CMP 610b Lecture 3
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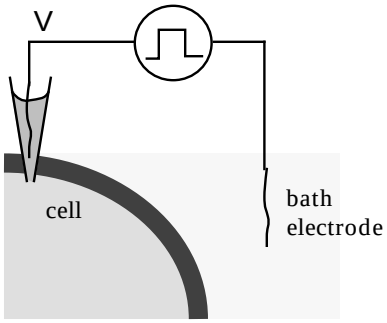
Two-Microelectrode Voltage Clamp

With the background of the first two lectures we can now look at the principles and the implementation of a two-microelectrode voltage clamp amplifier. We will take as an example the problem of recording from *Xenopus* oocytes.

PRINCIPLES OF THE VOLTAGE CLAMP

We saw in the first lecture the problem of trying to enforce a membrane potential on an oocyte through a microelectrode. Suppose we impose a potential V on the microelectrode, like this:



The membrane potential E will follow the equation

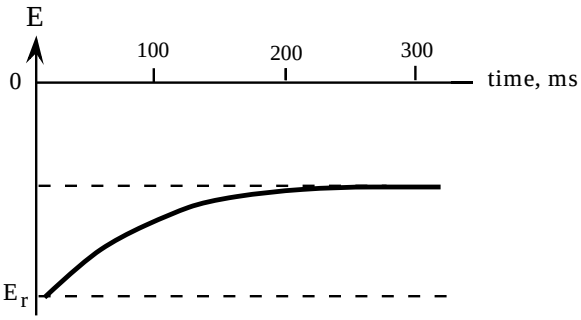
$$E + \frac{C}{g_m + g_e}\frac{dE}{dt} = \frac{g_m E_r + g_e V}{g_m + g_e}. \qquad (1)$$

This is eqn. (8) from the first lecture. It can be rewritten in the standard form

$$E + \tau\frac{dE}{dt} = E_\infty,$$

where the time constant τ and the final value E_∞ can be read from the equation above.

If we suppose that V suddenly changed from being the resting potential E_r to a new potential of zero, the cell's membrane potential will change very slowly, with a time constant on the order of 100 ms in the case of a 1 MΩ electrode in a *Xenopus* oocyte.



We could change *E* more quickly if we briefly made *V* very large. You can see this in eqn. (1): d*E* / dt is proportional to *V*. This would quickly charge up the membrane capacitance of the cell. Then if we kept *V* at the right value, we could force the steady-state level of E to be the value we wanted. The only problem is to find the right values to set *V* to.

From what we saw last time, the idea of feedback suggests itself. We saw that an operational amplifier changes its output voltage to until a particular condition–the equality of two input voltages–is met. What we need is a feedback system that changes *V* (or, equivalently, changes the injected current) until the membrane potential reaches the desired value.

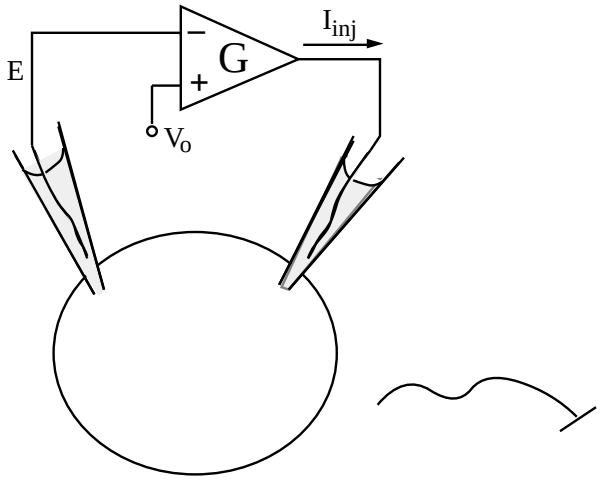


Fig. 1

One way to do it is to have a sort of amplifier that makes a current I_{inj} that depends on the difference of input voltages, for example as

$$I_{inj} = G\,(V_0 - E) \qquad (2)$$

where *G* is a scaling factor that has units of conductance (amperes per volt). Now let us write an equation for the membrane potential. From the 'fundamental equation'

$$I_{ionic} + C\frac{dE}{dt} = I_{inj} \qquad (3)$$

we obtain

$$I_{\text{ionic}} + C\frac{\mathrm{d}E}{\mathrm{d}t} = G\,(V_0 - E). \qquad (4)$$

Control of membrane potential

Let us now consider two cases. First, how well does the membrane potential E follow changes in the command potential V_0? To do this, we suppose that the ionic current is a simple membrane conductance, i.e.

$$I_{\text{ionic}} = g_{\text{m}}(E - E_{\text{r}})$$

where E_{r} is the resting potential. Then (4) becomes, with some rearrangement,

$$E + \frac{C}{g_{\text{m}}+G}\frac{\mathrm{d}E}{\mathrm{d}t} = \frac{GV_0 + g_{\text{m}}E_{\text{r}}}{G+g_{\text{m}}} \qquad (5)$$

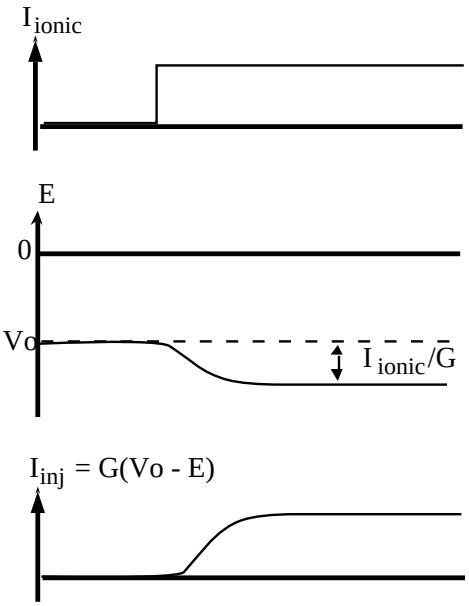
Here the time constant is $C/(g_{\text{m}}+G)$ and the final value is a weighted average of V_0 and E_{r}. If G is much larger than g_{m}, then the final value becomes very close to V_0, and the time constant becomes much shorter than otherwise.

Measurement of current

Now the second case. The injected current I_{inj} will be related to the ionic current across the membrane, I_{ionic}. If it is similar enough, then we could somehow measure I_{inj} and use it as our estimate of I_{ionic}. How closely do they correspond? Equation (3) can be rewritten as

$$E + \frac{C}{G}\frac{\mathrm{d}E}{\mathrm{d}t} = V_0 - \frac{I_{\text{ionic}}}{G} \qquad (6)$$

where here we assume that I_{ionic} doesn't depend directly on E, but instead makes a sudden change, e.g. a channel opens. There will be a change in E, with a final value displaced by I_{ionic}/G from V_0; the change will be small if G is large, and the change will occur with a time constant C/G. We can compute the time course of I_{inj} from Eqn. (2), and find that I_{inj} makes an exponential relaxation with the same time constant and has a final value exactly equal to I_{ionic}, as shown below.



Thus if we make *G* large enough, there will be little perturbation in membrane potential when an ionic current turns on, and the injected current will follow I_{ionic} with good fidelity.

PRACTICAL IMPLEMENTATION OF THE VOLTAGE CLAMP

1. How do you make an amplifier that creates an output *current* proportional to an input *voltage*? It is possible, but in our case it is not really a problem. Suppose we simply apply a voltage V_e to the current-injecting microelectrode. A current

$$I_{inj} = g_e(V_e - E) \qquad (7)$$

will pass through the microelectrode. If V_e is much larger than E, then we could approximate the situation by

$$I_{inj} \approx g_e V_e \qquad (8)$$

which, as it turns out, is a reasonable approximation. Then we need to have a more conventional amplifier that gives an output voltage

$$V_e = A(V_0 - E)$$

to put into the circuit, where we have chosen the gain A of the amplifier so that $G = g_e A$. (As an exercise, you can try working out Eqn. (5) using Eqn. (7) explicitly, instead of using the approximation.)

2. How do you make it sensitive to the *difference* of input voltages? One approach is to hook up two inverting amplifiers in the following way:

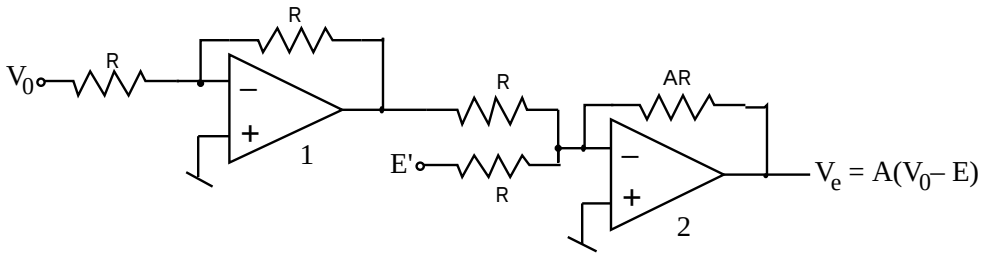


Fig. 2

Here amplifier 1 takes the voltage V_0 and inverts it, i.e. creates an output voltage of $-V_0$. Meanwhile amplifier 2 produces an output that is –A times the sum of its two inputs. The result is the desired difference.

The same thing can be accomplished with a single operational amplifier in a circuit that is a hybrid of the inverting and non-inverting amplifiers we discussed last time. If R2/R1 = *A*, then the following circuit does the same thing:

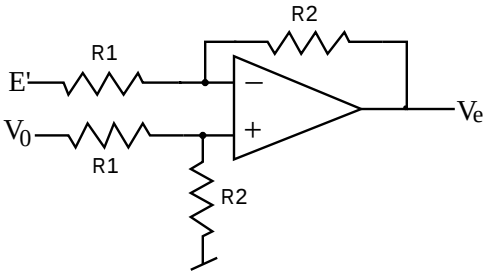


Fig. 3

3. How do you measure the potential without drawing current through the microelectrode? If you were to hook one of the circuits in Figs. 2 or 3 directly to a microelectrode to measure *E*, you would have a problem, because they rely on resistors that cause currents to flow that are proportional to the input voltages. Such currents flowing through the voltage recording electrode will cause an error in the measured potential. We can use a voltage follower to isolate the microelectrode from the other amplifiers:

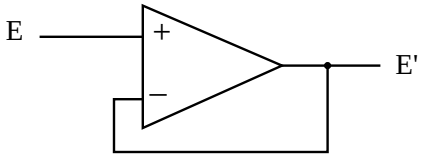


Fig. 4

Here no current flows into the input terminal.

4. How do you deal with electrode offsets? As you remember from the first time, if the Cl^- concentration is not the same at the wire in the microelectrode as at the bath electrode, there can be a considerable electrode offset potential, maybe 50-100 mV. This has to be subtracted from *E'*, the estimate of membrane potential, before it is used for the feedback. This can be done with yet another

amplifier stage that has two inputs: one receives E' and the other receives a variable voltage (set by a potentiometer, i.e. a variable voltage divider); these are added together in the amplifier.

5. How do you measure the current? The basic idea is to use the current-to-voltage converter we discussed last time. We could measure the current being injected into the microelectrode, but equivalently we can measure the current flowing into the bath electrode. The latter is simpler to do. The operational amplifier keeps the potential of the bath electrode at zero while allowing the current to be measured with the resistor R.

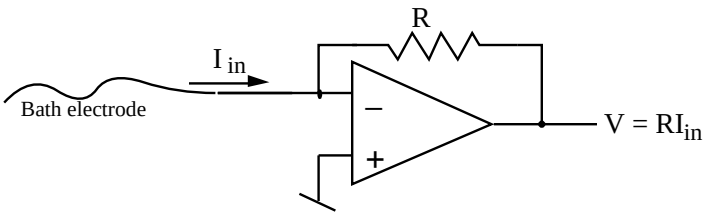


Fig. 5.

You will recognize these building blocks, sometimes in embellished form, in the diagram of the oocyte voltage clamp in the appendix to these notes.

LIMITS ON VOLTAGE-CLAMP SPEED AND ACCURACY

From our earlier analysis it appears that the larger we make the gain G (or A) of the voltage-clamp amplifier, the better things will be. There are limits, of course. We consider two of the limits here. Another limit, concerning the dynamics of the feedback loop, involves solving higher-order differential equations. This is not all that hard, but is beyond what can be covered in this lecture.

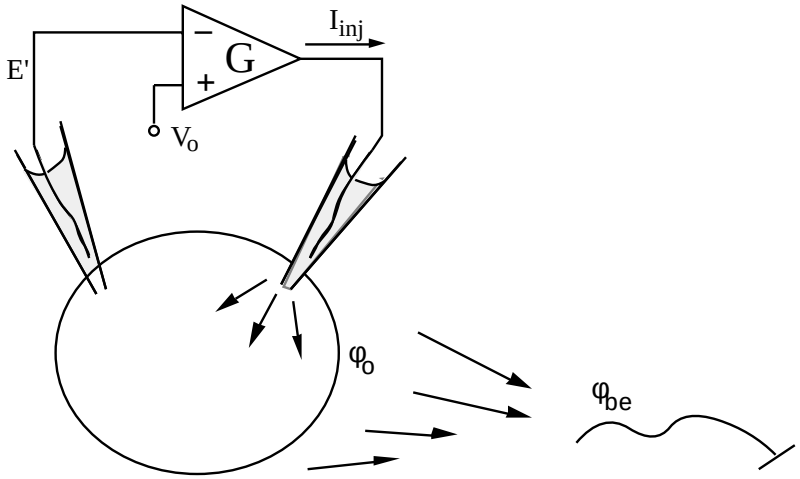
1. Maximum voltage available for V_e. The maximum rate of change of membrane potential is seen, by inspection of (3), to be equal to I_{inj}/C, or by the approximation of Eqn. (8), is about equal to $V_e g_e/C$. For an oocyte, where $C = 2\text{x}10^{-7}$F and $g_e = 10^{-6}$S one obtains a maximum dE/dt of only about 5 volts per second when V_e is 1 volt. This is quite slow when one wants to, say, look at a voltage-gated channel by changing E by 100 mV in less than 1 ms (>100 V/s). In oocyte voltage clamps it is therefore common to use special amplifiers that produce output voltages in excess of 100 V, allowing maximum dE/dt on the order of 500V/s. There is also little point in using gains much larger than 1000 since the amplifier will not be able to provide a linear response to commanded changes larger than about 100 mV without exceeding its maximum output voltage of 100 V.

2. Coupling capacitance between microelectrodes. This is an interesting example of a spurious feedback pathway. Consider what happens when the voltage clamp amplifier is commanded to make a step in potential, e.g. V_0 suddenly changes by 100 mV. Then due to the large gain ($A \approx 1000$) of the amplifier, there will, in the first instant, be a 100 V jump in the voltage on the current passing microelectrode. If there is on the order of 0.005 pF of capacitance between the microelectrodes, this will result in a 50 mV jump at the voltage recording microelectrode, due simply to capacitive coupling "through the air", and assuming a capacitance of 10 pF at the voltage microelectrode. Note that this voltage jump has nothing to do with true changes in membrane potential. The clamp

amplifier, seeing the apparent 50 mV jump in *E*, quickly reduces the output voltage, and therefore the injected current, to half. The result is that while the amplifier sees very rapid changes in the apparent *E*, the true changes in *E* are much slower than what you would expect, and also slower than what you will observe with an oscilloscope connected to the membrane voltage monitor signal! The only hint that something is wrong is that the current monitor signal will show a slowly-decaying capacitive current transient.

The moral of this story is that it is important to place some sort of electrostatic shielding between the microelectrodes, in the form of some sort of grounded metal object. A good technique is to wrap aluminum foil around the current passing electrode, and to connect the foil to ground with a clip.

3. Series resistance. This is probably the most insidious artefact in voltage-clamp measurements. Recall that the goal of implementing the voltage clamp in the first place is to force the membrane potential to a particular value, regardless of the amount of ionic current flowing. We saw in eqn. (6) that, by making the gain large enough, we can come close to this ideal. However, this assumes that the electrodes provide us with a reliable estimate of the membrane potential. In addition to the transient errors due to coupling capacitance between electrodes just discussed, there also can be steady-state errors due to current flow through the bath solution and cytoplasm. Let us first consider the effects of current flow through the bath solution. Suppose a substantial current I_{inj} is being applied, due to a large ionic current through the cell membrane. Then φ_o, the potential at the outside surface of the cell membrane, will be different from the potential φ_{be} at the bath electrode (taken to be zero in the situation diagrammed below).



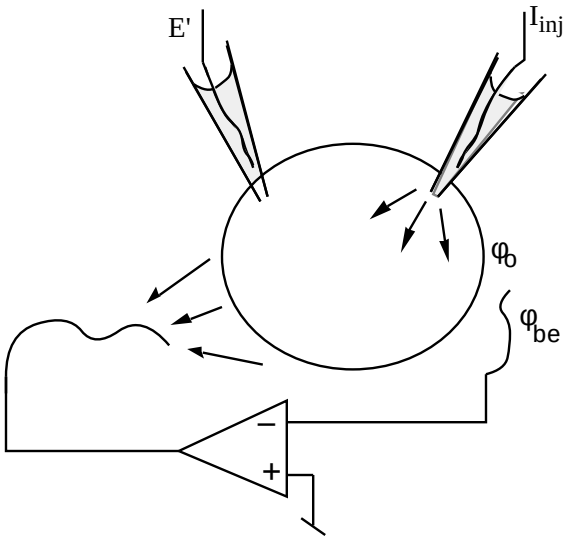
This means that the voltage *E'* recorded with the voltage microelectrode will not be the true membrane potential *E*, but rather be

$$E' = E + \varphi_o.$$

The discrepancy is given by

$$\varphi_o = R_s\, i_{inj}$$

where R_s is the "series resistance" of the path through the bath solution from the membrane surface to the bath electrode. This effect becomes serious when I_{inj} is large. For the oocyte voltage clamp R_s is often on the order of 1000 Ω. It is not uncommon to have channels expressed in an oocyte yielding currents of tens or hundreds of microamperes. With R_s = 1000 Ω this translates into membrane potential errors of tens or hundreds of millivolts, which are usually intolerable. As a first step toward reducing the effective series resistance, one can try to maintain the potential at the surface near zero, for example by using a *second* voltage clamp circuit for the bath electrodes:



Here an operational amplifier (having essentially infinite gain) keeps the potential φ_{be} at zero, while all the current flows through the left-hand electrode. Note that there will still be series resistance problems, since because of the asymmetrical current flow φ_o will not be the same everywhere on the membrane surface. But this arrangement helps the situation somewhat.