Voltage Clamping a Patch
Measuring Macroscopic Currents through Channel Populations

Goals of this Tutorial:

- To plot currents in response to individual depolarizing voltage steps and to plot families of voltage steps.
- To plot the conductance increases (due to the opening, closing, and inactivation of the channels) in response to these voltage steps.
- To experiment with "tail currents" (which give information on the time course of the closing of the channels when the voltage is returned to rest).
- To use the voltage clamp to demonstrate that a portion of the Na channels are inactivated at rest.
- To experiment with the effect of temperature on the kinetics of the conductance changes.

Experiments and Observations:

*Observe the Na and K currents in response to a step depolarization.*

10 mV steps of voltage were applied between voltages -40mv and +80mv. Currents iNa and iK (in mA/cm^2) were plotted separately, then superimposed to yield a graph very similar to the results posted by Cole and Moore in 1960.
Observe the conductance increases (due to the opening, closing, and inactivation of the channels) in response to these voltage steps.

To the left is the evolution of Na conductance relative to Na current from 0 – 120 mV.

Below is a snapshot of both Na and K conductance at a step to 0 mV.
Observe families of currents

Some questions:

- Why do the inward currents grow larger with depolarization, then smaller?
• What is special about the voltage at which the Na current is zero?

• Why are the currents outward at large de-polarizations?

  \textit{Observe "tail" currents.}

\textbf{What dictates the magnitude of a tail current?}

The magnitude of the tail current for an ion depends both on how many of the ion's channels are open at any given time (the conductance) and on the driving force on the ion. If the ion's equilibrium potential is also changing as a function of time (for example, when K has accumulated in a restricted extracellular space during a prolonged depolarization and then is being transported back into the cell) the interpretation of tail currents can be quite complex! The typical Na action potential is fast enough, however, that during its duration $E_{Na}$ does not change appreciably.

\textbf{A brief pulse evokes the maximum Na tail current.}

We observe that the Na tail currents after a short depolarization of approximately the duration of an action potential ($<$1 ms) and large enough (to $>$0 mV) to open a large proportion of the channels. This pulse evoked the maximum conductance not only because most of the Na channels were activated but because there was not time for them to be inactivated appreciably (a key feature of Na channels).
Tail currents as a function of pulse duration (0.25s intervals from 0.25 to 3.0):

[ note the conductance mirroring in the current spike peaks ]

Tail currents as a function of driving force (10mV increments from -30mV to 30mV):
Tail currents are strongest when both the driving force and conductance components are at full force. Since conductance is a complex function that reaches a plateau before falling off exponentially, there is a “sweet spot” of duration for a given cell’s parameters at which conductance reaches a maximum. The driving force component does not seem to have any real limit (beyond physiologically imposed ones) – the higher the test voltage, the greater the subsequent spike in current.

**Demonstrate inactivation of the Na conductance**

![Graph showing membrane currents vs time and membrane conductance vs time](image)

**How does inactivation of the Na channels depend on voltage?**

At the normal resting potential of –65 mV, about 40% of the HH Na channels are inactivated. Hyper-polarization removes inactivation with time; at a much greater resting potential (for instance, –100 mV), none of the channels will be in the inactivated state. On the other hand, a depolarized holding (conditioning) potential will shift more of the channels to the inactivated state.

![Graph showing membrane conductance vs time](image)
Observe the effect of temperature on the Na and K conductance kinetics.

For every 10 degree step in temperature, the rate constants increase by 300%

The rate constants determine how quickly the values of these variables can change. These rate constants are not really "constant" but are strong functions of both temperature and membrane voltage. In particular, they change 3-fold, or 300%, with a change in temperature of 10 ºC.

The temperature sensitivity of the rate constants is characteristic of kinetic reactions but enormous compared to that associated with the increase in conductance of the bathing solution or cytoplasm. Because the temperature sensitivity of solutions is proportional to the change in absolute temperature, it is relatively quite small, only about 3% per 10 ºC at room temperature.

Closing Questions:

- How do holding (conditioning) potentials influence the alpha function over time?
- How are tail currents affected by semi-permanent changes in a cell’s equilibrium potential?
- Are tail current spike amplitudes calculable as a function of time, or strictly measurable?