

VOLTAGE CLAMP.

So far we have discussed how the action potential develops and terminates in terms of Na activation, Na inactivation, and K activation and their time courses. How can we determine activation and inactivation and the time courses? How do we get the activation plots such as the one I showed you earlier? We get them with a technique called the **voltage clamp**. This technique allow us to determine the response when the membrane voltage is “clamped” to a fixed value.

1 The technique is outlined in this overhead. I will describe the technique as it is used on the squid axon. Two electrodes are inserted into the axon. One records voltage and the other is used to inject current. The experimenter decides on a “command voltage” that the cell should be clamped to. The voltage recorded by the recording electrode is compared to this command voltage. If these voltages differ, then current is injected into the cell (positive or negative as needed) to make the membrane voltage equal the command voltage. (The principal is much like a thermostat. You set the thermostat to a particular temperature. The air temperature is compared to desired temperature and heat (or air conditioning) is sent into the room to bring the room temperature to the desired temperature.)

The current injected to maintain the clamped voltage must be equal in magnitude and opposite in sign to the ionic currents in the membrane. Because the voltage is held constant, there is no capacitive current and the injected current truly reflects the ionic currents in the membrane. (Note, voltage clamp at a point (soma) will not clamp the voltage at distant locations (distal dendrites, axon branches). To get true currents we need “space clamp” as well. In squid axon, a wire is extended down the length of the axon to give voltage clamp at all spatial locations (space clamp).

2 If we clamp the voltage to various steps we see that the current needed to maintain the command voltage looks like this (Fig. 5.6, 7.2 and 7.3, 7.13 and 7.15 all show this so it must be important. We will look at Fig. 7.2 and you will generate something similar to this in the next computer lab.

For small voltage steps, we see an early, inward, negative current followed by a late outward current. For large voltage steps we see only outward current. Since you know that the ions present are K and Na, why is the current purely outward at large voltage steps? Ans. we are above E_{Na} so the sign of the Na current reverses.

It is clear (as shown here from Fig. 7.2b) that at early times, the net current is inward and at later times, the net current is outward. Nevertheless, what we see here is a mixture of all currents present. How can we separate out individual currents?

3 We can separate the Na and K components of these currents by using ion substitution experiments or by blocking Na or K pharmacologically with TTX or TEA.

This time I will show you Fig. 5.6 (although Fig 7.3 and 7.15 show the same thing) and again you will see this in computer lab. How does this work? You can substitute choline for Na in the seawater. Choline will not go through Na channels. Then in the voltage clamp experiments, the only current you will see is potassium as shown here. If you now subtract the K current obtained in Na-free seawater from the total current obtained in normal seawater, you get the Na current.

Pharmacological blockers are more direct. Applying TTX (tetrodotoxin) will block Na channels and give you the K current alone in the voltage clamp experiments. Similarly, applying TEA (tetraethylammonium) will block K channels and leave you with only the Na current.

4 Let's first analyze the K current, obtained here by blocking the Na current with TTX (Fig. 5.6.b.2). We see that the current rises to a steady-state value over about 10 ms. So if we have an I_K value for a given voltage step, what is the conductance g_K ? We get this from $I_K = g_K(V_{\text{clamp}} - E_K)$. (see eq. 7.9 and 7.10) We know I_K , we know V_{clamp} and we know E_K , so we use this information to compute the steady-state g_K at a particular voltage. This is shown in Fig. 7.5.B. As the voltage clamp voltage becomes larger, g_K reaches a maximum (here all K channels are open). We plot the steady-state (final) conductance values (in 7.5.B) as a function of the voltage clamp voltage, relative to this maximum value. The result is a function that describes the open probability of a K channel at a particular voltage (as we saw earlier—the curve for “n”).

We can also do curve fitting to determine the time constant of K activation at each voltage. And this is what Hodgkin and Huxley did in their quantitative analysis of the ionic basis of the action potential.

5 Quantifying the Na conductance is more difficult. If we clamp to various voltages we can get g_{Na} as a function of time (Fig. 7.5) or I_{Na} as in Fig 5.6B. We see that the Na conductance activates and then inactivates when the voltage is clamped to a particular potential. But this does not quantify the activation and inactivation processes. How can you characterize the activation and inactivation processes?

The trick is to clamp the voltage to potentials where we have no inactivation or where we have maximum activation.

If we clamp the voltage to a very hyperpolarized value, we can remove Na inactivation. Then if we step the voltage to a positive step and measure the peak I_{Na} we can be confident that this peak I_{Na} can be used to get Na activation at that voltage. (We cannot use the steady-state I_{Na} because inactivation will develop after a few milliseconds.) Again we can use $g = I/V$ and we fit the time course as we did with K assuming that the peak is the steady-state value.

6 To study inactivation we need to measure the amount of steady-state inactivation at various voltages and also measure the time course of de-inactivation. To get steady-state inactivation, we clamp the voltage to various holding potentials (-100, -70, -50, etc) until we are confident that inactivation is as a steady-state. Then we change the voltage to a depolarized level where activation will be 100% and measure the peak current. The maximum peak current should be at -100 mV. The peak current when you start at -50 mV will be less because of inactivation. The ratio of the peaks will tell you the level of inactivation at -50 mV (see graph).

To measure the time course of de-inactivation we can clamp the cell to a depolarized potential to induce inactivation, then hyperpolarize the neuron to various voltages for various times and again depolarize to measure how much inactivation has disappeared.

The important point is all of this is that the voltage clamp technique can be used to quantify the activation and inactivation of the Na conductance and activation of the K conductance.

ACTION POTENTIAL FIRING PATTERNS. (Numbers refer to OC overheads)

1 Information can be transmitted in the nervous system by the frequency of action potentials or patterns of action potential firing. Cells in different parts of the nervous system may respond quite differently to brief or long current injections, and other neurons may not need current injection to fire. They fire tonically.

Here are some examples (Fig. 5.10). Most cortical pyramidal cells and CA1 hippocampal pyramidal cells (among others) fire regularly with depolarization, although with some adaptation (A). Others like CA3 hippocampal pyramidal cells, fire bursts of action potentials (B). Cerebellar Purkinje cells can generate high-frequency trains of action potentials as seen here (C). Thalamic relay cells can exist in two modes. In burst mode, the resting potential is -75 mV and depolarization will produce a burst of action potentials (D). In transfer mode, the resting potential is more depolarized and the neuron operates in "transfer" mode (E).

Why do we see such different firing patterns? Hodgkin and Huxley were lucky that the squid axon had only 2 types of voltage dependent conductances. Most cells have other conductances (although at nodes and along most of the axon, only Na and K are usually present).

The voltage clamp procedure outlined above for characterizing Na and K conductances with voltage clamp recordings and pharmacological manipulations, together with single channel measurements are also used to characterize other types of voltage-dependent ion channels, some of which we will discuss now.

2 OTHER CURRENTS (Many of the other currents that I will discuss are in [Table 5.2](#) and you will examine these further in the computer lab)

Na Currents. There seem to be two types of Na currents.

- 1) I_{Na} . The transient, rapidly activating and inactivating Na that is responsible for action potential generation
- 2) I_{Nap} . The persistent Na current. These channels do not support action potentials because of their low unitary conductance, but may contribute to the generation of plateau potentials (prolonged depolarization in response to a brief input) with action potential discharge. The threshold for activation is about -65 mV or below threshold and below the activation threshold for I_{Na} . The plateaus can end with hyperpolarizing inputs or hyperpolarizing conductances (slow K conductances).

Their response to a voltage clamp to -10 mV is shown here.

Ca currents (**LOWER HALF OF 2**) Besides Na currents there are also Ca currents. Calcium concentration is very small inside the cell and the gradient is such that E_{Ca} is very positive (+125 in Table 5.1) so calcium currents are always inward and depolarizing.

Why are Ca currents important? In some cells (cardiac cells) Ca and not Na is the primary ion for the action potential. 2) Increased Ca concentration is needed for neurotransmitter release as we will discuss in two weeks and Ca channels provide this calcium 3) calcium acts as a second messenger for many metabolic processes as you will discover later in the course and 4) calcium can activate certain K channels.

3 Here are some type of calcium currents.

I_L (L for long-lasting. i.e., it does not inactivate with V, inactivates with Ca slowly) This is a high threshold calcium channel that is activated by very depolarized voltages such as those seen with action potentials. It is the basis of the cardiac action potential.

I_N (N for neither long-lasting or transient) It does inactivate. It is a high threshold channel activated at very depolarized potentials, but maybe not as high as for L. This channels is the most common channel for neurotransmitter release at axon terminals.

I_T (T for transient) A low threshold calcium channel. Activates with relatively small depolarizations. May be involved in rhythmic burst firing in some cells (see Fig. 5.13)

How these currents respond to a voltage clamp is shown here.

4 K Currents

- 1) I_K (or I_{KDR}) (K current or delayed rectifier). Activated by depolarization. Does not inactivate. Repolarizes the action potential
- 2) I_A . A fast activating and fast inactivation (5-30 ms) K current. Activated positive to rest near threshold. Delays the onset of firing following a brief depolarization and slows the firing rate when a neuron is persistently depolarized. May be involved in action potential repolarization in some cells.
- 3) I_M (M for muscarine sensitive). A depolarization-activated current that only slowly (10s-100s of ms) activates and deactivates. Does not inactivate. Reduces responsiveness to prolonged depolarization (spike frequency adaptation), but changes the neuron's response to brief excitation only little because of its slow activation. May contribute to ending plateau potentials and the depolarizing phase of spontaneously oscillating neurons.
- 4) I_{K2} (maybe I_D) A slowly inactivating voltage sensitive current. Long inactivation kinetics, but activates relatively quickly. It induces a long delay to firing during a persistent depolarization.
- 5) I_{AHP} (AHP stands for AfterHyperPolarization). There are slow and fast AHP currents. The slow is also called I_{SK} A calcium dependent (not voltage dependent) current that is activated by increased levels of intracellular calcium. This current induces a long lasting afterhyperpolarization following a train of action potentials due to increased intracellular calcium that accumulates during a train.
- 6) I_C (also I_{BK}) A calcium activated current that is also voltage dependent. This current increases quickly with depolarization and increased calcium. May be the main current involved in action potential repolarization in some cells. Lengthens the interspike interval
- 7) I_{leak} Contributes to the resting potential. Not voltage dependent.

5 Let's see what some of these other conductances may do to the action potential train. We will also look at this in Thursday's computer lab.

Starting with just the Na and K conductances (A), we see if we add the I_C we get enhanced repolarization (B). On the other hand, if we add I_A we see a delay to firing (C). If we add M we still get the first spike (as M activates) but then M can reduce or shut down the response (D). The I_{AHP} will turn on with the spike train (as calcium enters) and will produce spike train adaptation and may ultimately shut off the spike train.(E)

In F and G we see the example of the thalamic cell which can exist in two modes, one in which I_T is activated and the other in which I_T is inactivated.

6 Multi-ion current

I_h H-current sometimes called I_q . The book incorrectly lists this under K currents although they do say in the description that it is a mixed Na and K current. The reversal potential is -20 to -40 mV. It is voltage dependent, but is activated by hyperpolarization instead of depolarization. It closes with depolarization. Activation is relatively slow and will cause a voltage sag (depolarization) when the cell is hyperpolarized. It is somewhat activated at rest and contributes to the resting potential (along with I_{Kleak} .) In spontaneously oscillating neurons with a pronounced afterhyperpolarization after the burst, this current can cause a depolarizing ramp (Fig. 5-13) to bring the cell to threshold for the next burst.

Chloride currents

Chloride currents in general are not well characterized, primarily because the Cl concentration is not actively maintained by pumps in many neurons and the E_{Cl} tends to be close to or equal to the resting potential. A class of inhibitory synapses tends to increase Cl conductance to make neurons less responsive to any stimuli (+ or -)

Synaptically activated currents (to be discussed later in the course)