } I_{K} = -0.034\ \mu A$$

$$I_{leak} = -(I_{Na} + I_{K}) = -(-0.009 + 0.034) = -0.025\ \mu A$$

$$g_{leak} = \frac{I_{leak}}{V_m - E_{leak}} = \frac{-0.025 * 10^{-6}}{[-70-(-60)] * 10^{-3}} = 2.5\ \mu S$$

4. What physical processes are modelled by the leakage pathway?

The leakage pathway models the "leaky" or passive channels on the cell membrane. The neuron cell membrane is partially permeable to Na+ ions, and K+ leak channels are always active at some level. These leak channels allow Na+ to slowly move into the cell and K+ to slowly move out, following their concentration gradient.

H. Accommodation

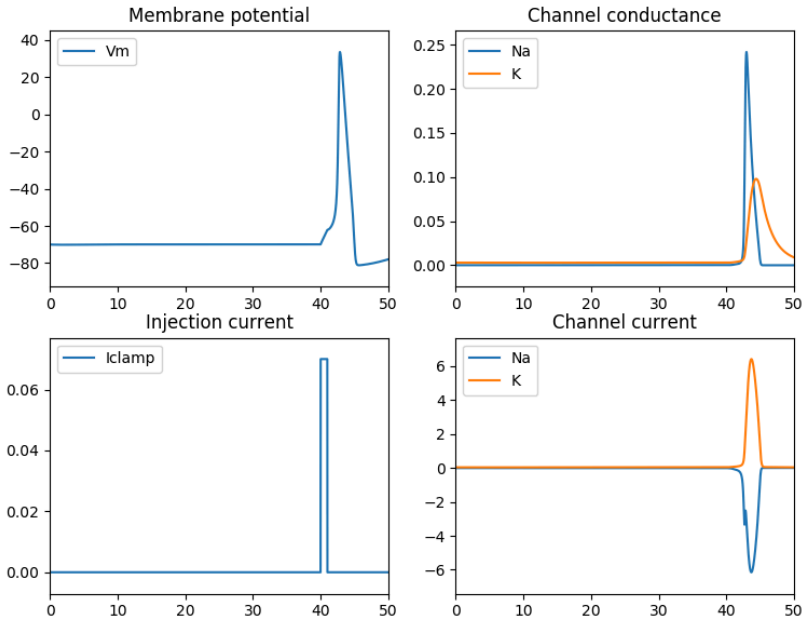

Figure 14: Action potential generated with default parameters (Base Level Current set to 0.0 μA)

Now set the Base Level Current to 0.03μA and now observe the membrane's reaction.

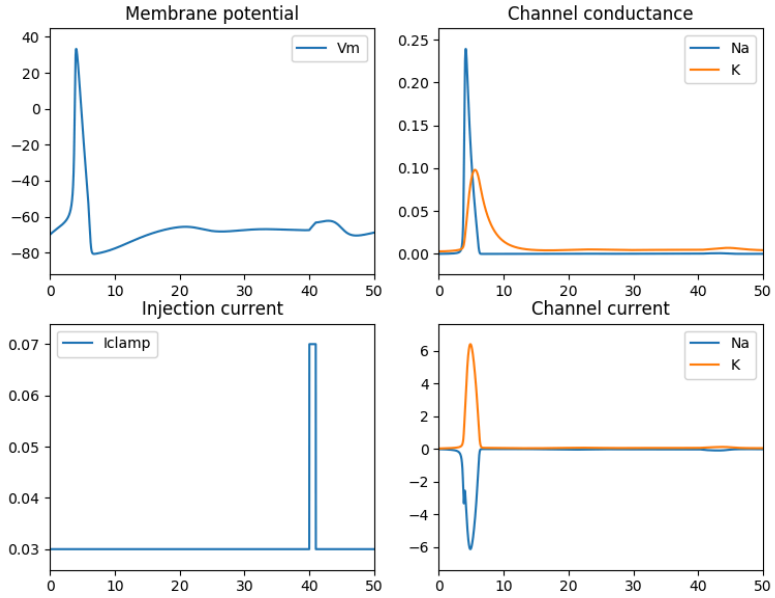

Figure 15: Action potential generated with Base Level Current set to 0.03 μA

1. Describe the change in Vm as the Base Level Current is changed from 0.0µA to 0.03µA

In *Figure 14,* as the base level current is 0, the cell doesn't fire an action potential until the first pulse current is injected at 40 ms. In *Figure 15,* since the Base Level Current is non-zero, the cell fires an action potential without any other external stimuli at time 0. Due to this low amplitude and long duration Base Level Current of 0.03µA, when the pulse current of 0.07µA is applied at t=40ms, the membrane potential cannot reach the threshold to fire an action potential, since the excitability of the membrane is reduced due to accommodation.

2. Using m, n and h to help you, why does the pulse that is normally above threshold fail to produce an action potential? What is a plausible physiological occurrence of such Accommodation?

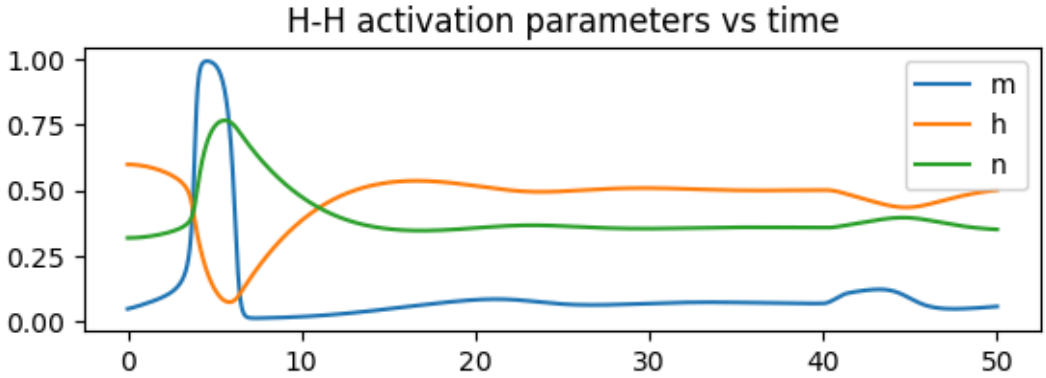

Figure 16: H-H activation parameters during Accommodation

Due to low and persisting stimuli, the neuron undergoes a slowly developing or prolonged depolarization, which causes the threshold for an action potential to increase. On an ionic level, this threshold increase is caused by a rise in the K+ conductance and an increase in the degree of inactivation. During accommodation, the pulse that is normally above threshold fails to produce an action potential due to the increased threshold, which the stimuli is now unable to meet.

Physiologically, accommodation is plausible as it prevents excessive and constant firing of action potentials under persisting stimuli, serving as a protection mechanism for the neurons.

3. Set the base current level to 0µA. Choose a -0.1µA stimulating pulse of 5ms duration and observe the plot.

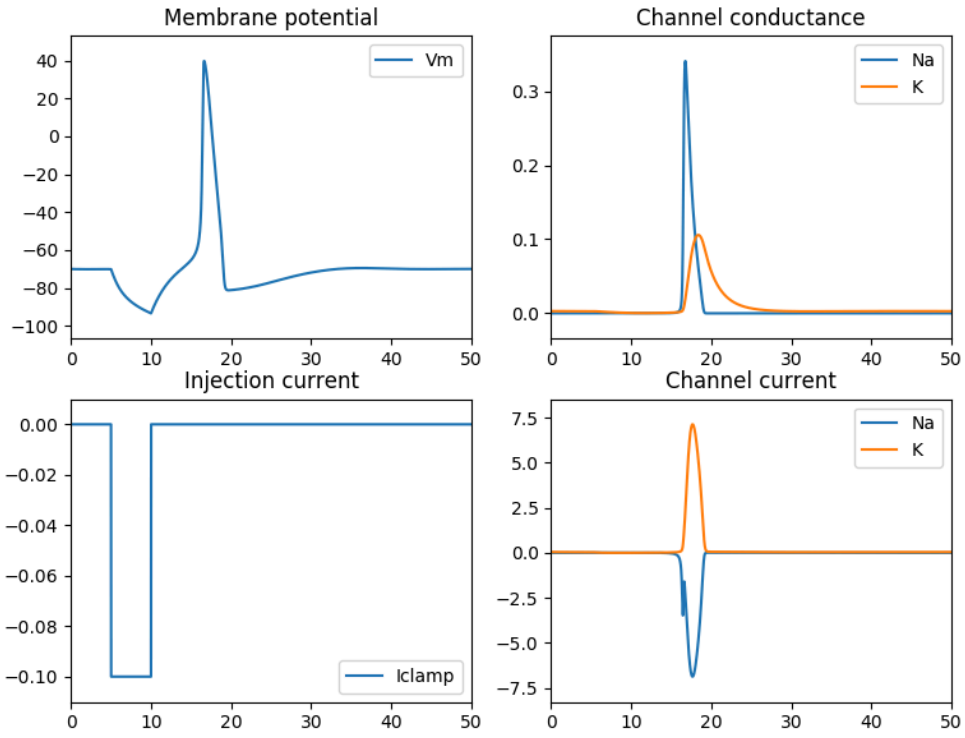

Figure 17: Action potential generated with a -0.1µA stimulating pulse of 5ms duration

What happens when the stimulus is turned off and why?

In *Figure 17*, by applying a negative stimulus pulse, we cause the membrane potential to be hyperpolarized in the beginning, as it drops below the resting potential. When the stimulus is turned off, the cell rapidly returns back to its resting potential by activating the Na+ channels. The influx of Na+ ions depolarizes the membrane and initiates an action potential.

I. Oscillatory Behaviour

A. With no current injection, for [K+] out = [15, 16, 17, 18, 20, 22, 24] mM, plot the relationship between [K+] out and the following:

1. AP firing frequency

2. AP resting level

3. AP amplitude

Table 6: AP firing frequency, resting level & amplitude with respect to various K concentrations

[K+]out (mM)	15	16	17	18	20	22	24
AP firing frequency (Hz)	0.04	0.051	0.054	0.058	0.069	0.081	0.092
AP resting level (mV)	-69	-67.8	-66.14	-66.13	-63.9	-62.23	-60
AP amplitude (mV)	78.83	78.7	76.57	73.27	65.53	55.109	43.42

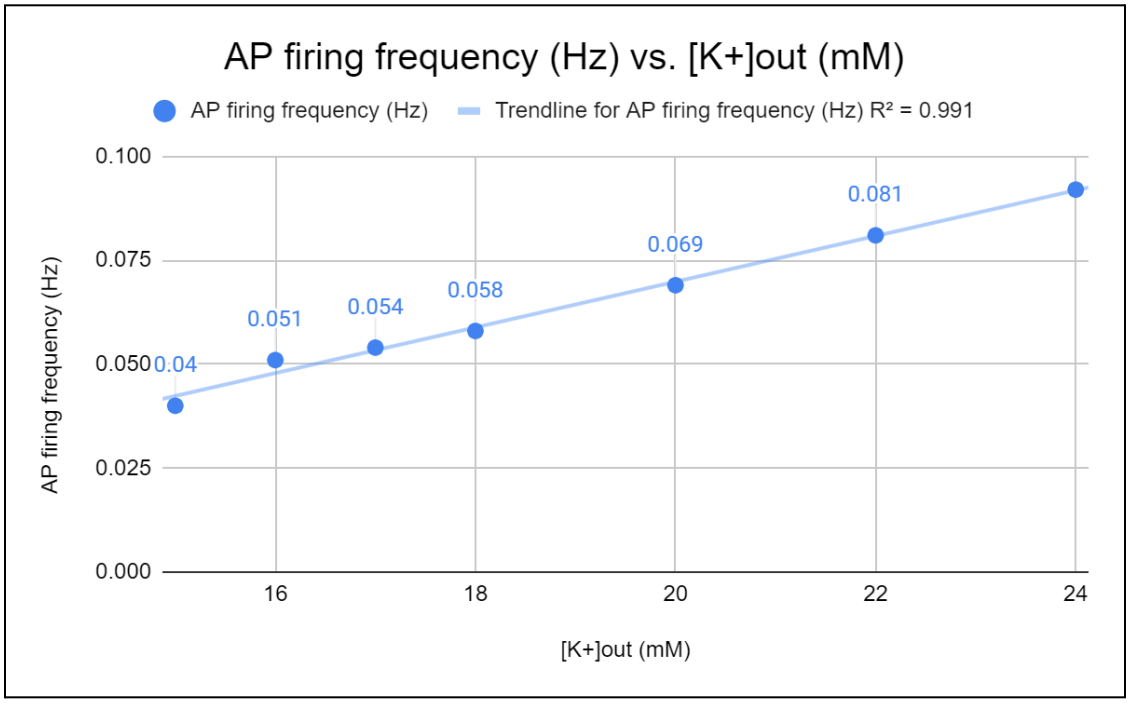

Figure 18: AP firing frequency vs. extracellular potassium concentrations

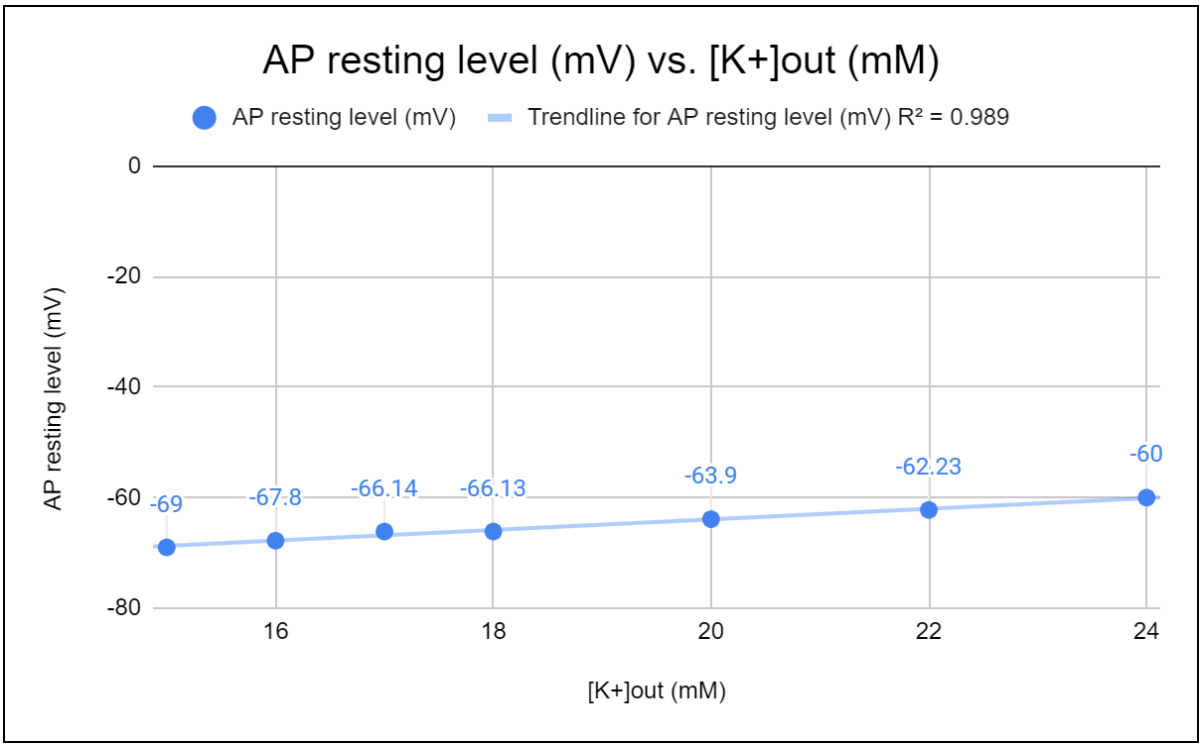

Figure 19: AP resting level vs. extracellular potassium concentrations

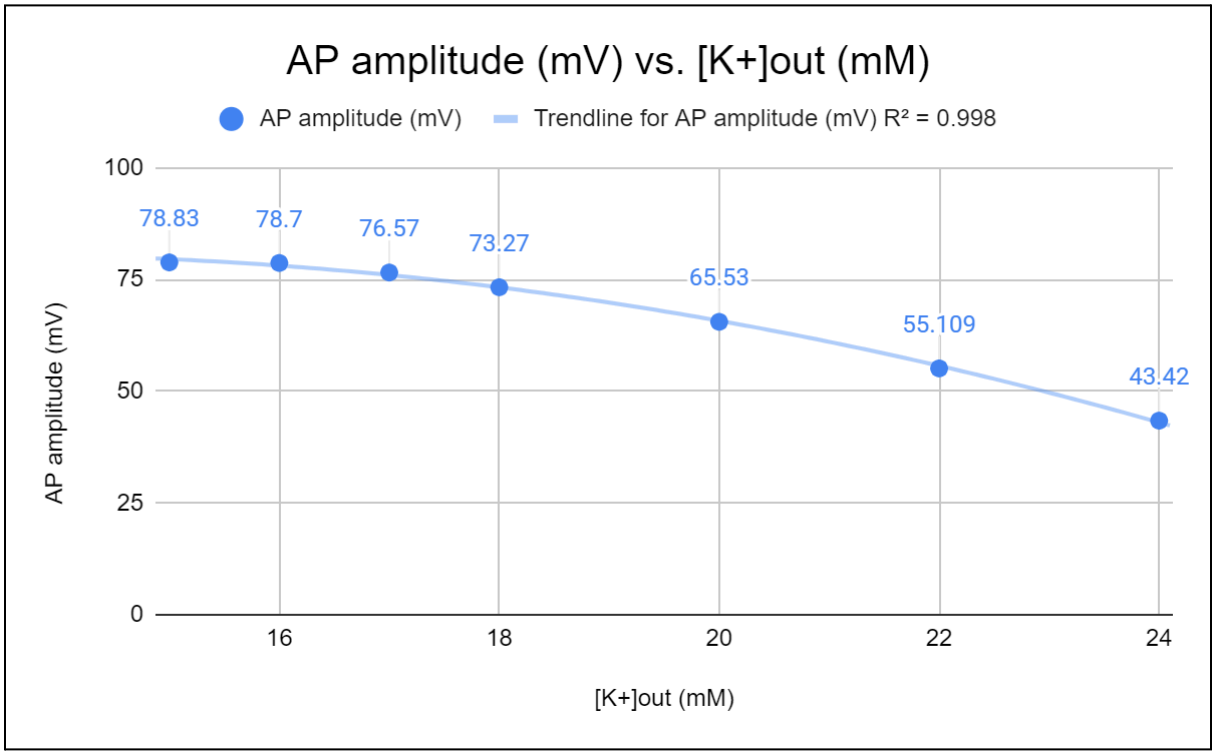

Figure 20: AP amplitude vs. extracellular potassium concentrations

B. For first pulse current amplitude = [0, 0.1 ... 1.2] µA, plot stimulus strength versus the following:

1. AP firing frequency,

2. AP resting level,

3. AP amplitude.

Table 7: AP firing frequency, resting level & amplitude with respect to various currents

Current Amplitude (µA)	0	0.1	0.2	0.3	0.4	0.5	0.6	0.8	1	1.2
AP firing frequency (Hz)	0	0.075	0.093	0.106	0.117	0.127	0.132	0.145	0.156	0.163
AP resting level (mV)	0	-70	-68.92	-66.7	-66.69	-63.9	-62.7	-60	-56.08	-54.97
AP amplitude (mV)	0	24.58	18.27	12.01	6.3	0.797	-4.157	-13.24	-20.35	-26.36

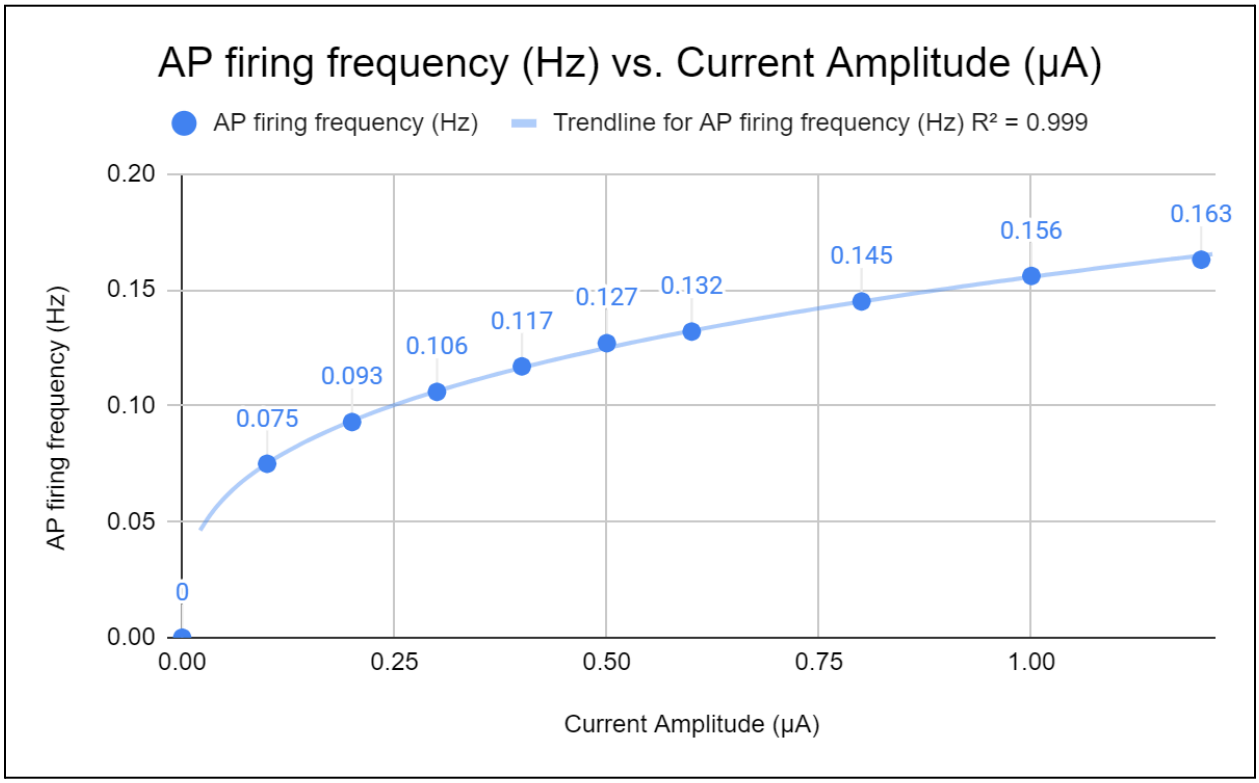

Figure 21: AP firing frequency vs. current amplitude

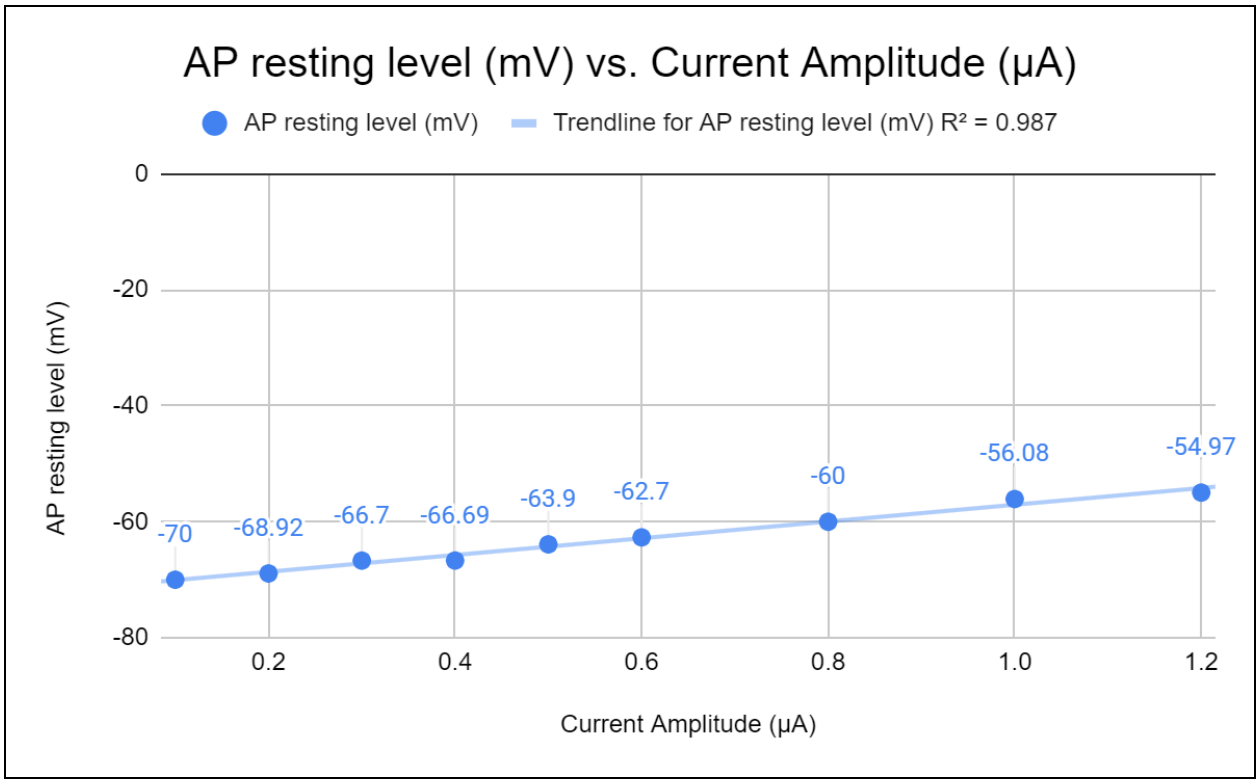

Figure 22: AP resting level vs. current amplitude

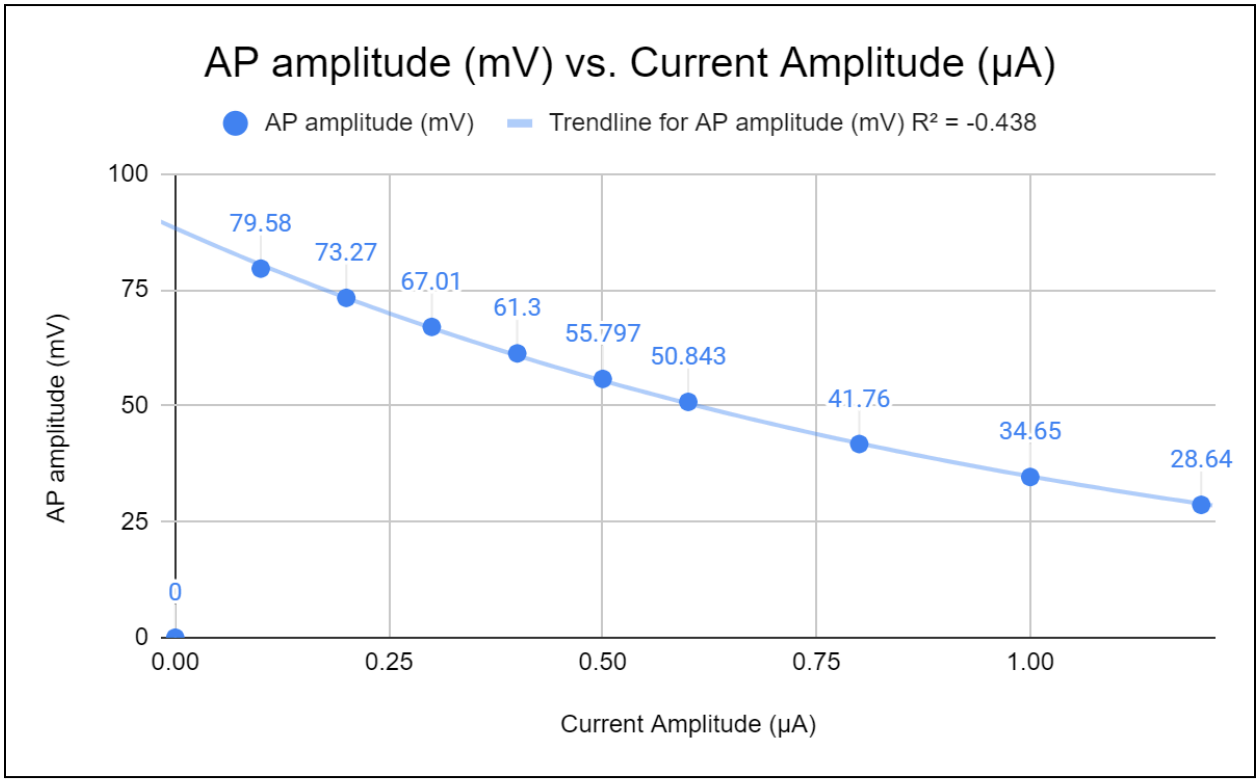

Figure 23: AP amplitude vs. current amplitude

Compare the effects of the above two stimuli (increased [K+]out and increased First Pulse Current), taking care to explain any similarities and differences. How do these frequency curves relate to the refractory & latency properties (from Parts B and C)? If you notice any differences between amplitudes of AP, are they what you would expect? Why or why not?

Both with increased [K+]out and increased First Pulse Current AP firing frequency and AP resting level increase whereas AP amplitude decreases. However, while AP firing frequency increases linearly with increased [K+]out, we observe faster changes with increased First Pulse Current as the relation is best represented as a function of power series. The AP resting level changes linearly for both cases, and by comparing *Figures 19 and 22*, we can observe that the increased First Pulse Current has a greater impact. AP amplitude decreases with both stimuli, and by comparing *Figures 20 and 23,* we observe that it decreases more rapidly with increased First Pulse Current. Therefore, we conclude that the increased First Pulse Current has a greater impact on the AP firing frequency, resting level and amplitude.

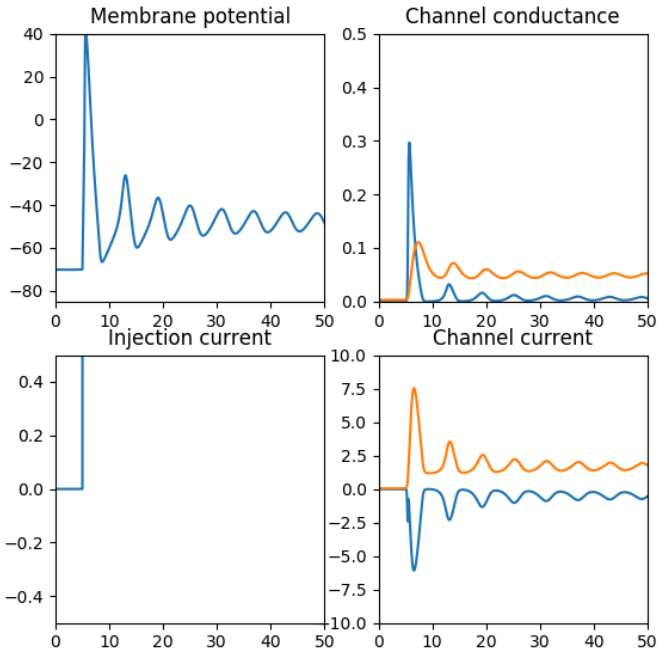

Figure 24: Effects of increased extracellular K+ concentration on the cell

With increased stimuli, we observe an increase in the frequency and a decrease in the amplitude of the action potentials as seen on *Figure 24*. Latency decreases with increased stimuli and as a result, the frequency of firing of the action potentials increases. Due to the relative refractory period after an action potential is fired, the cell is unable to achieve the same amplitude as before, with the next stimuli. As a result, we observe damped oscillations as the amplitude of the action potentials decrease over time.

C. Keeping everything at default values (and with no current injection), vary individually [K+] in , [Na+] out , and [K+] out . Which ion has more influence on cell's ability to fire an action potential?

Extracellular sodium ion concentration [Na+]out has the most influence on a cell's ability to fire an action potential. At resting, the [Na+]out is significantly greater than [K+]out. On the other hand, the K+ ions are more concentrated on the inside of the cell. To initiate an action potential, the membrane voltage needs to increase to the threshold value, and this requires an influx of positive Na+ ions. Therefore, as the [Na+]out concentration is increased, action potentials are rapidly initiated.

Set the standard current injection (pulse width 1ms, amplitude 0.1µA). Run the model with the default channel parameters, then change the parameters. Compare the two action potentials.

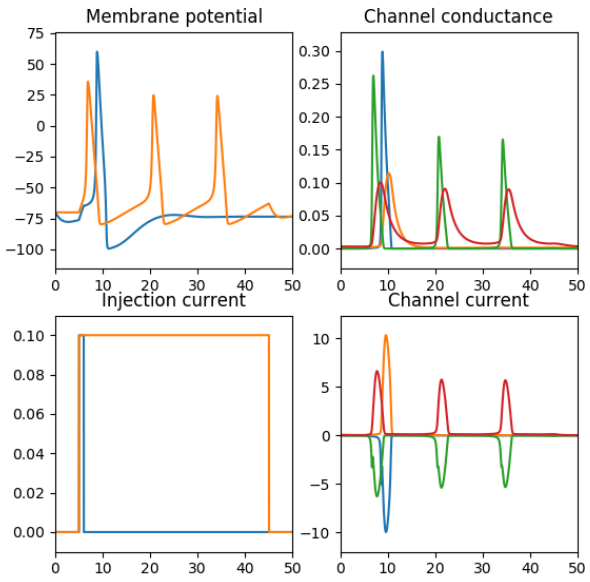

Figure 25: Comparison of action potentials in human and squid axons in overlay view

From the Membrane Potential graph in *Figure 25,* we observe that the amplitude of the action potential and the hyperpolarization effects are higher in the human neurons (shown in blue) than in the squid giant axon (shown in orange). We also observe a longer latency at the beginning as it takes longer time to reach the threshold in the human neuron, and a slower recovery after hyperpolarization in comparison to the squid giant axon. We observe that the squid neurons are able to recover rapidly and fire more action potentials due to their shorter refractory periods.

J. Final Thoughts

1. The Hodgkin-Huxley model and models like it have several assumptions inherent in their design. List at least two assumptions. What could be the potential limitations of the Hodgkin-Huxley model?

The Hodgkin-Huxley model assumes that an elementary step in the gating was a simple transition between two discrete on and off states, and that these reversible transitions were voltage dependent. They also assumed that the channels became conductive only if all of the gates were in the "on" state.

The Hodgkin-Huxley model was developed using a squid giant axon, which limits our understanding of the biophysics and evaluation of ion channels. Today, we know that most other neurons are much more complex, especially with regard to the number and diversity of potassium conductances in comparison to the squid giant axon. Therefore, considering the complexity of the real biological mechanisms, the Hodgkin-Huxley model may be insufficient in accounting for this level of complexity.

2. If an appropriate computer model is available, would it be a viable alternative for animal experimentation? Explain.

Yes, if an appropriate computer model is available it should be preferred over conducting an experiment on an animal. Even if the animal will be alive at the end of the experiment, the computer model would be preferred since keeping the animal in a laboratory environment requires enrapturing it which is likely to cause them stress, decreasing their quality of life. The care and maintenance of the animal would also add additional responsibility to the lab personnel. In addition, computer models are often easily accessible and more convenient for the use of education and research purposes. Unless no other alternative is available, experimentation on animals should be avoided.

3. A marketing research question: Given a live frog on which to experiment, would you
a. Whisk the frog away in your bag and set it free near a stream?
b. Snap its neck, decapitate it, pithe the head and cheerfully proceed to experiment?
c. Give the frog to the overworked T.A. to prepare?

I would whisk the frog away in my bag and set it free near a stream.

Summary of Results & Discussion

In this lab, we recreated Hodgkin and Huxley's original experiments on the giant squid axon using a computer simulation in order to enhance our understanding of the typical neuronal behaviour. We applied stimuli with different strengths and durations to observe its effects on the firing of the action potential in the neuron. We observed the latency and refractoriness properties of the neuron, and how the action potential is affected by the ion concentrations and channel conductances. We observed the Na+ and K+ conductance of the cell with respect to various voltages, by recreating Hodgkin and Huxley's original Voltage and Current Clamp techniques. By applying low and persisting stimuli, we observed that the excitability of the membrane was reduced due to the Accommodation property of the neuron. Finally, we observed the oscillatory behaviour of the nerve cells by increasing the extracellular K+ concentration or depolarization of the membrane by injecting an electrical current pulse.