CMP 610b Lecture 5
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Patch and Whole-Cell Recording

This lecture concerns the theory of several practical aspects of single-channel and whole-cell recording. We consider some issues of external electrical interference and capacitive transients, and then consider the series-resistance problem of whole-cell recording.

ELECTRICAL INTERFERENCE

Last time we considered at some length the sources of random noise due to the thermal motion of ions and electrons. Now we turn to the practical matter of dealing with the pickup of nonrandom electrical signals, such as interference from the 60 Hz power lines or from a computer monitor. There are two main principles to consider: electrostatic pickup through unshielded surfaces, and magnetic pickup through ground loops.

Electrostatic pickup. The principle behind this is simply capacitance. Given a capacitance C between a metal surface with some voltage V on it and the input to the patch clamp there will be a current

$$I = C\frac{\mathrm{d}V}{\mathrm{d}t}$$

For example, if V is a sine wave voltage

$$V = V_0 \sin(2\pi \mathrm{f t})$$

Then the current will be

$$I = 2\pi f C V_0 \sin(2\pi f t + \pi/2) \qquad (1)$$

which means that the current will be also sinusoidal, just shifted in phase and scaled by the factor in front. For $f = 60$ Hz, $2\pi f$ is about 400 sec^{-1}.

Suppose we have a 'capacitor' consisting of a conducting surface of about 1 cm^2 (the pipette and holder) that is some distance (let's say an effective distance of 10 m) away from a conductor carrying 110 V ac power. An example of such a conductor would be the plasma inside the tube of a fluorescent lamp. In this case the capacitance will be on the order of

$$C = \frac{10^{-13}\ \mathrm{F/cm} \times 1\ \mathrm{cm}^2}{10^3\ \mathrm{cm}}$$

or about 10^{-16} farads, i.e. 10^{-4} pF. Plugging this into eqn. (1) with $V_0 \approx 110$ V one obtains about 6 pA of induced current. This is of course very large, even with the source of interference so far away! Such interference can be greatly reduced by interposing a piece of metal between the source and the patch pipette. If this conductor is grounded, it will shield the pipette from the interference. If it is not grounded, it can act as an "antenna" and actually increase the interference substantially. Thus for example the parts of a microscope can act either as antennas or shields, depending on whether you have grounded them carefully. Some people make recordings without even using a metal-screen "Faraday cage" around their setups because they have very carefully grounded all of the metal parts of the microscope and other things that surround the patch-clamp probe.

Magnetic pickup. The phenomenon here is that if you connect two things together with a wire, they are not necessarily at the same potential. This can be demonstrated by forming the wire into a loop: if the loop encloses a changing magnetic field, a voltage will appear between the ends of the wire. Everything is fine if you connect things together using only one wire, or if you run all the wires together, say, from your electronics rack to the setup table. But if the ground wire runs in one path and the wire that carries the command potential to the patch clamp probe runs in another path, you will have the effect that they will actually have potentials that differ according to the magnetic fields nearby. In a typical setup the differences can be on the order of several millivolts, or more if power transformers or computer monitors are nearby.

The most serious effect of a potential difference of this sort is when it appears between the patch pipette and the bath ground electrode, or between the pipette and nearby "grounded" surfaces. Then the potential difference appears across a capacitance, which results in an interfering current being injected into the pipette.

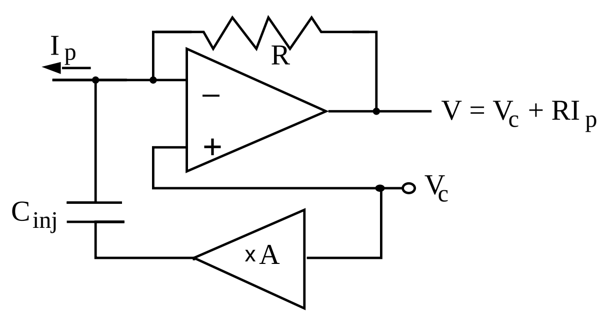
TRANSIENT SUBTRACTION IN THE PATCH CLAMP

What current flows in the pipette when a sudden potential change is commanded? Suppose that the patch clamp makes a 100 mV step in 10 µs. Suppose further that the total capacitance of the pipette, patch membrane, etc. is 1 pF. Then a current

$$I_p = C_p \frac{dV}{dt}$$

of 10^{-12} F x 10^4 V / s = 10^{-8} amperes will flow. This is some 10,000 times larger than the current of ~1pA that one would like to record through a voltage-activated channel, and is also much larger than the current that can be passed through the large resistances (typically 10-50 GΩ) used in patch clamp I-V converters.

The best way to deal with this huge current flow is to somehow predict what the necessary current will be, and inject it into the pipette just as it is needed. This can be done with a circuit like this:



Here an amplifier with variable gain A scales V_c before it is applied to the injection capacitor. If there is a change in the command voltage V_c, a current

$$I_{inj} = (A\text{-}1)\, C_{inj} \frac{dV_c}{dt}$$

will flow into the input. If we choose A just right, so that $(A\text{-}1)C_{\mathrm{inj}} = C_{\mathrm{p}}$, then the injected current will just equal the pipette current, and none of this capacitive current will flow through the resistor to be measured.

In practice the gain A is adjustable with a knob (e.g. "C-Fast") which you can turn to get rid of most of the hugh capacitive transient current. A similar circuit allows you to cancel transient currents that flow more slowly, for example from the gradual charging of the membrane capacitance through the pipette resistance ("C-Slow").

TRANSIENT SUBTRACTION IN THE COMPUTER

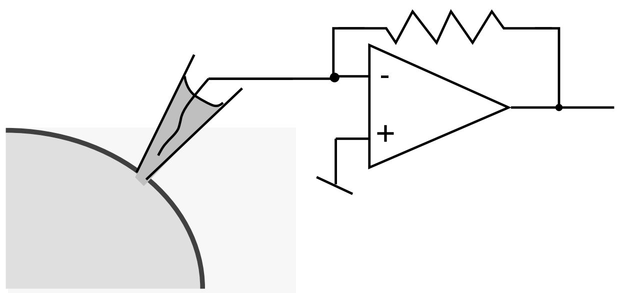
The transient cancellation in the patch clamp is not perfect. To be rid of the residual capacitive artifacts, as well as the "pedestal" in the trace that arises from the leakage conductance of the seal and membrane, some sort of <u>leak subtraction</u> is used. The most elegant subtraction method is to accumulate the average of sweeps in which no channel activity occurs, and then subtract this average from each sweep to be analyzed. Clearly this technique can be used only for patch recordings in which (1) single channel events can be readily resolved, and (2) when frequency of channel openings is low enough that blank sweeps are observed. Since the average is accumulated from a finite number of sweeps, it will contain some noise which will contribute to the total noise in the subtracted traces. If n sweeps are used in the average the rms noise will be increased by the factor $\sqrt{1+1/n}$; this noise increase is negligible for $n \approx 10$ or so. If there are not many null sweeps, one could go to the length of averaging just those *portions* of sweeps that lack channel activity.

A useful alternative to averaging null sweeps is to use <u>P/4 subtraction</u>. This technique was introduced by Bezanilla and Armstrong (1977) to remove artifacts from their recordings of the small gating currents in squid axons. It can be used for macroscopic currents as well as single-channel current recordings. The idea is to give a number of small, scaled-down copies of the test pulse and to use the scaled average response to these control pulses for subtraction. Traditionally pulses are given with a scaling of one fourth; hence the name "P/4". The control pulses are given in a potential range too negative to cause the activation of voltage-dependent conductances. For example, in an experiment in which the control pulse might go from -80 mV to 0 mV, the P/4 pulses might start at -120 mV and step to -100 mV. For subtracting artifacts in single-channel recordings you will want to accumulate the average of a large number of P/4 pulses. Since the average must be scaled up by a factor of 4 (which scales the noise variance by 16) the rms noise, after subtracting the scaled average of n P/4 responses, is increased by the factor $\sqrt{1+16/n}$. It is a good idea to average 100-200 responses to obtain the subtraction trace, which can then be used for subtraction from a large number of sweeps.

The P/4 subtraction relies explicitly on the responses being a linear function of the voltages applied. This requirement is definitely *not* met when some element of the recording system goes into saturation, i.e. receives such a large signal it cannot amplify or filter it linearly. Some patch clamps have a "clipping" light which shows when an internal amplifier goes into saturation; this is valuable because sometimes the patch clamp amplifier saturates on very large, fast current transients that are not visible once the signal is filtered. It is therefore important that all transient signals stay within the linear range (typically $\pm$ 10 volts) of all the electronic devices in the recording system.

WHOLE-CELL RECORDING

Although the patch-clamp techniques were originally developed in order to record single-channel activity, the "whole-cell" recording configuration has become of equal if not greater importance. To obtain a whole-cell patch recording, you seal the pipette against the membrane, establishing the "cell-attached" recording configuration, and then break the patch membrane under the pipette to obtain electrical access to the cell interior. The result is equivalent to impaling a cell with a microelectrode. However, the access resistance is typically much lower than what one would obtain with an appropriately narrow conventional microelectrode, and the induced leakage conductance is also lower. These features allow one to obtain lower noise and higher-fidelity recordings with the whole-cell patch technique.



There are various ways to "break" the patch membrane to obtain access to the cell interior. In the "conventional" whole-cell technique the membrane is disrupted with extra suction or with a brief, high-voltage pulse (say 1000 mV for 0.1 ms). In either case a diffusional pathway is created that allows quite effective dialysis of the cell. An alternative is the "perforated patch" technique, in which a low-resistance pathway is created in the patch membrane without breaking it. Instead, the pipette is filled with a solution containing nystatin or amphotericin B, either of which introduces a high density of cation-selective channels into the membrane. Under good conditions the access resistance through these channels in an intact patch membrane is comparable to the that of the "disrupted" membrane. This access resistance is usually not lower than about three times the resistance of the patch pipette when it is open to the extracellular solution.

SERIES RESISTANCE

The access resistance through the whole-cell recording configuration is so low--it can be only 1 or 2 megohms under the best conditions--that one often obtains good voltage control and current recording from small cells without having to worry about the errors due to this series resistance. However, it is worth considering once again these errors before we discuss the idea of series resistance compensation. Recall from the first and third lectures of this series, if we have a membrane conductance g_m and electrode (access) conductance g_s, in a cell with membrane capacitance C and resting potential E_r then the membrane potential E will satisfy

$$E + \frac{C}{g_m + g_s}\frac{dE}{dt} = \frac{g_m E_r + g_s V_p}{g_m + g_s}, \qquad (2)$$

where V_p is the potential on the pipette. Thus the membrane potential will follow changes in V_p with a time constant

$$\tau = \frac{C}{g_m + g_s}$$

which will usually be well approximated by $\tau = CR_s$, where the series resistance $R_s = 1/g_s$. The steady-state value of the membrane potential will differ from V_p with an error on the order of R_s/R_m.

What about the fidelity of current measurement? Let us model the membrane as having a conductance g_m but also allow a current I to be time varying, say representing a channel current that can change suddenly. What will the measured pipette current I_{inj} be? The fundamental equation of electrophysiology says

$$I + C\frac{dE}{dt} = I_{injected}$$

while the access conductance of g_s means that

$$I_{inj} = g_s(V_p - E)$$

If we now let V_p be a constant, so $dV_p / dt = 0$ then

$$\frac{dE}{dt} = -\frac{1}{g_s}\frac{dI_{inj}}{dt}$$

and one obtains

$$I_{inj} + \frac{C}{g_s}\frac{dI_{inj}}{dt} = I. \qquad (3)$$

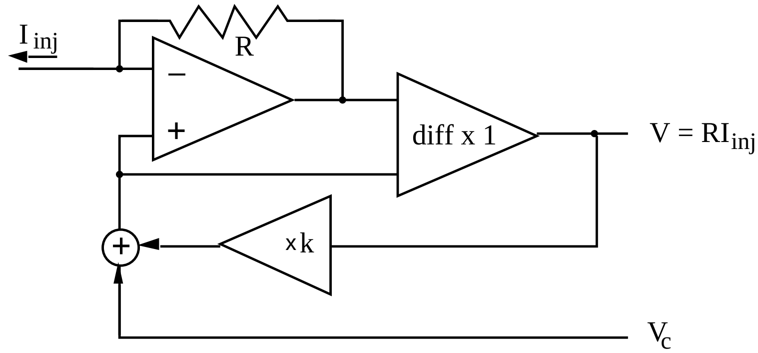
This says that the injected (and therefore measured) current will not necessarily reflect all of the true membrane current, and will respond to sudden changes with a time constant essentially identical to the one in the equation (2) above for the voltages. [Actually the time constant would be exactly the same except that we are using a different model for the current I in the derivation of eqn. (3) than in that for eqn. (2).]

SERIES RESISTANCE COMPENSATION

If you know what the value of the series resistance is, you can predict its effects and in principle can apply a correction to the commanded potential to compensate for the effects. Since we know that

$$V_p = E + I_{inj}R_s$$

we should be able to just subtract $I_{inj}R_s$ to the pipette potential to estimate E. Or, equivalently, we could just add the voltage $I_{inj}R_s$ to the command voltage V_c and apply this to the pipette: this will cause E to equal V_c. Here is a circuit that will do this:



The pipette potential will be given by

$$V_p = V_c + kRI_{inj} \qquad (4)$$

so if we adjust the scaling factor k appropriately, i.e. so that $kR = R_s$, then we should be able to correct away all the effects of the series resistance. There are at least two important limitations to this scheme.

1. It is impossible to correct away the series resistance perfectly, because that would require infinite pipette voltages. Consider the case in which we are imposing a step change on the membrane potential. In the first instant after the step change in V_c, the membrane potential will not have changed from its previous value. If we set $k = R_s / R$ then the solution to eqn. (4) along with

$$I_{inj} = \frac{V_p - E}{R_s}$$

with fixed E results in an infinite value for V_p, all because E refuses to change instantaneously.

2. The compensation fails if currents other than I_{inj} are measured by the patch clamp. Specifically, if there are also currents from the pipette capacitance, for example, these will be interpreted as part of I_{inj} and will result in serious errors or (very commonly) oscillations in the feedback loop.

TRANSIENT PREDICTION (SUPERCHARGING)

As in the case of subtraction the fast capacitive transient currents (page 2 of these notes), it is possible to predict and subtract the slower transient currents arising from the charging of the membrane capacitance. A subtle issue arises however when this subtraction is combined with series-resistance compensation. Since part of the membrane charging current is not being measured, the circuit shown above will not properly compensate for the voltage drop across the series resistance resulting from that current. However, the extra current that is injected by the transient subtraction circuit can be calculated (e.g. by an appropriate "analog computer" circuit) and the appropriate correction can be made to the pipette potential. When a step stimulus is applied, the effect of this correction is to provide an overshoot in the voltage applied to the pipette, which causes the membrane capacitance to be charged more quickly than it would be otherwise. This technique has been called "Rs-prediction" or "supercharging", and is part of the series-resistance circuitry on recent patch clamp amplifiers, such as the EPC-7 and 9, and the Axon 200.

REFERENCES

Several chapters in the book *Single Channel Recording* (2nd Edition, B. Sakmann and E. Neher, eds., Plenum Press 1995) give further details on the topics covered here. Chapters 1 and 2 give an overview of the patch-clamp setup and the issues of whole-cell recording, respectively. Chapter 4 gives a more detailed technical description of the transient-cancellation and series-resistance compensation circuitry.