Neurobiology 360: Ion Channels, Passive Properties, and the Patch Clamp

1a) Using the following equation, which is a derivation of Ohm’s Law \( I_{Na} = g_{Na}(V_m - E_{Na}) \), estimate the magnitude of the current for voltage dependent Na\(^+\) channels when the membrane potential is voltage clamped at -70 mV and then is stepped up to -20mV? 0mV? 20mV? 50mV? 70mV? Specifically indicate if the current is very large, large, small, very small or none and indicate whether or not it is an inward or an outward current. Assume \( g_{Na} \) reaches its maximum value at all voltages more positive than -25 mV.

\( E_{Na} = 50 \) mV

\( V_m = -20 \text{mV}; \) Very Large inward current of Na\(^+\)

\( V_m = 0 \text{mV}; \) Large inward current of Na\(^+\)

\( V_m = 20 \text{mV}; \) Small inward current of Na\(^+\)

\( V_m = 50 \text{mV}; \) No current flow of Na\(^+\)

\( V_m = 70 \text{mV}; \) Small Outward current of Na\(^+\)

When the membrane potential is clamped at a voltage below the equilibrium potential for Na\(^+\), Na\(^+\) will flow into the cell. As the membrane potential approaches the equilibrium potential of Na\(^+\), no current will flow. You have reached equilibrium. Once the membrane potential has exceeded that of the equilibrium potential of Na\(^+\), Na\(^+\) will flow out of the cell towards its concentration gradient.

To calculate this out mathematically use: \( I_{Na} = g_{Na}(V_m - E_{Na}) \)

At voltages more positive than -25mV, \( g_{Na} \) is maxed out, so this is negligible (this value will not affect \( I_{Na} \)); the units, however, are still necessary to properly calculate \( I_{Na} \), so do not ignore \( g_{Na} \) in your calculation.

1b) Assuming you are recording from the same cell as in 1a, explain why the magnitude of current is smaller when the cell is voltage clamped at -30mV compared to -20mV as depicted below. As in question 1a, Assume \( g_{Na} \) reaches its maximum value at all voltages more positive than -25 mV.

At -30 mV, \( g_{na} \) is not maximal and the Na\(^+\) current will be less than at -20 mV. \( I_{Na} = g_{Na}(V_m - E_{Na}) \)
2) Explain why myelin increases conduction speed of the action potential. Be as specific as possible.

Myelin influences the speed of conduction of the action potential by affecting both the time (τ) and length (λ) constants. Myelin increases membrane resistance (Rm) and decreases membrane capacitance (Cm). The length constant (λ) is defined as the square root of Rm/Ri; since myelin increases Rm, it also increases λ, leading to the electrical signal from the action potential spreading further down the axon before it decrements to zero. The time constant is defined as τ = RmCm. Because myelin decreases Cm more than it increases Rm, the net effect of myelinization is a decrease in τ, hence a change in voltage will take less time to reach maximal value. Thus the effect of myelin is to increase the length constant and decrease the time constant, both of which result in an increase in the speed of conduction of the action potential.

3) During a study of Na⁺ currents in the squid giant axon, experimenter’s voltage clamped the cell to 0 mV from a holding potential of either -70 mV or -100 mV as depicted below. They found that the inward Na⁺ current was greater from the more hyperpolarizing holding potential. Why?

At -100mV, nearly all (~99%) Na⁺ channels are closed and ready to open. Thus when the cell is depolarized to 0 mV, all of these Na⁺ channels will open, leading to a large inward Na⁺ current. In contrast, when the cell is held initially at -70mV, there is a smaller number of Na⁺ channels in the closed and ready to open state because a significant fraction of Na⁺ channels are in the inactivated, not able to open state. Thus, when the cell is depolarized from -70mV to 0mV, a smaller number of Na⁺ channels open leading to a smaller inward Na⁺ current compared to the Na⁺ current elicited when the cell was held at -100mV.

4a) It is 1986 and you developed the patch clamp technique. You obtained the following data from your first experiment.
What does this tell you about the ion channel in this recording? Provide a rationale for your answer.

The downward deflection of current indicates that this is an inward current. This recording also suggests that the time a channel is open is variable in duration (some are open for a short period of time while others are open for a longer period of time). This is shown by the variation in the duration of the current events. Each response has two conductance states, open or closed. This is shown by the sharp rise and sharp decline of the traces. They all pass the same amount of current because all of the first 3 traces are of the same magnitude. The fourth depolarization suggests that two channels have opened simultaneously since the size is twice the size of the first three. The conclusion based on this recording is that this channel could be either a sodium, chloride, or calcium channel.

4b) Through various experiments, you determined this channel was passing Ca\(^{2+}\). Taking into account that the membrane potential in the diagram above is clamped at -25mV, what would happen to the amplitude of the current if the membrane potential was clamped at 0mV? +50 mV? +70mV? Hint: Calculate the Nernst potential for calcium if the concentration of Ca\(^{2+}\) on the outside is 1mM and the concentration of Ca\(^{2+}\) on the inside is 100nM.

The equilibrium potential of Ca\(^{2+}\) is: 
\[ E_{\text{Ca}^{2+}} = \frac{58}{2} \log \left( \frac{[1 \text{mM}]}{[0.00001 \text{mM}]} \right) = +116 \text{mV}. \]

When we compare the equilibrium potential to the holding potentials indicated above, we can determine that with each successive increase in the holding potential, the amplitude of the current will continue to decrease because we have not yet reached the equilibrium potential. There is still a substantial driving force for Ca\(^{2+}\), and thus, Ca\(^{2+}\) will continue to flow into the cell. (See question 1).