

Density Estimation and the Analysis of Ion Channel Recordings

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Abstract

The patch clamp recording technique measures current flowing through single ion channels in cell membranes and produces very long time series which must be analyzed statistically. We present background information on ion channels and the nature of the measurements and then discuss some of our attempts to use density estimation methods to produce informative statistical summaries.

1 Introduction

Ion channels are pores in cell membranes formed by transmembrane proteins. In certain conformational states of the protein the pore opens, allowing ionic current to pass across the membrane. There is a large variety of ion channels whose inter-relationships shape the electrical signals of the neuromuscular system. In addition to being fundamentally important to the operation of the neuromuscular system, the involvement of ion channels in various neuromuscular disorders provides additional motivation for studying their structure and function. Hille (1992) provides a broad overview of the current state of knowledge.

In patch clamp recording, a micropipette is placed against a cell membrane and suction is applied to form a high resistance seal. Current flowing through ion channels in the tip of the pipette can be recorded. The diameter of the pipette is typically of the order of one micrometer whereas the diameter of a channel is of the order 100 Angstroms. The development of this ingenious methodology, referred to as single-channel patch clamp recording, earned B. Sakmann and E. Neher the Nobel Prize in Medicine and Physiology.

Such recordings reveal that the current flowing through a single channel is on the order of picoamperes and that channels randomly alternate between being open and closed on a time scale of milliseconds. The recordings are subject to noise and degradation due to such causes as noise in the electronics, leakage around the seal, baseline drift, lowpass filtering, and finite bandwidth of the recording apparatus. A good experiment can last several minutes and with a typical sampling interval of 100 microseconds very large quantities of data are produced. Various aspects of patch clamp recording are discussed in Sakmann and Neher (1983).

We have been involved in analyzing data gathered in patch-clamp recordings from NMDA receptors in cells in rat hippocampal slices in the laboratory of Professor David Colquhoun of University College, London (Howe et al., 1991). The NMDA receptor is an ion channel in the central nervous system that is activated by glutamate: Glutamate binding enables channel opening. The NMDA receptor is believed to play an important role in learning.

For illustrative purposes, we will consider in this paper 25 seconds of a single experiment, sampled with an interval of 50 μ sec, thus yielding 500,000 points. Figure 1 shows two short segments of the recording, each of length 100 milliseconds (ms). Current is measured in picoamps (pA) and negative

current corresponds to ionic flow across the membrane. From the figure we see that the channel alternates between being open and closed and that when it is open it tends to remain open at a constant conductance level before closing or making a transition to another conductance level. We note that the recording also contains long stretches of time during which the channel is closed, so that the segments in figure 1 should not be viewed as typical 100 ms snapshots of the record. Rather, we chose these segments to exhibit that there are a number of conductance levels.

Figure 1 about here

In the following sections of this paper we demonstrate how nonparametric smoothing techniques are useful in elucidating the structure of the conductance levels and the kinetics of the channel in this recording.

2 Identifying Conductance Bands with the Persistence Function

We first consider the problem of identifying conductance levels. Although many channels have only one conductance level, it is apparent from figure 1, and it is well-known, that the NMDA receptor exhibits a much more complex pattern of activity. Initially, under the assumption that there was a well-defined, discrete collection of conductance levels, we employed methodology based on hidden-Markov models (Fredkin and Rice, 1992) to identify those levels. After considerable experimentation, trying more and more levels, it became quite clear that the situation was not this simple: there appeared to be a continuum of conductance levels. This is probably partly due to the fact that in the recording we are seeing the activity of many channels. The patch probably encloses a large number of channels; during the course of the experiment, glutamate binding and dissociation from one receptor produces an activation, followed by an activation from a different receptor etc. Since the concentration of glutamate is deliberately quite low, it is rare to see more than one channel open simultaneously. Even if these receptors are identical proteins, they may well be located differently in the cell membrane, causing variation in conductance levels. As will be shown below, it is more accurate to

describe conductance exhibited in this recording in terms of “bands” rather than in terms of a small, discrete collection of levels.

It is thus desirable to develop a descriptive measure that identifies conductance bands, if they exist, and that also describes how long a channel tends to remain open in each band. The estimated one dimensional marginal density of the process, figure 2, fails to resolve distinct bands and is in any case incapable of providing information about characteristic time durations.

Figure 2 about here

We have, however, found collections of conditional probability density estimate useful for these purposes. If X_t is a stationary process, with marginal density $f(x)$ and joint density of X_0, X_t denoted by $f_t(u, v)$, we define the “persistence function”:

$$\begin{aligned}\phi(x, t) &= \frac{f_t(x, x)}{f(x)} \\ &= f_t(x|x).\end{aligned}$$

The idea is simply that if the value x is observed merely because of instrumental noise, which has a much shorter correlation time than the physical and chemical processes of interest, the persistence function will be small for small t , while if x reflects a genuine state of the channel the persistence function at small t will be large and will decay slowly.

We estimate the persistence function by estimating the corresponding joint and marginal densities. Rather than use a kernel method directly, it is much faster to bin the data on a fine grid and then smooth the bin counts (Scott, 1992). Figures 3 and 4 show estimates of the persistence function formed in this fashion. The initial grid was 80×80 , and a kernel $w(x) \propto (1 - x^2)^2$ with bandwidth 0.3 pA was used to smooth the counts. This bandwidth was chosen interactively to provide a moderate amount of smoothing without obscuring detail. Each computation took about two minutes on an IBM RS-6000.

Figures 3 and 4 go about here

Figure 3 shows the persistence function for short times, out to eight ms and figure 4 shows the function out to 50 ms. The ridge at 0 pA corresponds

to the channel being closed. The figures reveal the existence of conductance bands at -1.0, -1.8, -2.3, -2.9 and -3.7 pA, with the longest lived of these bands at -3.7 pA. Openings at -1.0 pA are comparatively short lived.

3 Measures of Time Dependence

The kinetics of conformational change of the channel protein are reflected in time dependence in the patch clamp recording. Figure 4 shows that the persistence of the longest lived conductance band is of the order of 25 ms. However, this measure of time dependence does not take into account transitions between different conductance bands. In this section we describe some attempts to use smoothing techniques to develop alternative measures of time dependence.

Figure 5 shows the estimated autocorrelation function of the series—the standard measure of dependence. According to this measure also, the time scale of the kinetics is of the order of 25 ms.

Figure 5 goes about here

Since the covariance function is such a simple and crude measure of dependence, it is natural to ask if more detailed information can be obtained. We thus consider decomposing the covariance function:

$$\gamma(t) = E[(X_0 - \mu)(X_t - \mu)] = E[(X_0 - \mu)E[(X_t - \mu)|X_0]]$$

The hope is that $E[(X_t - \mu)|X_0]$ as a function of both X_0 and t will reveal more detailed aspects of the kinetics than will the covariance function. It might be the case, for example, that when the channel is at some conductance levels it relaxes more slowly to equilibrium than at others. Such information might be lost in the covariance function, which averages over all initial values x_0 , including those corresponding to the very probable closed channel.

Figure 6 shows the estimate of $E[(X_t - \mu)|X_0]$ (the figure actually shows the negative of this function). It was obtained by estimating the conditional density $f(x_t|x_0)$ as discussed in the previous section. Ridges corresponding to conductance levels are perceptible, but not as clear as in the persistence function, and there is no striking inhomogeneity in the decay of dependence. (Calculations over larger ranges of t reveal little of additional interest).

Figure 6 goes about here

Since this time series is highly non-Gaussian, it might be hoped that measures of dependence other than the covariance function would be useful in revealing kinetic behavior. We have estimated information-theoretic measures of dependency (Cover and Thomas, 1991), in particular, the mutual information between X_0 and X_t :

$$\mathcal{I}(t) = \int \int f(x_0, x_t) \log \frac{f(x_0, x_t)}{f(x_0)f(x_t)} dx_t dx_0$$

by estimating the joint and marginal densities. (We note that Robinson (1991) proposed a test of independence in a time series context based on estimating $\mathcal{I}(t)$ nonparametrically.)

$\mathcal{I}(t)$ measures the decay to equilibrium of the joint density of X_0 and X_t . Figure 7 shows two estimates of $\mathcal{I}(t)$, both obtained from histogram estimates of the densities, one with 20×20 bins and one with 40×40 bins.

Figure 7 goes about here

It is initially striking that the curve based on 40 bins lies above that based on 20 bins. Upon reflection, it is clear that such an estimate of $\mathcal{I}(t)$ is biased. Our estimate is essentially the value of a likelihood ratio test statistic for testing independence in a two-way table and its expected value under independence (large t in our case) depends upon the number of degrees of freedom in the table (the number of bins in the histogram in our case). In a standard test for independence in an $m \times n$ table, the expected value of a chi-squared statistic under the hypothesis of independence is $(m - 1)(n - 1)$. However, closer examination of the results reveals that not all the bias is explainable in this way—the apparent limiting values are greater than suggested by this degrees of freedom analogy. Dependence in the series, which does not occur in the multinomial analogue, contributes to the limiting value of the estimate for large t (we thank Peter Bickel for this observation). At any rate, the time scale of dependence shown in Figure 7 is essentially that shown by the covariance function, so the results do not justify the additional complications of density estimation.

Finally, we considered a decomposition of $\mathcal{I}(t)$ similar to that of the covariance function:

$$\mathcal{I}(t) = \int \int f(x_0, x_t) \log \frac{f(x_0, x_t)}{f(x_0)f(x_t)} dx_t dx_0$$

$$= \int f(x_0) \int f(x_t|x_0) \log \frac{f(x_t|x_0)}{f(x_t)} dx_t dx_0$$

which suggests that we estimate

$$\mathcal{D}(x_0, t) = \int f(x_t|x_0) \log \frac{f(x_t|x_0)}{f(x_t)} dx_t.$$

This statistic, the Kullback Leibler distance or relative entropy between $f(x_t|x_0)$ and $f(x_t)$, measures how fast the process decays to equilibrium from an initial value x_0 . Figure 8 shows an estimate based on a kernel smoothing of a finely binned histogram. Its resemblance to Figure 6 is remarkable and disappointing. At least in this context, information-theoretic measures of dependence have shown us nothing more than have the simpler measures.

Figure 8 goes about here

4 Concluding Remarks

We have shown how density estimation is a useful tool in revealing patterns that occur in patch clamp recordings. The persistence function has been particularly useful in analyzing recordings from the NMDA receptor. As will be described elsewhere, we have used it to study how conductance patterns change as experimentally controllable conditions such as voltage are varied.

Effective analyses of very long non-Gaussian time series, such as those produced by patch clamp recordings, must go beyond estimation of covariance functions and power spectra. Study of the joint density of $\mathbf{x} = (x_0, x_t)$ both as a function of \mathbf{x} and t is one path. Rosenblatt (1970), Robinson (1983) and others have studied the statistical properties of density estimates from stationary time series. Although we have not done so, it seems to us that, ideally, estimates of such joint densities should take advantage of smoothness in both \mathbf{x} and t .

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Figure Captions

Figure 1 Two segments of a patch clamp recording.

Figure 2 The marginal density function, estimated from the recording by smoothing a finely binned histogram with a kernel $k(x) \propto (1 - x^2)^2$ with bandwidth .3pA.

Figure 3 Estimated persistence function for times up to 8 ms.

Figure 4 Estimated persistence function for times up to 50 ms.

Figure 5 Autocorrelation function.

Figure 6 Estimate of $-E((X_t - \mu)|X_0)$; the current axis corresponds to X_0 .

Figure 7 Estimated mutual information, $\mathcal{I}(t)$, based on histograms with 20×20 bins (solid curve) and 40×40 bins (dashed curve).

Figure 8 Estimated Kullback-Leibler distance, $\mathcal{D}(x_0, t)$, between the conditional, $f(x_t|x_0)$, and marginal, $f(x_t)$, densities as a function of x_0 (current) and time.















