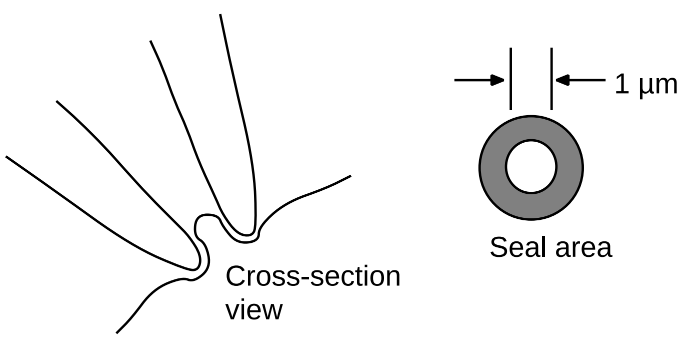
CMP 610b Lecture 4
F. Sigworth

# Thermal Noise in the Patch Clamp

The original impetus for the development of the patch clamp was to be able to resolve the small currents that flow through single channels. The advances that allowed these currents to be recorded mainly involved increasing the resistance of the "seal" between the glass pipette and the membrane. An obvious advantage of having a high seal resistance is that a greater fraction of the membrane current will be forced to flow into the pipette (with its access resistance) rather than to leak out through the imperfect seal. Another important advantage of a high seal resistance is the reduction in electrical "noise" introduced by the thermal motion of ions in the seal region. When expressed as a current, this noise is lower when the seal resistance is high.

**"LOOSE" AND "TIGHT" SEALS**

As far as I know, no one really understands the nature of the tight seal between the membrane and pipette. It seems to be a direct apposition of the glass surface and the membrane, because the resistance would be much lower if some water were in between. For example, suppose the area of contact between the pipette tip and the membrane were an annulus about 1 µm in diameter and 1 µm thick:




Suppose the aqueous pathway between the membrane and the glass is very narrow, only 20Å in thickness. The resistance of the pathway from the pipette interior to the bath solution would be roughly

$$R = \frac{\rho l}{A} \approx \frac{100\ \text{ohm}\ \ \text{cm} \times 1\mu\text{m}}{2\pi \times 1.5\mu\text{m} \times 20\text{Å}}$$
$$\approx 50\ \text{megohms}$$

This resistance is what would be obtained in a good "loose patch" recording. It is clear that the gigohm resistances that are routinely obtained in "tight seal" recording must represent a much closer approach of the two surfaces, probably direct adhesion between them.

## THERMAL NOISE AND CONDUCTANCE

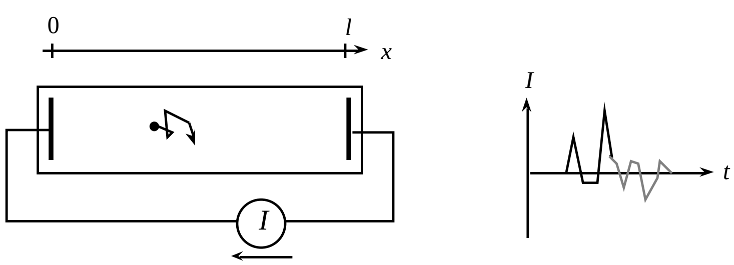
Various electrical noise sources place limits on the resolution of current recordings. An important one arises from the seal resistance, but there are other significant noise sources as well. I will give here an overview of the general theory of the electrical noise due to the thermal motion of charged particles. Nearly all of the noise sources in the patch clamp can be described using this theory.

In this theory I will make use of the idea of random variables. A random variable $x$ is a quantity that takes on a different value every time we go to measure it. We can talk about a particular *realization* of the variable, i.e. a particular measured value, but it is more informative to talk about average values. For example, we can write $\langle x \rangle$ which is the average value--formally, the *Expectation value*--of $x$. You can imagine that the expectation value is the value you would get by averaging over an infinite number of measurements of $x$. Similarly, we can take the expectation value of functions of $x$. The expectation of $x^2$ is written as $\langle x^2 \rangle$. The variance of the random variable is just $\langle x^2 \rangle$ if the mean value happens to be zero. In general, the variance

$$\sigma^2 = \langle x^2 \rangle - \langle x \rangle^2$$

is given by the expectation of $x^2$ minus the square of the expectation of $x$.

We can compute the size of random current fluctuations from the brownian motion of a charged particle. Suppose we place a charged particle inside a container with electrodes at each end (the particle might be a single ion in very pure water, or an electron in a vacuum):




Whenever the particle moves in the positive $x$ direction we observe a positive current $I$ flowing in the external circuit. We can describe this by a charge movement $Q$ in the circuit that reflects the position and charge $q$ on the particle,

$$Q = \frac{qx}{l} \tag{1}$$

and the current will be

$$I = \frac{dQ}{dt}. \tag{2}$$

How can we describe the random motion of the particle? Einstein's equation for Brownian motion says that average squared displacement of the particle during a time $\delta t$ is

$$\langle x^2 \rangle = 2D\,\delta t. \qquad (3)$$

Thus if we were to take "snapshots" of the particle position at time intervals $\delta t$ we would see that the square of the difference in the $x$ position will, on the average, be given by eqn. (3). Because of the "random walk" nature of the particle motion, the average difference in $x$ itself will be zero. But the square of the difference in $x$ will always be a positive number, and gives us a measure of how far the particle moves.

Now let us define the time-averaged current $\bar{I}$ as the average current during a time interval $\delta t$. Thus the analogue of eqn. (2) is

$$\bar{I} = \frac{Q(t+\delta t) - Q(t)}{\delta t}. \qquad (4)$$

Now what we would like to do is to obtain a value for the magnitude of $\bar{I}$. Again, $\bar{I}$ itself is going to average to zero, but we can say something about $\langle \bar{I}^2 \rangle$, using eqns. (1) and (3):

$$\langle \bar{I}^2 \rangle = \frac{q^2 \langle x^2 \rangle}{l^2\,\delta t}$$

$$= \frac{2q^2 D}{l^2\,\delta t}.$$

What if we had more than one particle giving rise to our current? We can calculate $\langle \bar{I}^2 \rangle$ in this case on the basis of a fundamental theorem in statistics. Let **r** and **s** be random variables having zero mean, i.e. $\langle \mathbf{r} \rangle = 0$ and $\langle \mathbf{s} \rangle = 0$, and also let them be statistically independent. Then if we have a new random quantity

$$\mathbf{z} = \mathbf{r} + \mathbf{s}$$

then the mean squared value (variance) of **z** will be simply

$$\langle \mathbf{z}^2 \rangle = \langle \mathbf{r}^2 \rangle + \langle \mathbf{s}^2 \rangle$$

i.e. the sum of the variances of **r** and **s**.

By this argument we can say that if there were $N$ identical particles, and that these particles did not interact (thus having statistically independent motions) then the time-averaged current will be given by

$$\langle \bar{I}^2 \rangle = \frac{2Nq^2 D}{l^2\,\delta t}, \qquad (5)$$

That is, the variance of $I$ increases linearly with the number of particles.

It is now of interest to compare the size of these current fluctuations with the conductance of our system. This system is just a resistor; if we impose a voltage $V$ between the electrodes, an average current $\langle I \rangle$ will flow as the particles are pushed along in the electric field. In this case, the average velocity of a particle is

$$\frac{d\langle x\rangle}{dt} = \text{mobility} \times \text{force}$$

$$= \frac{D}{kT}\,\frac{Vq}{l}.$$

The average current is then given by, using eqns. (1) and (2),

$$\langle I\rangle = \frac{Nq^2 D\, V}{l^2 kT},$$

which means that we can write the conductance $g = \langle I\rangle/V$ of the system as

$$g = \frac{Nq^2 D}{l^2 kT}. \qquad (6)$$

This formula is interesting because of its striking similarity to eqn. (5). Since our model of our system is quite general--it can represent a resistor, or an ionic solution, or the leakage pathway in a pipette-membrane seal--we can say that for any system of this sort the current fluctuations will be related to the conductance according to

$$\langle \bar{I}^2\rangle = \frac{2kTg}{\delta t} \qquad (7)$$

This is a variant of the formula derived by Nyquist in the 1920s to explain the electrical noise measured by his contemporary, Johnson, in a resistor. It is commonly called "Johnson noise".

**SPECTRAL DENSITY**

In actual experiments the time scale of a current measurement is determined by a filter. We use "low-pass" filters which pass all frequencies up to a "cutoff" or "corner" frequency value $f_c$. The relationship between corner frequency and $\delta t$ is, for the purposes of the present discussion,

$$\delta t = 1/2f_c$$

This means that if we use a filter with $f_c = 1$ kHz, it is roughly equivalent to making measurements on the time scale of 0.5 ms.

We can now rewrite (7) as

$$\langle \bar{I}^2\rangle = 4kTgf_c.$$

Further, we can define a quantity $S$, the spectral density, such that

$$\langle \bar{I}^2\rangle = Sf_c.$$

$S$ has units of Amperes$^2$ per Hertz, and is given by

$$S = 4kTg \quad (8)$$

This is the spectral density of the current in a conductance $g$.

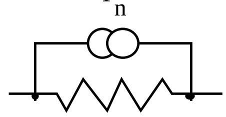
**NOISE SOURCES IN THE PATCH CLAMP**

Now we can turn to thinking about actual noise sources in the patch clamp situation. How do we work from a value of the spectral density to a measurable quantity? First we compute the noise variance, based on the corner frequency $f_c$ of the filtering in the recording system. Then we can take the square root of the variance to obtain the standard deviation $\sigma_i$ of the random current. This is also called the "rms" current, which means the "square root of the mean of the square" of the current. This is the sort of quantity measured by an rms voltmeter, for example.

The other main result that we must keep in mind is the idea that if you have independent random values, the variance of the sum of these is equal to the sum of the variances. In practice this is an important rule because if we have several noise sources in a circuit, all of which contribute, say, to a fluctuating current, the variance of the total current can be computed and then we can take the square root as above to get to the measurable value.

**VOLTAGE NOISE IN A RESISTOR**

Equation (8) says that the random current in a resistor will inversely with the resistance (and proportionately to its conductance). This is the opposite of what you would expect if you ever made voltage recordings with high-resistance microelectrodes. It is well known that there is more noise with higher-resistance electrodes than with low-resistance ones. The resolution of this paradox comes from the distinction between current noise and voltage noise. To introduce this distinction let us make an artificial representation for a resistor. We represent it as a noiseless resistor (e.g. one cooled to near absolute zero temperature) and then represent the randomly-moving charge carriers by a little current generator that makes the random current $i_n$.




If we were to connect this thing to a current-to-voltage (I-V) converter which keeps the voltage across the resistor at zero, the I-V converter will measure $i_n$. If instead we connect a voltmeter across this thing, and this voltmeter draws no current into itself, all of $i_n$ will flow through the resistor and we will measure a voltage

$$v_n = R\, i_n. \quad (9)$$

Now what is the variance of $v_n$? If $v_n$ fluctuates around zero (which we will assume) then

$$\langle v_n^2 \rangle = \langle (Ri_n)^2 \rangle$$
$$= R^2 \langle i_n^2 \rangle$$

i.e.

$$\sigma_v^2 = R^2 \sigma_i^2 \qquad (10)$$

Since $\sigma_i^2$ is proportional to $1/R$, we find that $\sigma_v^2$ is proportional to $R$.

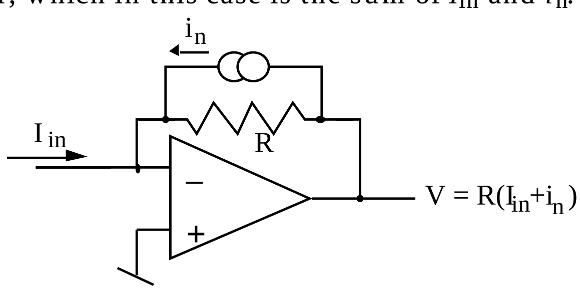
**NOISE SOURCES IN PATCH RECORDING**

1. The seal resistance. This is like a shunt conductance at the input to the I-V converter. We have already worked out this noise. Suppose we have the rather low resistance of 1 GΩ. This will result in a noise current, assuming $f_c$ = 1 kHz, having the standard deviation

$$\sigma_i = \sqrt{4kTf_c/R}$$

Since $4kT \approx 1.6 \times 10^{-20}$ joule, one gets $\sigma_i \approx 0.13$ pA. This noise source alone would be enough to prevent you from seeing channel events smaller than about 1 pA at this filter setting.

2. The feedback resistor. The resistor in the current-to-voltage converter also has current noise. Due to the action of the amplifier, the output voltage is proportional to the total current flowing through the resistor, which in this case is the sum of $I_{in}$ and $i_n$.




Thus the noise current in the feedback resistor makes the same sort of contribution to the total variance as a shunt resistance connected to the input. Although this is not usually the main noise source in patch recording, some patch clamp designs now use a capacitor (which is essentially noiseless) instead of a resistor as the feedback element in the I-V converter.

3. Dielectric relaxation in the pipette glass. Here is a remarkable noise source. Earlier we considered that the physical origin of electrical noise in a conducting medium is the random motion of the charged particles that carry current. In glass there are trapped ions which however can change their positions by a limited extent and on a fairly slow time scale, on the order of 1 ms. The thermal motion of these ions in the glass wall of a pipette induces a considerable noise current flow. If the pipette is made from "soft" glass which has a high density of mobile ions, the induced current can be a noise source that is much larger than the other sources mentioned here. The coupling of the ion motion to current in the pipette can be reduced by coating the

pipette with an insulating material such as a silicone polymer (e.g. Sylgard). Also reducing the amount the pipette is immersed into the solution helps. Some glasses have much less "dielectric relaxation" than others. For example, a favorite in my laboratory is Corning 7052 glass.

It is interesting to note that the same mechanism that gives rise to excess noise also gives rise to a slow capacitive transient in response to voltage steps. That is because the voltage change causes rearrangement of the same charges in the glass that induce thermal noise current. Glasses giving less noise also give smaller transients. This reflects a general physical principle called the fluctuation-dissipation theorem.

<u>4. Induced current noise from voltage noise.</u> Finally we consider the effect of a fluctuating command voltage applied to the I-V converter. This will cause the I-V converter to impose this fluctuating voltage on the pipette; the currents necessary to charge and discharge the pipette capacitance and other capacitances will be measured by the I-V converter and appear like another current noise source. The same effect occurs even when the command voltage does not fluctuate, because the operational amplifier itself has an intrinsic voltage noise, such that it forces the voltage between the inputs to be not exactly zero, but to be equal to a small random voltage $e_n$. Clearly it is best when $e_n$ is small.

**DEDUCING CONTRIBUTIONS FROM NOISE SOURCES**

Most commercial patch clamps now incorporate a display that reads the rms current noise. This measurement, along with the rule that variances add, can allow you to deduce which noise sources are the main ones. For example, suppose the noise meter reads 100 fA with nothing connected to the input of the patch-clamp probe, but 150 fA with the pipette holder inserted. Converting these reading to variances you obtain $10^{-26}$ and $2.25 \times 10^{-26}$ $A^2$, respectively. The numbers imply that the pipette holder contributes more (1.25 times as much) to the variance than the amplifier does.