A stimulus current applied to an axon delivers a quantity of charge \( Q \) that charges the membrane capacitance and tends to depolarize the intracellular space. A \textit{supra-threshold} stimulus depolarizes the membrane such that the sodium channels open and eventually an action potential is generated. A \textit{sub-threshold} stimulus does not provide enough charge to sufficiently depolarize the membrane, resulting in graded potentials along the axon.

The refractory period is the time of reduced excitability immediately following an action potential. No action potential can be generated during the \textit{absolute} refractory period, regardless of the stimulus strength. During the \textit{relative} refractory period a second action potential can be generated, but the excitability of the membrane is reduced (i.e., the excitation threshold is increased) so more stimulus current is required.

This purpose of this study is to characterize and quantify the excitation threshold; that is, to determine the charge density needed to elicit an action potential, and how the amount of needed charge is related to the electrical properties of the membrane. In addition, this study will quantify the absolute and relative refractory periods in the Hodgkin-Huxley model.

**Base Hodgkin-Huxley Model**  Start by running the base (“standard”) Hodgkin-Huxley that you developed in Homework 9. Generate plots of the membrane potential, currents, and the gates versus time. Compute the action potential amplitude, duration at 90% repolarization (\( \text{APD}_{90} \)) and the maximum upstroke velocity, \( dV/dt_{\text{max}} \).
Strength-Duration Relation  Begin this experiment by applying a stimulus current $J_m$ for
0.1 millisecond. Increase the amplitude of the stimulus current until an action potential is generated; this
is the threshold current for this duration. Increase the stimulus duration in increments until you reach the
maximum duration of 20 milliseconds. For durations less than 1 millisecond, you should increase the
duration of $J_m$ in 0.1 millisecond increments. For durations over 3 milliseconds, try 1 or 2 millisecond
increments. At each duration, determine the new threshold current (to within $\pm 0.2 \mu A/cm^2$), the
maximum upstroke velocity ($dV/dt_{\text{max}}$), and the duration at 90% repolarization ($\text{APD}_{90}$).

Estimate the rheobase $J_R$ for the Hodgkin-Huxley model. Study the relation between the threshold
current density $J_{\text{th}}$ and duration $T$; this relationship is given by

$$J_{\text{th}}(T) = \frac{J_R}{1 - e^{-T/\tau_m}}$$

What value of the membrane time constant $\tau_m$ makes the equation best fit your data? If $\tau_m = R_mC_m$,
how does this relation involve the parallel conductance model of the membrane? How does the total
amount of charge density delivered by the threshold current vary with the duration?

Refractory Period  This experiment is performed using an $S_1 - S_2$ stimulus protocol. The stimuli are
external currents applied to membrane. The first stimulus $S_1$ is applied at $t_1$ and elicits an action
potential. The second stimulus $S_2$ is applied at $t_2$; depending on the delay $t_2 - t_1$ and the magnitude of
$S_2$, the second stimulus may or may not elicit a second action potential.

Begin by applying $S_2$ for 0.15 milliseconds, starting at $t_2 = 8$ milliseconds after $S_1$. Find the $S_2$
amping required to generate a second action potential. Using the same $S_2$ amplitude (and duration),
decrease $t_2$ until no action potential is generated. Now double the $S_2$ amplitude (that you found above),
and set $t_2 = 8$ milliseconds (keep the duration at 0.15 milliseconds). Decrease $t_2$ until no action potential
is generated; this is the end of the absolute refractory period, and the beginning of the relative refractory
period. Determine, to within $\pm 0.1$ millisecond and $\pm 0.5 \mu A/cm^2$, the absolute and relative refractory
periods. The tricky part is deciding if an action potential is generated. You can define a “generated action
potential” as one where $V_m$ reaches reaches zero millivolts (or higher) in response to the $S_2$ stimulus.

What is the relation between the $S_2$ amplitude and the delay? How do the membrane conductances (or
the activation and inactivation gates) affect the response to $S_2$?

Combined Effects  Does the absolute refractory period change with the strength of the initial $S_1$
stimulus? For example, if the $S_1$ stimulus amplitude is increased by 10%, does this lengthen the absolute
refractory period? Provide simulation results to support your conclusion.