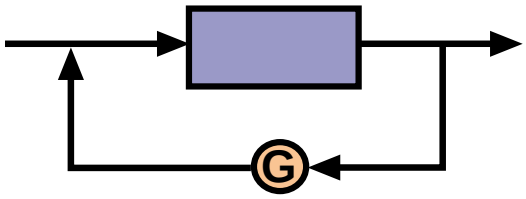
THE VOLTAGE CLAMP

Objectives

1. To understand the classical voltage clamp experiments, and to illustrate how these measurements were used to formulate the kinetics of voltage activated ion channels responsible for excitation (Na^+, K^+, fast gates, slow gates, etc.)

2. To simulate a feedback system on a realistic model and to illustrate:

- ❑ negative (and positive) feedback
- ❑ gain
- ❑ steady-state error
- ❑ stability and oscillations



Background

Excitation of a "normal" axon involves the rapid, but transient increase in sodium conductance, g_{Na}, followed by a slower, more prolonged increase in potassium conductance, g_K. These changes in membrane permeability are governed by the opening and closing of ion channels:

- fast Na^+ "m gates",
- slow Na^+ "h gates", and
- slow K^+ "n gates".

Study of the axon in its natural state is complex because the opening and closing of each gate depends on both time and membrane potential, and the membrane potential, in turn, is changing in response to the state of the gates. This mutual interaction between gates and membrane potential endows the axon with excitation properties allowing it to respond in an explosive manner when stimulated.

To simplify analysis of the gates it is expedient to interrupt this cycle by making the membrane potential independent of changes in the gates. This is accomplished by "forcing" the membrane potential to remain constant at a preset level throughout the axon's response and then measuring the current (ion flow) through the channels while the measurement is underway. (If the current is large, there must be many channels open. To find what portion of the current is due to Na^+, remove the Na^+ or poison the Na^+ channels; what remains must be due to either K^+ or to other ion leaks.

A Voltage Clamp Experiment

For simplicity we illustrate the voltage clamp experiment in an axon with poisoned Na channels and we ignore the small leakage currents. In this special case all current is carried by K^+.

At rest the concentration gradient (gray arrow) causing K^+ to diffuse out of the cell is balanced by the electrical force (membrane voltage , black arrow) acting in the opposite direction. Consequently, very little K^+ leaks out (**Figure 1**).

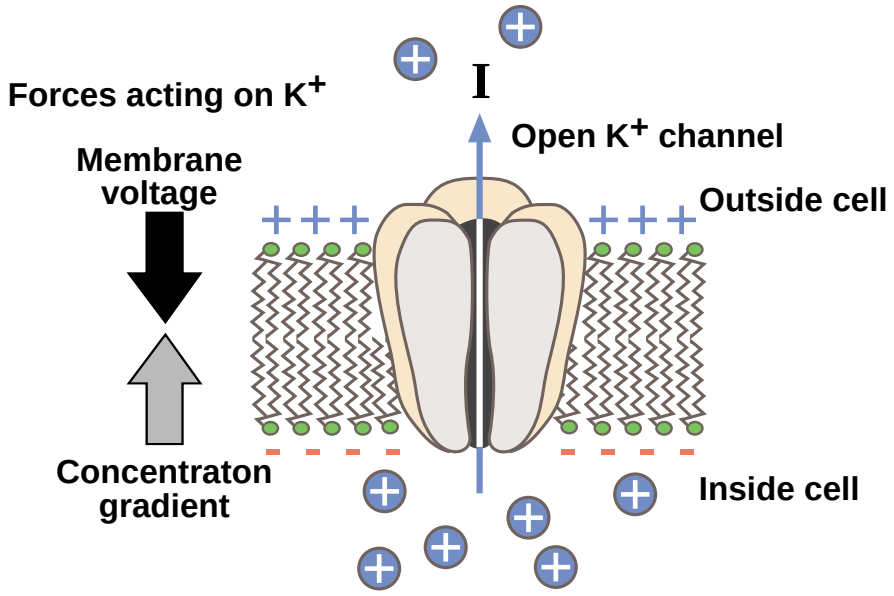


Figure 1. Forces acting on the K^+ ions.

When the voltage clamp is turned on, a small pulse of negative charge is delivered to the external membrane surface and an equivalent positive charge is delivered to the internal surface. This new charge is just sufficient to jump the membrane potential from -65 mV to -20 mV (Figure 2).

This new -20 mV membrane potential tending to force positive charge into the cell is too weak to balance the tendency of K^+ to diffuse out of the cell. In addition, the depolarization of the membrane opens more K^+ channels. K^+ diffusing out of the cell would add positive charge to the outside and change the membrane potential, but the voltage clamp monitors E and prevents any change by adding one negative charge for each K^+ that crosses the membrane out of the cell (Figure 3).[1]

The value of the voltage clamp is due to the fact that with modern technology it is not possible to chemically measure the small amounts of K^+ that enter or leave the cell within a fraction of a millisecond, but the *charge* delivered by the voltage clamp can be measured easily. If both Na^+ and K^+ channels are open then the measured voltage clamp current equals the sum of the individual Na^+ and K^+ currents.

[1] The negative charge added to the solution is an ion, not a bare electron. The identity of the ion depends on the type of electrode, and need not concern us here

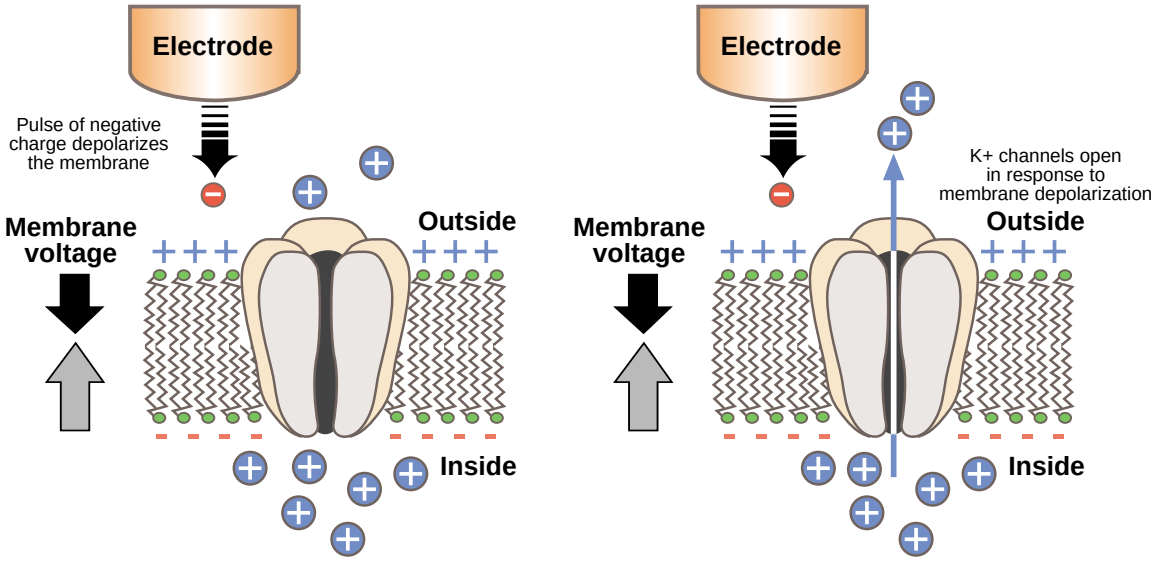


Figure 2. **Left:** The voltage clamp has been turned on. We have not shown the intracellular electrode which acts in a similar way by "absorbing" the excess negative charges (ions) left behind by K^+ when it moves to the outside.
Right: K^+ channels open in response to depolarization. Charge delivered by the electrode compensates for the K moving out of the cell preventing any change in membrane potential

Procedure

To impose a constant membrane potential:

1. Choose a desired membrane potential; call this E_{set}.

2. Add charge to the membrane until the membrane potential is equal to the desired amount. This is accomplished by adding $(E_{set} - E_{rest})C_m$ to the membrane (be careful with + and - signs on the E's: remember E_{rest} is negative).

One good way to set this up is to use the PULSE function with the desired charge as the "volume" part of the pulse. For example, if you wanted to start the voltage clamp after 0.2 msec have elapsed, use the following stimulus:

Istim = CHARGING CURRENT = PULSE((Eset - Erest)*Cm, 0.2, 100)

The electrical current containing this pulse of charge is called the "charging current". Although this will create the desired change by giving E a good boost to get it close to E_{set}, it will not hold it there because ions will immediately commence to move. To maintain E close to E_{set}, you will have to monitor E at all times and keep adding or taking away charge via the stimulating electrodes to stop E from drifting. This compensating current used to maintain E close to E_{set} is called the "feedback current".

3. Measure the *error* defined as the difference between E (the membrane potential) and E_{set}. Keep adding charge proportional to this error (via the stimulating electrodes) so that the error is always close to zero. If the error is positive, you will have to take + charge away from the internal membrane

surface to compensate (i.e. inject a negative current), and vice versa.[2] The proportionality constant that relates the feedback current to the error is called the "GAIN".

FEEDBACK = - GAIN*(E - Eset) = - GAIN*(error)

Increasing the GAIN increases the sensitivity of the feedback—with a large GAIN, a small error will induce a large feedback response.

To clamp the voltage for a prolonged period of time, the feedback current should be added to the charging current (again via the stimulating electrodes. However, our feedback current is not used to create a new potential, but only to maintain it. Therefore it is best to add the feedback current just *after* the pulse has ceased.

Suppose you begin the clamp after 0.2 msec. You charge up the membrane with a pulse starting at 0.2 msec and lasting for only DT additional msec. A short time later, say 0.02 msec (greater or equal to DT msec) you turn on the feedback, i.e. at 0.22 msec.

Istim = CHARGING CURRENT + FEEDBACK

= PULSE ((Eset - Erest)* Cm , 0.2, 100) - GAIN*(E - Eset)* STEP(1, 0.22)

The feedback current is easily measured, but what does it tell us? Since the voltage is held constant, the membrane charge cannot be changing. Therefore, the only time we can add a charge from our electrodes to the inner surface of the membrane is when a charge of like sign has left this surface. In other words, *the feedback current measures the net charge flowing through the channels*. It is just what we need to compute the opening and closing of channels: if the current is large, there must be many channels open. To find what portion of the current is due to Na^+, take the Na^+ away (or poison the Na^+ channels), and what remains must be due to K^+ or to leaks.

4. Try E_{set} = -20 mV and experiment with different values of GAIN to see how close you can hold (clamp) the membrane potential to -20 mV. Set the duration at 8 msec.

5. Record both E and Istim. The feedback of I_{stim} is a measure of the net charge flowing through the channels.

6. Compare the magnitude of I_{stim} in the voltage clamp measurements with the same quantity in last weeks excitation experiments. This gives some idea of the magnitude of the relatively small stimulus (charging current necessary to depolarize the axon to some threshold value) and the huge response of the intact nerve (charge flowing through channels).

7. The steady state error is given by the deviation of E from -20 mV in the TABLE. This quantity is important in feedback systems. Can you reduce it by increasing the GAIN? Can the GAIN be made as large as you please?

8. Separate the contributions of Na^+ and K^+ to I_{stim}. You can accomplish this by recording I_{stim} after poisoning (removing) one channel type and studying the other. To a good approximation, you can ignore the contribution of the leakage current I_L. Verify this by plotting the leakage current on the same scale as I_{stim}. Note that both I_{Na} and I_K have a measurable delay before changing at their maximal rate. This is not characteristic of a simple exponential (linear) process, and it is what prompted the Hodgkin and Huxley to consider raising n and m to a power (i.e. n^4 and m^3). Try measuring I_{Na}, and IK at several different voltages (E_{set}).

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[2] Note that if you make a mistake in the sign of your current, you will change a negative feedback into a positive one, and the system will go unstable!

9. Try changing the system into a positive feedback—you will see the system go unstable very quickly!

10. Plot the steady state values of n, m, and h—given by $a_x/(a_x+b_x)$—as a function of E and compare them in terms of opening or closing with depolarization (E becoming more positive). The "apparent time constants" of n, m, and h are given by $1/(a_x+b_x)$. Plot these as a function of voltage and compare them in terms of fast and slow gates.