**Lecture 4: Voltage Clamping**

## I. Leak subtraction (Fig. 2)

### *A. In most textbook figures (e.g., Fig. 1) you don't get a realistic view of the data as it comes off of the voltage clamp*

**1. The raw traces are processed to remove leak and capacitative currents**

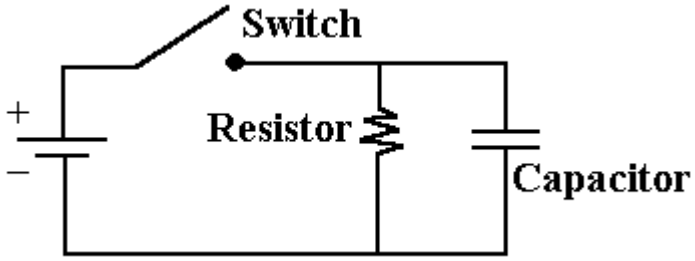
**2. The leak currents are produced by the flow of ions through leak channels – but since we're only interested in voltage-dependent channels, we don't care about the leak currents**

***a) But they're there & we have to deal with them***

### *B. Capacitative currents are currents that flow through the membrane capacitance*

**1. RC circuits and time constant (Fig. 3)**

***a)***




***b) Draw circuit with battery, switch; capacitor and resistor in parallel***

***c) As switch is closed, current first flows primarily through capacitor***

***d) As capacitor becomes fully charged more current flows through the resistor***

(1) When capacitor is fully charged all current flows through resistor

*(a) Amount of current determined by Ohm's law*

*(b) The current that still passes through the membrane following charging of the capacitor is the current flowing through the channels*

## II. Determination of ionic currents during voltage clamping

***A. Voltage step produces a transient inward current followed by a prolonged outward current***

***B. Now repeat experiment but in saline with an equal amount of sodium as inside the cell (that means that $E_{Na}$ = 0 mV)***

**1. Transient inward current is gone leaving only outward current (Note that the voltage step <u>MUST</u> be to $E_{Na}$ or you will have a sodium current flowing**

**2. Subtract this record from the record in normal saline to obtain sodium current**

***C. What carries the outward current?***

***D. How could you determine this?***

***E. Can't remove potassium because most of it is in the cell***

***F. Clue: generate family of curves to different voltage steps***

***G. Sodium current reverses at a step to +55 mV***

*a) Sodium current reverses at $E_{Na}$*

**2. Outward current is getting larger as steps become more positive**

*a) Obviously wrong direction step to get this current to reverse*

***H. You might think "Let's try negative going steps"***

**1. Can't do this because the outward current is voltage sensitive – the current isn't present unless the cell is depolarized**

**2. Change $E_K$ by changing $[K^+]_{OUT}$ and see where outward current reverses**

*a) Squid giant axon is so large that we can replace the cytoplasm with a fluid of known composition*

***I. Currents can also be isolated using drugs or poisons which selectively block a particular current***

***J. The only way to characterize a particular drug or toxin is to know what currents are present in a cell & see if the particular drug blocks a particular current***

**1. The electrode gods are not so nice to tell you what currents a new drug might or might not block**

*a) Drugs that block a particular current will sometimes have a different action in a different animal*

### *K. Common drugs used to block currents*

**1. Some of these poisons are venoms produce by animals for subduing prey or for defense**

**2. Tetraethylammonium (TEA)(not a venom) placed in the external medium blocks $K^+$ conductance**

*a) Extensive voltage clamp analysis was needed to show that TEA actually blocked $K^+$ channels*

*b) TEA treatment produces a voltage clamp record of just the sodium current*

**3. Tetrodotoxin (TTX) is derived from puffer fish and a few species of frogs and salamanders (actually produced by symbiotic bacteria)**

*a) TTX blocks sodium conductance and leaves only the potassium conductance*

(1) Fugu is Japanese sushi dish prepared from puffer fish

*(a) TTX is concentrated in the liver and skin*

(i) Chef must be licensed to prepare fugu so that he doesn't kill the diner

*(b) However, all tissue contain some TTX*

(i) Some of the appeal of fugu is apparently the buzz the diner gets from the TTX

**4. Cesium is used to block the delayed rectifier potassium channel (the one that underlies the repolarization of the action potential), but doesn't block a number of other potassium channels**

**5. Cobalt and barium can be used to block calcium channels**

**6. There are lots of other compounds that will block particular channels**

*a) Some companies primary business is selling such drugs*

## III. I-V Curves

### *A. I-V curves (current-voltage curves) are a common way of analyzing voltage clamp data (Figs. 4 & 5)*

1. **The most common procedure is to measure the peak current value of a current & than plot it versus the membrane potential of the step**

2. **Voltage-gated channels for sodium & calcium commonly produce a "N-shaped" I-V relationship**

   a) ***The N-shape is produced by the large increase in inward current that occurs as the channels reach threshold***

3. **N-shaped I-V curves can also be generated when there is more than one current activated by the voltage steps**

   a) ***Example: Meech & Standen J. Physiol 249:211-239, 1975***

   b) ***In a particular neuron in Helix aspersa (our local pest land snail) there are two potassium currents***

   c) ***The delayed rectifier***

   d) ***A calcium-activated potassium channel***

      (1) Calcium enters the cell via voltage-gated calcium channels & binds to calcium-activated channel at a site on the internal surface

      (2) Calcium-activated potassium channels also show voltage dependence & inactivate when depolarized enough

         *(a) Mechanism appears similar to inactivation gate on voltage-gated sodium channel*

## IV. Analysis of synaptic potentials using voltage clamping

### *A. The currents passing through ligand-gated channels can be analyzed using voltage clamping*

### *B. Why use voltage clamping to analyze synaptic potentials?*

1. **The current flowing through the ligand-gated channel and the induced voltage response (EPSP or IPSP) do not occur simultaneously (Fig. 8)**

   a) ***The reason the voltage change is delayed is that the synaptic current charges the membrane capacitance, which slows the rate at which the voltage changes***

2. **The time constant ($\tau$) is equal to $\tau = R_m C_m$**

   a) ***$R_m$ and $C_m$ are the membrane resistance & capacitance***

3. **Because many synaptic currents are short duration, they do not charge up the membrane capacitance fully**

4. **Because channels are opening to mediate the synaptic current & voltage, there can be significant distortion of the voltage response depending upon the number of channels opening, which changes $R_m$**

### *C. Voltage clamping overcomes this shortfall by charging the membrane capacitance so that we can look at the current through the channels in isolation*

1. **In other words, synaptic currents are a more accurate measure of synaptic events than are synaptic potentials**

### *D. Example of use of synaptic currents during voltage clamping*

1. **Postsynaptic neuron is voltage clamped while presynaptic neuron is activated**

2. **NMDA glutamate receptor passes more current when phosphorylated by Src tyrosine kinase (Fig. 9)**