Single-Channel Analysis

SINGLE-CHANNEL RECORDING--WHAT GOOD IS IT?

Many things that one would like to find out about channels–their selectivity, relative probability of opening, the shape of the current-voltage relationship–can be found out without going to the trouble of single-channel recording and analysis. If you include the technique of fluctuation analysis you could almost say that the recording of single channels is superfluous. In the early days it was interesting to see single-channel events to be certain that the currents were actually rectangular pulses of current, with a well-defined current amplitude and random durations. Now we know that and expect that general sort of behavior of any ionic current.

So what is the advantage of observing the unitary events directly? First, one obtains a direct measure of the single-channel current. This is interesting for theories of permeation. It is also useful because different kinds of channels can be distinguished quite easily by their differing conductances. Secondly, one can obtain some direct information about the way the channel current is switched on and off. No one has yet resolved the time course of turning on and off of a channel, which likely reflects the fact that when something happens in a system the size of a single protein molecule, it happens on a time scale of picoseconds or perhaps as slow as a few nanoseconds. These times are far below the ~10µs resolution of present recording technology. However, the dwell times between openings of a channel, and the dwell times of a channel in its open state, are interesting. For example, one can apply a channel-blocking drug to a membrane and watch little interruptions in the current appear on the oscilloscope screen. It is also useful to analyze those interruptions, because if you assume they represent the direct action of the channel blocker, they tell you immediately the association and dissociation rates of the blocking molecule. It will not do, however, to look at only a few events, because the events are random in duration, reflecting the random nature of molecular motion and diffusion. You have to use statistics on large numbers of events if you want to make quantitative kinetic generalizations from the behavior of a single channel.

FILTERING

In a previous lecture we saw that the measured size of random noise depends on the time scale (or equivalently, the bandwidth) of the measuring system. Some filtering of the current-monitor signal from the patch-clamp amplifier is necessary in order to be able to recognize single-channel events in the presence of background noise. The process of filtering is perhaps best described in terms of a digital filter. A typical digital filter is a computer program that forms output values y_i from input values x_i by forming a weighted sum as diagrammed below.
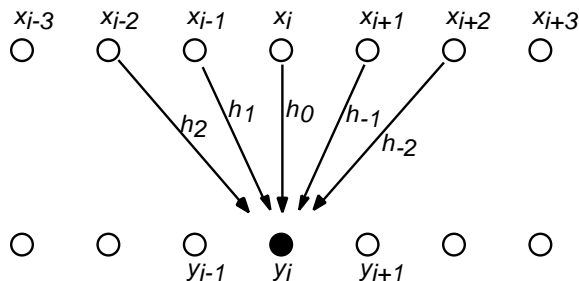
We can write the operation in this way,

$$y_i = \sum_{j=-n}^{n} h_j x_{i-j} \qquad (1)$$

where the h_j sum to unity. The set of coefficients h_i uniquely determine the response characteristic of such a filter. If h_0 and its neighboring coefficients are relatively large, while the more distant coefficients vanish, the filtering will be relatively light, because in effect relatively few input points are averaged to produce each output point. If the h_j are zero for all negative values of j, the filter is said to be <u>causal</u>, because it does not make use of future x values to compute the present y value; any filter that operates in real time must be causal, but of course a digital filter operating on stored data need not be causal.

Gaussian Filter

A useful type of filter for single-channel analysis is the Gaussian filter. It can be realized by a digital filter, and it is also very similar to the Bessel analog filters that are used in practical recording. Because of the close similarity, analytical results that we will obtain here will also be applicable to Bessel filters. The coefficients of the Gaussian filter are values of a Gaussian function of time; if we consider continuous time rather than discrete time points, we can write the function $h(t)$ corresponding to the coefficients as

$$h(t) = \frac{1}{\sqrt{2\pi}\,\sigma_g} \exp\left(\frac{-t^2}{2\sigma_g^2}\right), \qquad (2)$$

where σ_g is the "standard deviation" of this Gaussian function in time. The frequency response of this filter can be calculated by applying a sinusoidal input function of frequency f and asking, what is the amplitude $B(f)$ of the sinusoidal output. Here we use the continuous-time analog of eqn. (1):

$$B(f)\cos(2\pi ft) = \int_{-\infty}^{\infty} \cos[2\pi f(t-\tau)]h(\tau)\,d\tau$$

Evaluation of the integral yields

$$B(f) = e^{-kf^2}$$

with

$$k = 2\pi^2 \sigma_g^2.$$

The Gaussian function has the remarkable property that its Fourier transform is also a Gaussian function. This is reflected here that the filter's frequency response is a Gaussian function of frequency. For comparison with more conventional filters, one is typically interesed in the "corner" or "cutoff" frequency of the filter, traditionally defined as the frequency f_c where

$$\left|B(f_c)\right|^2 = 1/2.$$

In this case, the width parameter σ_g is then found to be

$$\sigma_g = \frac{\sqrt{\ln 2}}{2 \pi f_c}. \tag{3}$$

Using this value for σ_g along with eqn. (2) we can derive various properties of the filter. For example, its response to a unit step is given by the function

$$H(t) = \frac{1}{2}\left[1 + \text{erf}\left(\frac{t}{\sqrt{2}\,\sigma_g}\right)\right]. \tag{4}$$
$$= \frac{1}{2}\left[1 + \text{erf}\left(5.336 f_c t\right)\right]$$

A particularly useful description of a filter is its risetime. This is traditionally measured as the time it takes the step response of the filter to go from 10% to 90% of the full amplitude of the input step. An alternative definition of the risetime T_r is the inverse of the maximum slope of the step response,

$$T_r^{-1} = \max\left(\frac{dH(t)}{dt}\right)$$

and represents the time for the step transition in the case that the slope remained at its maximum value during the entire transition. For a Gaussian filter, these two definitions of the risetime give nearly identical values of

$$T_r \approx 0.33 / f_c.$$

Thus the risetime is about $1/3$ of the period of a cycle at the frequency f_c. Given the risetime it is easy to calculate things like the duration of the shortest event that will cross a threshold, etc.

Of special interest for single-channel analysis is the property that the frequency response of two Gaussian filters in cascade is itself Gaussian, with the effective corner frequency f_c given by

$$\frac{1}{f_c^2} = \frac{1}{f_1^2} + \frac{1}{f_2^2} \tag{5}$$

where f_1 and f_2 are the corner frequencies of the two filters. This property allows repeated filtering to be done on the signal with a predictable result.

THRESHOLD ANALYSIS OF CHANNEL EVENTS

To characterize single-channel open and closed times, the simplest technique is simply to set a threshold value halfway between the closed and open channel amplitudes, and count all times spent above the threshold as open times, and all those below the threshold as closed times. We know, from consideration of the filter risetime, that the shortest events that will cross the threshold will be about $T_r/2$ in duration. Thus it is best to use the shortest risetime (highest corner frequency) possible for good time resolution. We also know, however, that there is a finite probability of background noise signals becoming large enough to cross the threshold. It turns out that the average frequency of these false events depends on the standard deviation σ_n of the background noise according to

$$\sigma_f = k_s f_c \exp\left(-\frac{\Phi^2}{2\sigma_n^2}\right) \tag{6}$$

where the factor k_s depends on the filter response characteristic and the form of the spectrum of the background noise, but for our purposes can be taken to be equal to 1. If $f_c = 1\text{kHz}$ and σ_n is $1/5$ of the threshold value, σ_f is only about 0.003 s^{-1}; however, if σ_n is slightly larger, $1/4$ of the threshold value, the false event rate is much greater, about 0.3 s^{-1}.

THE MEANING OF RATE CONSTANTS

We turn now to the mechanistic interpretation of channel open and closed dwell times. In this I will follow the traditional Markov-process framework of thinking about channel kinetics. There are popular theories involving 'chaotic' or 'fractal' behavoir in channels, but the relationship between these theories and the detailed behavoir of proteins is, if not more difficult to understand, at least much less concrete than the Markov view.

When we make a kinetic scheme, where a closed and open state are connected by rates α and β,

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O \tag{7}$$

what we are really imagining is this: a molecular entity (like a channel) has two fairly well defined and stable conformations, and once it is in one of these 'states', it stays there for a *very* long time. Considering that the main modes of molecular vibrations have frequencies of about 10^{13} Hz, the fact that a channel molecule might stay in an open state for a whole millisecond is quite remarkable. It says that the open state is very stable, and the chance that a particular vibration or collision can push it out of that state is only about 10^{-10}. It is clearly going to be unpredictable exactly when the particular constellation of unlikely events will come together to force the transition out of that state. Instead about the best we can do is to define a number

$$\alpha = \lim \frac{\text{Prob}\{O \to C \text{ during } (t, t+\Delta t) \mid O \text{ at } t\}}{\Delta t} \tag{8}$$

where we take the limit as Δt becomes very small (but still larger, say, than 10^{-13} seconds, if we wish to keep physical reality in view). This is the definition of a rate constant: it is the probability per unit time of a transition occurring.

Now we can write down an equation for the probability F' of a channel being found in the open state from time zero up to t. It is

$$\frac{dF'(t)}{dt} = -\frac{\text{Prob}\{O \text{ at } t \text{ and } O \to C \text{ during } (t, t+dt)\}}{dt}$$

The right-hand side is closely related to eqn. (8): all that is lacking is the condition that the channel is in state O at time t. We can take care of that by multiplying by the open probability F' and obtain

$$\frac{dF'}{dt} = -\alpha F'. \tag{9}$$

Supposing that we start out knowing that the channel was in O at time zero, we can write that the probability that the channel stayed open continuously from 0 to t is given by

$$F'(t) = e^{-\lambda t}. \qquad\qquad (10)$$

This is sometimes called the <u>survivor function</u>. It tells what fraction of the channels 'survive' in the open state after a time t.

HISTOGRAMS AND THE PDF

In collecting together experimental data we typically form histograms. We measure the durations of many events and collect them into bins. For example we count how many open times fell in the range 0..1 ms, how many into 1..2 ms and so on. If we measured a total of N events we would expect that the number n_1 of events falling into the 0..1ms bin would be

$$n_1 = N [F'(0) - F'(1\text{ms})]$$

If our bins have a width w that is quite narrow, we could approximate the number n_b of entries in a bin by

$$n_b \approx Nw \left. -\frac{dF}{dt} \right|_{t=t_b}$$

where t_b is the time corresponding to the center of the bin. The function $-dF'(t)/dt$ has a name. It is called the <u>probability density function</u>,

$$f(t) = -\frac{dF'(t)}{dt}$$

For the special case considered in eqn. (10) we have

$$f(t) = \lambda\, e^{-\lambda t}.$$

This is another decaying exponential function. Its initial amplitude is λ but it has unity area.

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