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STATISTICAL ANALYSIS OF MULTIPLE ION CHANNEL DATA[1]

BY ANDRÉ ROBERT DABROWSKI AND DAVID MCDONALD

University of Ottawa

Biological cell membranes contain molecular structures known as ion channels. The electrical activity of these channels regulates a number of cellular functions, such as heartbeat and neural transmission. Often the experimenter can only observe the combined current conducted through a cell patch containing an unknown number of ion channels, each channel of equal conductance. The observed current record is a step function with unit up or down steps occurring at random times. Based on such data, we obtain a goodness-of-fit test for the hypothesis that the channels in a portion of cellular membrane are operating independently, and that K types (K small) of channels exist in the membrane. If the hypothesis is accepted, estimates for the mean behaviour of each type of channel in the experiment are obtained. These techniques are applied to simulated and biological data.

1. Introduction.

Background. The outer membranes of biological cells frequently contain molecular structures known as ion channels. In response to specific stimuli, these complex structures can permit certain ions to cross the cell membrane. The current conducted by these ion channels governs a number of biological functions, such as heartbeat and neural transmission. These currents can be observed by the experimeter, and can be used to develop hypotheses about the molecular structure of the ion channels. Because of their importance in many fields of biology, these ion channels have been the object of considerable research [cf. Hille (1984)].

An ion channel may assume one of several configurations in response to external stimuli. In some configurations the channel will transport ions across the cell membrane; in other configurations it will not. We will assume that each ion channel has two observable groups of configurations: open and closed. We say that a channel is open when it is conducting current, and closed when it is not. This difference in current level is a fixed constant determined by the structure of the channel and external conditions (such as voltage across the membrane), and it is readily discernable in practice. For simplicity, we will take the nonconducting level to be 0 current, and the conducting level as 1 unit of current. The precise manner by which the channel opens and closes is called the gating mechanism. A suitable stimulus for opening a channel may be a chemical agonist for drug-activated channels or a physical stress for stretch-

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activated channels. Unfortunately, ion channels are extremely small—small mounds when viewed by an electron microscope—and their activity cannot be studied directly. We may study the gating mechanism indirectly through the character of the current produced by a channel over a long period of time. The recorded current will alternate between 0 (a closed state) and 1 (an open state). A large number of papers dealing with the analysis and interpretation of such current records have appeared in scientific journals [e.g., Sakmann and Neher (1983)].

A widely accepted mathematical model for the gating mechanism is that of Colquhoun and Hawkes (1983) [see also Sansom, Ball, Kerry, McGee, Ramsey and Usherwood (1989)], who represent the conformational state of a single ion channel as a finite state-space continuous-time Markov chain. In this model the channel (Markov chain) shifts among a finite number of states. Some of these states are open (i.e., conduct 1 unit of current) and the rest are closed (i.e., 0 current observed). While in an open state, the channel emits the unit of current detectable by the experimenter, but it is impossible to distinguish one open state from another open state on the basis of this current. The same is true of the closed states. This reduced view of a Markov process is known as an aggregate Markov process. If the complete record of a single channel is recorded, an analysis of its gating mechanism may be possible, although some models are not identifiable—see Fredkin, Rice and Montal (1985), Fredkin and Rice (1986) and Kienker (1989). For identifiable models, the constants describing the Markov chain can be usefully estimated by decomposing the observed sojourn distributions into their exponential components. Examples of this type of analysis are the papers of Colquhoun and Hawkes (1982), Neher, Sakmann and Steinbach (1978) and Auerbach and Sachs (1983). These authors discuss the application of Markov chain techniques to the case where a single channel is isolated, and its current record is obtained.

In many cases channels cannot be isolated and can only be observed in small random groups found on cell patches. The researcher cannot obtain the current record of each channel individually, but can record the total current across the cell patch. That is, one can only observe the sum, $X(t)$, of the individual current records from an unknown number of channels. The activity of one channel will interfere (censor) the observations of a different channel. For example, if the current record is initially at 4, rises to 5, and returns to 4 one second later, we do not know whether a single channel opened for 1 second, or whether one channel opened, and a different channel closed 1 second later. Moreover, the behaviour of an ion channel in isolation may be different from its behaviour as a member of a group. For instance, if one is interested in whether ion channels cooperate, it is the joint behaviour of ion channels which is of interest.

The analysis of the general multiple channel current record is difficult since one is no longer certain of observing the full lifetime of a single open period of a single channel. One route to the analysis of the combined current record from such cell patches is to assume the channels are statistically independent and identically distributed, and that the stimulation applied to the membrane is so slight that only one channel will be stimulated into activity. Modified versions of the Markov chain techniques will then yield useful information—see Colquhoun and Hawkes (1990) and the experimental studies quoted by Jackson (1985). Even if only one channel is open at a given time, but there are several active channels in the patch, the analysis is not as straightforward as if there were only one active channel in the patch. Moreover, under even moderate stimulation several channels can be opened simultaneously. Further, this excludes the study of strongly stimulated ion channels or the dependence between channels. To study the dependence of one channel on another or, on the contrary, to establish that the channels are in fact acting independently, and to obtain results at higher levels of stimulation, we must study the current record produced by several channels operating simultaneously. This paper addresses data containing simultaneous openings of several ion channels and provides new techniques for their analyses.

Methods. A principle technique in multiple channel data has been to construct the dwell-time histogram, a histogram of the durations of occupation times of the current record at the different current levels [see Kirber, Singer, Walsh, Fuller and Peura (1985)]. Here we will use a simpler version of this idea described in Glasbey and Martin (1988), the histogram of proportions of time spent at each current level. To distinguish this object from the dwell-time histogram, we shall call it the occupation-time histogram of the current record. For example, after observing the current record for a long time, we may have that the proportions of time spent at current levels 0, 1, 2, 3 and 4 by $X(t)$ are 0.01, 0.05, 0.19, 0.40 and 0.35. If the channels are identical and independent, this histogram should resemble a binomial probability density function, $f(x\colon c, p) = \binom{c}{p} p^x (1 - p)^{c-x}$. Here c is the number of channels in the cell patch, and p is the stationary probability that a single channel is open. The current level is x, an integer between 0 and c. We may also interpret p as the ratio of the mean open time to the mean cycle time for a single channel. The mean open time is the expected length of an open period and the mean closed time is the expected length of a closed period for a single channel. The mean cycle time is the sum of these two values. By fitting a binomial density to the observed occupation-time histogram, one can estimate the number of channels in the patch and the probability that a single channel is open. For the example at the start of this paragraph, we might choose $c = 4$ and $p = 0.75$.

A number of authors have examined multiple channel data by essentially Markovian methods and variations on the above ideas [e.g., Dionne and Leibowitz (1982), Glasbey and Martin (1988), Horn and Lange (1983), Jackson (1985), Sine and Steinbach (1986), Yeo, Milne, Edeson and Madsen (1989) and Yeramian, Trautmann and Claverie (1986)]. We refer the reader to these papers for further references and examples. These analyses and others make qualitative judgements as to the significance of the discrepancy between the observed histogram and the ideal hypothesized form. For example, we may believe that a fit of the proportions stated previously by a binomial density with $c = 4$ and $p = 0.75$ is quite good. Is the discrepancy large or small? By concentrating on the renewal structure of the data, Dabrowski, McDonald and Rösler (1990) addressed the question whether or not this discrepancy was statistically significant.

Dabrowski, McDonald and Rösler (1990) developed a goodness-of-fit hypothesis test based upon the observed multichannel current record for the model of an unknown number of independent and identically distributed ion channels in a cell membrane. This test compares the binomial histogram for independent and identical channels to the observed occupation-time histogram. In a manner similar to that used in the well-known Hotelling T^2 statistic, a distance between histograms is computed from the data. If the distance is small compared to an upper percentile of a χ^2 distribution, the model is accepted, and the number of channels in the patch, the average open and closed periods for a single channel and the corresponding variances are estimated from the multiple channel data. The distribution functions of an open period and a closed period of a single channel can also be estimated. If the distance is large, the model of independent and identical channels is rejected.

The model of independent and identical ion channels is too restrictive. Although the independence hypothesis may be true, it has become clear that the ion channels in a cell patch may operate at different rates. The inner structure of an ion channel determines which ions may pass through the channel, and also the conductance of that channel. On the other hand, the gating mechanism seems to be part of the surface structure of the ion channel [see Hille (1984), Chapter 14]. It is quite possible that channels with the same conductance may have different gating mechanisms. For instance, stretch-activated channels may be activated by "anchors" attaching the channel to the outer cell membrane, and the sensitivity of the channel to pressure may depend on how many such anchors are attached to it—see Sachs (1990). Thus different channels will be independent, but will not be identical. Rather, the channels will form distinct independent groups corresponding to how many anchors are attached to the channel. In such cases, the test of Dabrowski, McDonald and Rösler (1990) will reject the hypothesis of independent and identically distributed channels, and no information on the behaviour of individual channels would be obtained.

In this paper we expand the hypothesis test of Dabrowski, McDonald and Rösler (1990) by including transitions between current levels as well as the durations of visits to those levels. The basic structure of the test is the same, and is easily modified to produce statistical tests for a variety of null hypotheses. For example, we can test the null hypothesis that the patch contains at most two independent but different groups of independent ion channels against the alternatives that these channels are dependent or more than two distinct groups of channels exist on the cell patch. If we conclude that all the channels are in fact independent and each channel belongs to one of K types of channels, point estimates are obtained for the number of channels, c^k, of type k, $k = 1, \dots, K$, in the cell patch, the ratio of mean open period to mean cycle length, p^k. Under an additional, but biologically reasonable, assumption on the parameter space, we also have consistent estimates of the mean open and closed periods, μ_F^k and μ_G^k, for each type of channel. In addition to these hypothesis tests, we develop techniques to examine the stationarity assumptions critical to all of the above results.

Guide to the paper. Section 2 starts with a simple illustration of the problem using simulated data. Section 2.2 states the model we will use, our estimates of c^k, p^k, μ_F^k and μ_G^k and our tests of hypotheses. We present tests on the independence of the channels, the first-order stationarity of the current record and the second-order stationarity of the record. We also have confidence intervals for the lag covariances of the current process, $X(t)$. It is also important to know the limits of these statistical procedures. Section 2.3 looks at an extreme case in the alternative where the independent ion channels have the same ratio p, have different mean open and closed times, but where the mean open and closed times for one channel are the same constant multiple of the mean open and closed times of another channel. Our test has no power against such (in practice, unlikely) alternatives.

Our techniques are applied to the simulated data in Section 3. Section 3 introduces data obtained on the stretch-activated potassium channels of *Lymnaea stagnalis* (pond snails). One such record is analyzed in detail. Our estimates of the mean open time obtained from a cell patch ignore the effect of time interval omission—the loss of data because events occurring within a short bandwidth cannot be resolved. Several authors have attacked this problem from the Markovian approach—see Roux and Sauvé (1985), Ball and Sansom (1988), Ball, Milne and Yeo (1991) and Hawkes, Jalali and Colquhoun (1990). Another analysis of this problem is presented in Dabrowski and McDonald (1990). The method used there was to artificially increase the bandwidth and to recalculate our estimates at these elevated bandwidths. The different estimates are then extrapolated back to 0 bandwidth. We have not attempted that additional analysis for this paper. In Section 4 we present the theorems and proofs of our procedures. We extend the methods of Dabrowski, McDonald and Rösler (1990) by considering jumps between states in addition to sojourns in a state.

2. Structure of the model and fundamental results.

2.1. *Illustrations.* Figure 1 illustrates simulations of short sections of typical observations (after filtering out background noise) of the type that interest us. Figure 1(a) arises from six channels operating independently and identically. Figure 1(b) arises from six channels of the same type, but where channel 2 is simply channel 1 delayed by 0.002 seconds. Figure 1(c) is generated by six independent channels of the same mean open time, but where the mean closed time of two channels is one-half of the mean closed time of the remaining four channels. Figure 1(d) concerns data from a patch clamp experiment on pond snails, *Lymnaea stagnalis*.

Our hypothesis test is applied to these four sets of data in Section 3. Here we just note that the hypothesis of independent identical channels is rejected

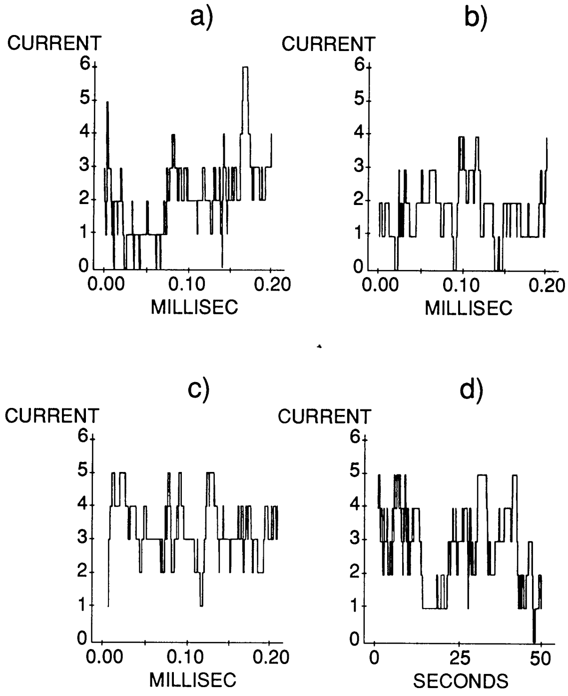

FIG. 1. (a) *A* 0.2-*second section of a simulated current record*, $X(t)$, *generated by six independent and identical channels is depicted. Each channel is an alternating renewal process with mean on time* 0.01 *seconds, and mean off time* 0.02 *seconds.* (b) *A* 0.2-*second section of a simulated current record*, $X(t)$, *generated by six channels is depicted. Each channel is an alternating renewal process with mean on time* 0.01 *seconds, and mean off time* 0.02 *seconds. Five of the channels operate independently. The remaining channel is exactly the first channel delayed by* 0.002 *seconds.* (c) *A* 0.2 *second-section of a simulated current record*, $X(t)$, *generated by six independent channels is depicted. Four channels are alternating renewal processes with mean on time* 0.01 *seconds, and mean off time* 0.02 *seconds. Two channels are alternating renewal processes with mean on time* 0.2 *seconds, and mean off time* 0.1 *seconds.* (d) *A* 50-*millisecond segment is presented of the current record*, $X(t)$, *taken from a cell patch of* Lymnaea stagnalis *heart tissue.*

for the data of Figures 1(b) and 1(c), but not for the data of Figure 1(a). The hypothesis test for two distinct groups of independent and identical ion channels is rejected for the second set of data, and accepted for the third set.

2.2. *Hypothesis tests.* This section contains three further divisions. The first describes our model and notation for ion channels in detail, the second presents hypothesis tests for the independence of channels and associated estimators, and the last subsection discusses hypothesis tests for stationarity of the data record.

Notation. Our model consists of a cell patch which contains c independent channels. Each channel is modelled as a finite state-space semi-Markov process. We will follow a notation similar to that found in Colquhoun and Hawkes (1983) and Dabrowski, McDonald and Rösler (1990). See also Ball, Milne and Yeo (1991) for an analysis of the embedded process in a semi-Markov model. The ith channel has state-space $\mathscr{S}^i$ and transition function $\mathscr{P}^i_{xy}$ (i.e., the probability the embedded chain makes a transition from x to y for $x, y \in \mathscr{S}^i$). Let π^i denote the stationary probability measure of this embedded chain. The subset of open (closed) states is denoted by $\mathscr{A}^i$ ($\mathscr{B}^i$). We now define a second embedded process (which we will call the $\mathscr{A}^i\mathscr{B}^i$ process) which models the time of entry into $\mathscr{A}^i$ or $\mathscr{B}^i$. Given that this $\mathscr{A}^i\mathscr{B}^i$ process is in $\mathscr{A}^i$ ($\mathscr{B}^i$), the probability it is in $a \in \mathscr{A}^i$ ($\mathscr{B}^i$) is

$$\rho^i(a) = \frac{\sum_{b \in \mathscr{B}^i} \pi^i(b) \mathscr{P}^i_{ba}}{\sum_{a \in \mathscr{A}^i} \sum_{b \in \mathscr{B}^i} \pi^i(b) \mathscr{P}^i_{ba}} \qquad \left(\delta^i(a) = \frac{\sum_{a \in \mathscr{A}^i} \pi^i(a) \mathscr{P}^i_{ab}}{\sum_{a \in \mathscr{A}^i} \sum_{b \in \mathscr{B}^i} \pi^i(a) \mathscr{P}^i_{ab}} \right).$$

Let θ^i_a denote the density of the time elapsed from an arrival (from $\mathscr{B}^i$) of the chain at $a \in \mathscr{A}^i$ until the chain hits $\mathscr{B}^i$ again. The mean of θ^i_a is μ^i_a. Similarly, let θ^i_b denote the density of the time elapsed from an arrival (from $\mathscr{A}^i$) of the chain at $b \in \mathscr{B}^i$ until the chain hits $\mathscr{A}^i$ again. The mean of θ^i_b is μ^i_b. Thus the $\mathscr{A}^i\mathscr{B}^i$ process jumps back and forth between $\mathscr{A}^i$ and $\mathscr{B}^i$ with the transition probabilities of the embedded chain and sojourn times with densities θ^i_a, $a \in \mathscr{A}^i$, and θ^i_b, $b \in \mathscr{B}^i$, respectively. The distribution, F^i (respectively, G^i), of a sojourn in $\mathscr{A}^i$ ($\mathscr{B}^i$) has density and mean (respectively)

$$\sum_{a \in \mathscr{A}} \rho^i(a) \theta^i_a \qquad \left(\sum_{b \in \mathscr{B}} \delta^i(b) \theta^i_b \right)$$

and

$$\mu^i_F = \sum_{a \in \mathscr{A}} \rho^i(a) \mu^i_a \qquad \left(\mu^i_G = \sum_{b \in \mathscr{B}} \delta^i(a) \mu^i_b \right).$$

We will require that for some state $\hat{a}^i \in \mathscr{A}^i$, the return time to that state $\hat{a}^i$ has a distribution R^i satisfying Condition 9 in Section 4.1. This smoothness condition is satisfied by finite state-space Markov processes and is reasonable for the biological models we are considering.

The probabilistic analysis of the cell patch current data is based on the following unobservable random processes:

$$X^i(t) = \begin{bmatrix} 1, & \text{if channel } i \text{ is open} \\ 0, & \text{if channel } i \text{ is closed} \end{bmatrix} \quad \text{at time } t,$$

$$\begin{aligned} N^i(t) &= \text{the number of transitions by channel } i \text{ from } \mathscr{A}^i \text{ to } \mathscr{B}^i \text{ in}]0, t] \\ &= \text{the number of times } X^i(t) \text{ stepped down in }]0, t]. \end{aligned}$$

We assume that we can observe the following processes for a long period, $]0, T]$. Since we are dealing with asymptotic results, T must be large compared to the μ^i_F. Define

$$X(t) = \sum_{i=1}^{c} X^i(t) \quad (\text{total current produced by the } c \text{ channels at time } t),$$

$$N(t) = \sum_{i=1}^{c} N^i(t) \quad (\text{number of times the } X(t) \text{ process steps down in}]0, t]).$$

The statistical tests of this paper are based on the following functions of $X(t)$ and $N(t)$. The time interval, $]0,T]$, is broken into equal pieces of length u, $\{](j-1)u, ju],\ j = 1,2,\dots,T/u\}$. For each subinterval define the following variables:

$$M_{sj} = \int_{(j-1)u}^{ju} I[X(t) = s]\,dt \quad \text{for } s \in \{0,1,\dots,c\},$$

which is the occupation time of s by $X(t); t \in](j-1)u, ju]$,

$$M_j = \langle M_{sj} \colon s \in \{0,1,\dots,c\}\rangle,$$

which is the vector of occupation times for $X(t); t \in](j-1)u, ju]$,

$$N_{sj} = \sum_{\mathscr{S}} 1 \quad \text{where } \mathscr{S} = \{t \in](j-1)u, ju],\ X(t) = s,\ N(t) - N(t-) = 1\},$$

which is the number of downsteps from $s; t \in](j-1)u, ju]$, and

$$N_j = \langle N_{sj} \colon s \in \{1,\dots,c\}\rangle,$$

which is the vector of downsteps; $t \in](j-1)u, ju]$.

For notational simplicity we assume that the units of time are such that $u = 1$. The authors have found that choosing u so that on average two downsteps are observed in each subinterval avoids computational difficulties in practice. Note that $X(t)$ is a step-function which takes its values among the integers, $\{0,1,\dots,c\}$, and $N(t)$ is an increasing nonnegative integer-valued process, where $N(t) = 0$. The random vectors M_j and N_j take values in $\mathbb{R}^{c+1}$ and $\mathbb{R}^{c}$, respectively.

Tests for independence of channels. The goodness-of-fit test of Dabrowski, McDonald and Rösler (1990) examines the temporal behaviour of $X(t)$ by looking at the proportions of time that $X(t)$ spends at each current level in $\{0,1,\dots,c\}$. If the channels are independent and identical, the observed occupation-time density will be close to a binomial density. The squared distance of the observed histogram from the best-fitting binomial histogram is compared to an upper $100(1-\alpha)$ percentile of a χ^2 density. This test is easily extended to independent, but possibly different, channels by fitting a density which is the convolution of independent Bernoulli densities to the observed density histogram. We will further expand the scope of this test by also looking at the number of times $X(t)$ steps down (experiences a downward transition) from each current level—for example, from current 6 to current 5. For any stated mixture of independent ion channels, we can compute the mean number of downward steps during $]j-1, j]$. Thus we can compare both the observed occupation-time density, and the observed downstep counts, to the best-fitting constants consistent with our model. If the discrepancy exceeds the upper $100(1-\alpha)$ percentile of a χ^2 density, we reject the model.

The goodness-of-fit test of Dabrowski, McDonald and Rösler (1990) is based on fitting a binomial density (with parameters c and p) to the occupation-time density alone. The mean open time of a single channel was estimated by comparing an estimate of $cp = c\mu_F/(\mu_F + \mu_G)$ to a separate estimate of

$c/(\mu_F + \mu_G)$. This test can be extended to test for at most K distinct types of ion channels. However, no analogue to the separate estimate of $c/(\mu_F + \mu_G)$ is available. Consequently, we cannot estimate the mean open time of a single channel from the occupation-time density and the total number of downsteps. The addition of the downward transitions to the goodness-of-fit test introduces elements which also depend on the values of the mean open times, μ_F^i, rather than only on the ratios, p^i. Thus we are also able to give estimates of these mean open times for each type of channel in the patch.

Let $\mathscr{M} \subseteq \{0, 1, \ldots, c\}$ and $\mathscr{N} \subseteq \{1, 2, \ldots, c\}$ be such that the covariance matrix T of the random vector $\gamma_j = \{\langle M_{kj}, N_{lj}\rangle \colon k \in \mathscr{M},\ l \in \mathscr{N}\}$ is invertible. Define $\mathbb{B}_K$ to be the set of all mean values of γ_j consistent with a model of K distinct groups of channels on the cell patch, where the channels within each group are identical. All the channels operate independently. The set $\mathbb{B}_K$ is described precisely by Theorem 13 and simple calculations. We require that $|\mathscr{M}| + |\mathscr{N}| \geq 3K + 1$. If not, there are enough free parameters in $\mathbb{B}_K$ to fit the observed occupation-time histograms and downstep frequencies exactly. A test of asymptotic level at most α for

H_0: The cell patch consists of K distinct groups
of independent channels of
unit current (thus $\mathbb{E}\gamma_1 \in \mathbb{B}_K$)

H_1: $\mathbb{E}\gamma_1 \notin \mathbb{B}_K$

rejects the null hypothesis when

$$n^{-1} \inf_{e \in \mathbb{B}_k} \left[\sum_{j \leq n} (\gamma_j - e)^T T_n^{-1} \sum_{j \leq n} (\gamma_j - e) \right] > \chi_\alpha^2. \tag{1}$$

The matrix T_n is the estimated covariance matrix as specified in Theorem 12, and χ_α^2 is the $100(1 - \alpha)$ percentile of a χ_ν^2 distribution with $\nu = |\mathscr{M}| + |\mathscr{N}| - 2K$.

Remark 2. One would hope that if we assumed that the channels were indeed independent and that there were at most K distinct types of ion channels, the minimization process inherent in (1) would yield consistent estimates for the number of channels of type k, c^k, in the cell patch, the ratio of the mean open to the mean cycle time, p^k, and the mean sojourn times, μ_F^k and μ_G^k, for each channel of type k, $k = 1, 2, \ldots, K$, in the cell patch. The example of Section 2.3 shows that this is not so. However, if we make the additional assumption that the channel patch does not contain any pairs (i, j) of channels for which $\mu_F^i = C\mu_F^j$ and $\mu_G^i = C\mu_G^j$, $C \in \mathbb{R}^+$, then this minimization process also yields consistent estimates of all our parameters.

Remark 3. We suggest that the test for at most K distinct and independent groups of iid channels be performed by fitting on $3K + 1$ occupation-time and downstep coordinates. If $X(t)$ varies between 0 and d, then $2d + 1$ coordinates are available. Thus we require $K < 2d/3$. As a practical concern, we also want to avoid nearly degenerate T by avoiding rarely visited levels and this will further restrict K. Considerable difficulties will arise from the estimation of the variance of occupations of rare current levels, and the inverse of the estimated covariance matrix containing such a rare level will be quite large for that component. The latter feature will result in our procedure tightly fitting that component at the expense of the other (better-estimated) components. Concern about the invertibility of T also influences our choice of time units. We need that n is large enough for the asymptotic theory to take hold, but if $]j-1, j]$ is too long, then the covariance matrix T_n will be quite close to 0 and will be harder to invert. We take n to be measured in small time units such that with respect to these time units there is a high probability that any unit interval $]j-1, j]$ contains a downstep. This is not necessary for our theory, but in a practical application it avoids near-singular covariance matrices.

REMARK 4. We can rephrase the test (1) as follows. Let Γ_j be the complete vector of occupation times and downstep frequencies for interval j, that is, $\Gamma_j = \{\langle M_{mj}, N_{nj}\rangle\colon m \in \{0,1,\dots,c\} \text{ and } n \in \{1,2,\dots,c\}\}$. Let P be a diagonal $(2c+1)\times(2c+1)$ matrix whose diagonal entries are all either 0 or 1, and such that the first $c+1$ are not all 1. Then (1) tests whether or not $\mathbb{E}(P\Gamma_1)$ belongs to a certain set by using the central limit theorem for dependent random vectors applied to the Γ_j. By requiring that $P\Gamma_1$ have an invertible covariance matrix, we avoid the use of generalized inverses, and we are able to state a test even though we do not know the number of channels in the patch, c. For example, although the patch may contain six channels, the probability that more than three channels open simultaneously may be so low that we do not observe such events in our finite record. In such a case P would be a diagonal matrix with a 1 for the first, second and fifth components, and 0 elsewhere. The $\gamma_j = P\Gamma_j$ are well-defined random vectors in $\mathbb{R}^3$, even though we do not know the size of the Γ_j. Clearly, similar tests can be constructed for more general matrices P, but their utility is not evident.

REMARK 5. If $\mathbb{B}$ is chosen so that it contains all mean vectors consistent with at most c independent, but possibly distinct, ion channels, then (1) yields a test of H_0: the channels are independent versus H_1: $\mathbb{E}\gamma_1 \notin \mathbb{B}$ and an estimate of the parameters in $\mathbb{B}$. However, $\mathbb{B}$ is so rich that we can fit the observed occupation-time histogram and downstep frequencies exactly for most cases. The test then has power against only gross deviations from our model. For example, $X(t)$ may skip level 4 entirely.

Tests for first- and second-order stationarity. In addition to the estimation of the parameters in our model and hypothesis tests on the independence of channels, the structure we develop also allows us to verify some of the assumptions critical to our model. One consequence of our model is strict stationarity of the current record, $X(t)$, and the downstep counts, $N(t)$. We can test for the presence of first- and second-order stationarity. These tests are useful from a practical point of view as well. Some authors have suggested that an ion channel may become "tired" or desensitized after a period of high activity, and become inactive for a protracted interval. Such a change would be perceived as a failure of stationarity by our tests. We also develop a heuristic check on another technical consequence of our methods, fast ϕ-mixing of the current record.

Recall that $X(t)$ is the total current being produced at t, and $N(t)$ represents the total number of times that $X(t)$ has made a downward transition in $]0, t]$. Either choose $Y_j = \int_{j-1}^{j} X(t)\,dt$ or choose $Y_j = N(j) - N(j-1)$ for $j = 1, 2, \dots, \infty$. For $\{Y_j\}$ as chosen, define

$$A = \{(i,j): |i-j| \le k(n),\, i,j \in \{1,2,\dots,n\}\},$$

$$\overline{Y}_n = n^{-1} \sum_{i=1}^{n} Y_i,$$

$$\overline{R}_n^{(\kappa)} = (n-\kappa)^{-1} \sum_{i=1}^{n-\kappa} Y_i Y_{i+\kappa},$$

$$U_n = n^{-1} \sum_{(i,j)\in A} \left(Y_i - \overline{Y}_n\right)\left(Y_j - \overline{Y}_n\right)$$

and

$$V_n^{(\kappa)} = (n-\kappa)^{-1} \sum_{(i,j)\in A} \left(Y_i Y_{i+\kappa} - \overline{R}_n^{(\kappa)}\right)\left(Y_j Y_{j+\kappa} - \overline{R}_n^{(\kappa)}\right).$$

Here $k(n) = o(\log(n))$ is a nondecreasing sequence of integers tending to ∞. Further, let $Z = \sup\{|B_b(t)|: t \in [0,1]\}$ for a standard Brownian bridge B_b, and $P[Z > z_\alpha] = \alpha$. Let Φ_α denote the $100(1-\alpha)$ percentile of a standard normal distribution. Under the assumptions of Theorem 12, we have the following hypothesis tests of (approximate) level α and (approximate) $100(1-\alpha)\%$ confidence intervals:

First-order stationarity:

H_0: $\mathbb{E}Y_1 = \mathbb{E}Y_j$, $j = 2, 3, \dots$.

(6) H_a: Expected values are not constant.

Reject the null hypothesis if $\sup\{|(nU_n)^{-1/2}(\sum_{i=1}^{r} Y_i - r\overline{Y}_n)|: r = 1, 2, \dots, n\} > z_\alpha$.

Second-order stationarity:

H_0: $\mathbb{E}Y_1 Y_{1+\kappa} = \mathbb{E}Y_j Y_{j+\kappa}$, $j = 2, 3, \dots$.

(7) H_a: Expected values are not constant.

Reject the null hypothesis if $\sup\{|(nV_n^{(\kappa)})^{-1/2}(\sum_{i=1}^{r} Y_i Y_{i+\kappa} - r\overline{R}_n^{(\kappa)})|: r = 1, 2, \dots, n\} > z_\alpha$.

Confidence intervals for $\mathbb{E}Y_1$ and $\mathbb{E}Y_1 Y_{1+\kappa}$:

(8) $$\overline{Y}_n \pm \Phi_\alpha \sqrt{nU_n}, \qquad \overline{R}_n^{(\kappa)} \pm \Phi_\alpha \sqrt{nV_n^{(\kappa)}}.$$

We chose Y_i above as either the average current or as the number of steps down in $]i-1, i]$. These procedures may also be applied to any real functions of the vectors of Theorem 11 with finite fourth moment.

The confidence intervals of (8) do not directly provide confidence intervals for $\mathrm{Cov}(Y_1, Y_{1+\kappa})$. The natural estimators are

$$C_n^{(\kappa)} = \frac{1}{n-\kappa} \sum_{j=1}^{n-\kappa} \left(Y_j - \bar{Y}_a\right)\left(Y_{j+\kappa} - \bar{Y}_b\right),$$

where $\bar{Y}_a$ and $\bar{Y}_b$ are the sample averages over the first and last $n-\kappa$ observations. Similar confidence intervals to those above are developed in Section 4.2. If our model is correct, the graph of $C_n^{(\kappa)}$ versus κ should decrease like a^κ for some $0 < a < 1$. This graph provides a heuristic check on a critical theoretical property of our model, ϕ-mixing.

2.3. *An extreme case.* It is not surprising that the test of Dabrowski, McDonald and Rösler (1990) has no power against certain alternatives. Here we present an example of an alternative for which it is somewhat surprising that (1) fails to work. Consider the case where the channels are independent and different, but where the ratio of the mean emitting time to mean nonemitting time, p, for the channels are all equal. The test of Dabrowski, McDonald and Rösler (1990) will fail to pick up the fact that the channels are not identical. Here the occupation-time histogram is identical to that obtained for independent and identically distributed channels with the same p. No test based on this histogram will be able to distinguish between c identical and c different channels with common p. What is surprising is that (mean number of downsteps from s)/(mean occupancy of s) $= \mathbb{E}N_{js}/\mathbb{E}M_{js}$ (see Section 4.1) is also equal to that obtained for identical channels, albeit for an intermediate value of μ_F. Since it is this ratio which permits the estimation of μ_F, the hypothesis test, (1), of this paper also cannot be used to distinguish between the case of two channels where one channel is simply running at C times the rate of the other, and the case of independent and identical channels.

As an example, we present here the calculation of the mean number of downsteps for two cases. Case 1 consists of four independent channels with $\mu_F = 5$ and $\mu_G = 10$. Case 2 replaces one of these four channels by a channel running at C, $C > 1$, times the speed of the other three channels. This fast channel has a mean open time of $5/C$ seconds and a mean closed time of $10/C$ seconds. Let $s \in \{1, 2, 3, 4\}$.

Case 1:

$$\mathbb{E}N_s = \frac{s}{5}\binom{4}{s}\left(\frac{1}{3}\right)^s \frac{2}{3}$$

by Theorem 13. Thus $\mathbb{E}N_{js}/\mathbb{E}M_{js} = s/5$.

Case 2:

$$\mathbb{E}N_s = \frac{s(3-s+1)+s(s+C-1)}{4\times 5}\binom{4}{s}\left(\frac{1}{3}\right)^s\left(\frac{2}{3}\right)^{4-s}$$

by Theorem 13. Thus $\mathbb{E}N_{js}/\mathbb{E}M_{js} = (3+C)/5s$.

This calculation shows that the expected values of the number of downsteps in Case 2 can be matched by a collection of four independent and identical channels, with the same p, and common mean open time $5/(3+C)$ which is between the mean open times for the two types of channels. At a practical level, this extreme example may have little influence. For example, it is widely believed that the mean open time of a stretch-activated ion channel does not change under stress, but that the mean closed time does change. Consequently, the ratio p would be different under different levels of stress. The calculations of this example would not apply.

3. Applications to data.

Simulated independent channels: *Figure* 1(*a*). Each channel in this simulation was generated by a two-state Markov process. The open state had a mean sojourn length of 0.01 seconds ($\mu_F = 0.01$). The mean closed state sojourn length was 0.02 seconds ($\mu_G = 0.02$). The common ratio, p, of these channels is $0.\dot{3}$. Each of six channels was simulated and the individual 0–1 current processes were combined into a single observable current record. This generated a total of 9853 downsteps over 50 seconds.

Simulated dependent channels: *Figure* 1(*b*). A 50-second record for each of five independent channels in this simulation was generated by a two-state Markov process exactly as given above. A sixth channel record was obtained by delaying the current record of the first channel by 0.002 milliseconds. The six-individual 0–1 current processes were then combined into a single observable current record. This generated 10,108 downsteps.

Simulated independent nonidentical channels: *Figure* 1(*c*). Each channel in this simulation was generated by a two-state Markov process. For four of the six independent channels, we generated 50-second records as for the first example above. For the remaining two channels, we took $\mu_F = 0.2$ and $\mu_G = 0.1$. The ratio p for these channels is $0.\dot{6}$. The six individual 0–1 current processes were combined into a single observable current record. This was observed over a 50-second time period. The record contained 6900 downsteps. The longest period of constant current was 0.05 seconds, and the shortest period of constant current was 0.00000065 seconds.

Analysis of simulated data. Here we present the results of our tests and estimation procedures. By construction, each process is stationary. The test for first-order stationarity, (6), was applied to the average current process, $X(t)$,

TABLE 1

Test	**Figure 1(a)**	**Figure 1(b)**	**Figure 1(c)**	**Mixed $\times 10^{10}$**	***Lymnaea stagnalis***	
					Full data	**800 ms**
First-order test on						
X	0.47	0.38	0.42	0.2	0.00005	0.13504
N	0.82	0.47	0.21	0.1	0.00008	0.63570
Second-order test on						
X at lag						
0	0.40	0.30	0.40	0.4	0.00064	0.14214
1	0.40	0.31	0.37	0.3	0.00059	0.13359
2	0.57	0.28	0.44	0.3	0.00055	0.12570
3	0.59	0.50	0.43	0.6	0.00052	0.11681
4	0.57	0.32	0.43	1.5	0.00051	0.11846
5	0.54	0.55	0.42	0.6	0.00044	0.12062
N at lag						
0	0.54	0.81	0.14	0.2	0.01439	0.81232
1	0.73	0.31	0.18	0.1	0.00578	0.82984
2	0.82	0.43	0.30	0.2	0.00472	0.75408
3	0.97	0.32	0.23	0.2	0.00301	0.84354
4	0.79	0.30	0.46	0.3	0.00253	0.61196
5	0.91	0.41	0.21	0.2	0.00293	0.52528

The p-values obtained for the tests of stationarity, (6) and (7), are tabulated for various data sets. The first three columns of numbers refer to the data of Figures 1(a), 1(b) and 1(c). These cases are strictly stationary by design. The fourth case, "mixed," corresponds to the 25 seconds of the Figure 1(a) record, followed by a further 25 seconds on the same data, but where the sixth channel has been dropped from the combined current record. That channels can arbitrarily start or stop functioning is an important problem in experiments. The last two columns refer to the data on *Lymnaea stagnalis* illustrated in Figure 1(d). The full record clearly fails the test of stationarity. The last column reports the p-values when our tests of stationarity are applied to an 800-millisecond initial segment of the complete record.

and the total number of downsteps process, $N(t)$. The test for second-order stationarity, (7), was also applied to these two processes for lags $\kappa = 0, 1, \ldots, 5$. A fourth case ("mixed") was created by taking 25 seconds of the data from Figure 1(a), followed by 25 seconds of the data from Figure 1(c). The test for independence of channels, (1), requires that for each K (number of types present) of interest, we choose which occupation-time densities, and which downstep counts, will be used to construct $\mathbb{B}_K$. For $\mathbb{B}_1$, we chose to fit on the occupation-time density at current levels 0, 1 and 2, and on the downstep counts from current levels 3, 4 and 5. For $\mathbb{B}_2$, we chose to fit on the occupation-time density at current levels 0, 1, 2 and 3, and on the downstep counts from current levels 2, 3, 4 and 5. The relevant numbers are presented in Tables 1, 2 and 3. A remark on how we chose the levels used in our tests is in order here. The overall occupation-time density in each case had a mode at 2 or 3. We chose the occupation-time levels and downstep levels so that, as far as possible, our algorithm tried to fit the observed occupation-time levels on one side of the mode, and the downstep levels on the other side. We avoided the

TABLE 2

Current level	Figure 1(a)			Figure 1(c)				Figure 1(d) (800 ms)		
	Obs.	$\mathbb{B}_1$	**Exact**	**Obs.**	$\mathbb{B}_1$	$\mathbb{B}_2$	**Exact**	**Obs.**	$\mathbb{B}_1$	$\mathbb{B}_2$
					Occupation time					
0	0.090	0.091	0.088	0.020	0.030	0.021	0.022	0.006	(0.008)	(0.003)
1	0.270	0.268	0.263	0.135	0.144	0.131	0.132	0.036	0.049	0.038
2	0.326	0.329	0.329	0.298	0.284	0.303	0.296	0.159	0.142	0.164
3	0.219	(0.216)	0.219	0.325	(0.300)	0.322	0.318	0.306	0.238	0.316
4	0.076	(0.079)	0.082	0.171	(0.178)	(0.173)	0.177	0.297	(0.257)	0.276
5	0.016	(0.016)	0.016	0.045	(0.056)	(0.046)	0.049	0.143	(0.185)	(0.141)
6	0.002	(0.001)	0.001	0.005	(0.007)	(0.005)	0.005	0.044	(0.089)	(0.048)
7								0.008	(0.027)	(0.012)
8								< 0.001	(0.005)	(0.002)
					Downsteps					
1	1.363	(1.33)	1.317	0.227	(0.371)	0.228	0.241	0.048	(0.028)	(0.106)
2	3.256	3.263	3.292	1.307	1.467	1.291	1.295	0.162	(0.158)	(0.150)
3	3.252	3.206	3.292	2.539	2.320	2.538	2.480	0.464	(0.400)	0.473
4	1.540	1.575	1.646	2.097	1.834	2.097	2.080	0.648	0.577	0.637
5	0.386	(0.387)	0.411	0.712	(0.725)	(0.769)	0.791	0.433	0.519	0.451
6	0.056	(0.038)	0.041	1.108	(0.115)	(0.104)	0.112	0.203	0.299	0.199
7								0.036	(0.108)	(0.060)
8								0.005	(0.022)	(0.013)

For the data sets of Figures 1(a) and 1(c), this table states the observed occupation-time densities and the observed downstep counts per unit of time. The fitted values are given when the null hypothesis is that there is only one kind of channel (noted $\mathbb{B}_1$) and when the null hypothesis is that there are at most two types of channel (noted $\mathbb{B}_2$). The test for $\mathbb{B}_1$ was carried out by fitting the model to the occupation-time densities at levels 0, 1 and 2, and to the downstep counts from levels 2, 3 and 4. If the hypothesis of only one type of channel was rejected, the test for $\mathbb{B}_2$ was carried out by fitting the model to the occupation-time densities at levels 0, 1, 2 and 3, and to the downstep counts from levels 1, 2, 3 and 4. For such columns, the fitted values found for components not involved in the test are enclosed by brackets. The theoretical values (to three decimal places) are given for the data of Figures 1(a) and 1(c). The results for the apparently stationary 800-millisecond section of data on *Lymnaea stagnalis* are also presented.

visits of the process to level 6 (or downsteps from level 6) since the rarity of these visits would lead to an estimated variance of nearly 0 for these components. In other simulations the authors have found that the estimate of c (or more generally c^i) may not be stable for short simulations on the order of 2000 downsteps. Nevertheless, the estimators of p and μ_F have performed well.

An application to potassium ion channels of Lymnaea stagnalis. Cathy Morris (Ottawa) and Wade Sigurdson (Buffalo) kindly provided us with data on stretch-activated potassium ion channels in the heart tissue of pond snails, *Lymnaea stagnalis* [see Sigurdson, Morris, Brezden and Gardner (1987)]. We applied the tests of stationarity and independence to these data following the

TABLE 3

	Figure 1(a)			Figure 1(b)		Figure 1(c)			Figure 1(d)	
	$\mathbb{B}_1$	$\mathbb{B}_2$	Exact	$\mathbb{B}_1$	$\mathbb{B}_2$	$\mathbb{B}_1$	$\mathbb{B}_2$	Exact	$\mathbb{B}_1$	$\mathbb{B}_2$
(1)	4.3	5.0	—	44.1	21.6	107.9	2.98	—	13.96	2.37
$\hat{c}^1$	6	3	6	16	16	6	4	4	9	17
$\hat{c}^2$	—	3	—	—	4	—	2	2	—	3
$\hat{p}^1$	0.329	0.339	$0.\dot{3}$	0.127	0.071	0.441	0.318	$0.\dot{3}$	0.419	0.069
$\hat{p}^2$	—	0.319	—	—	0.223	—	0.692	$0.\dot{6}$	—	0.779
$\hat{\mu}_F^1$	0.010	0.005	0.01	0.010	3×10^6	0.019	0.010	0.01	2.43	1.43
$\hat{\mu}_F^2$	—	4.58	—	—	0.004	—	0.148	0.20	—	3.64
$\hat{\mu}_G^1$	0.021	0.010	0.02	0.068	4×10^7	0.025	0.021	0.02	3.38	19.14
$\hat{\mu}_G^2$	—	9.76	—	—	0.015	—	0.066	0.10	—	1.03

For each of the data sets of Figures 1(a), 1(b), 1(c) and 1(d) (800 ms), this table states the observed value of the test statistic in the test of independence, (1), and the related estimates of several parameters: the number of channels of each type, the ratio p and the mean sojourn times for each type of channel. Exact values are given when appropriate. These values are given for two hypothesis tests: when the null hypothesis is that there is only one kind of channel (noted $\mathbb{B}_1$), and when two kinds of channel are allowed (noted $\mathbb{B}_2$). The tests were chosen so that approximate χ^2 distributions of our statistics have 4 degrees of freedom in each case. The 99th percentile of a χ^2 distribution with 4 degrees of freedom is 13.3.

style set out above. The mean open time for a single channel is known to be approximately 2.3 milliseconds. We do not attempt to make any biological statement here about ion channels; we only illustrate our theory.

Figure 1(d) and Tables 1, 2 and 3 summarize the results. Figure 1(d) gives a 50-millisecond section of the data. Note that jumps of absolute size larger than 1 are due to the problems of resolving the data, rather than difficulties with the semi-Markov model for ion channel behaviour. From Table 1 we see that the original data (2047 milliseconds long) are clearly not stationary. Based upon a graph of the current record, we chose the initial 800 milliseconds for further analysis. This short segment seems reasonably stationary by our test of stationarity. The subsequent tables indicate that these data lead to rejection of the hypothesis of a single type of channel, and that the hypothesis of two types of channels is not contradicted. Under the (apparently false) hypothesis of one type of channel, we found a mean open time per channel of 2.43 milliseconds—a value consistent with existing estimates. Under the hypothesis of two types of channels, we obtained mean open times of 1.42 and 3.63 milliseconds for the two groups. This suggests that ion channels are not in fact identical in a cell patch, or at least they are not all stressed equally by stretching the cell membrane. We must stress here that these data were not originally collected with our techniques in mind, and the 800-millisecond portion we selected for study is relatively short. It contains only 1172 downsteps, whereas the data of Figures 1(a), 1(b) and 1(c) contain up to 10,000 downsteps. Nevertheless, stationarity of real data will clearly be a problem.

4. Proofs.

4.1. *Model of independent channels.* Our theorems require that for each channel in the cell patch, the chain has a recurrent open state, a, whose return times have a distribution function, R, which satisfies the following condition.

CONDITION 9. *There exists a distribution function* K *with*

$$\frac{1 - R^i(t+s)}{1 - R^i(t)} \leq 1 - K(s)$$

uniformly for all $i \in \{1, 2, \ldots, c\}$, $s \geq 0$ *and all* $t \geq 0$ *with* $1 - K(t) > 0$. *Further, for some* $\alpha > 0$, $\int_0^\infty e^{\alpha t} K(dt) < +\infty$. *Finally,* R^i *is spread out—that is, some convolution power of* R^i *has a nontrivial absolutely continuous component.*

DEFINITION 10. Let $\{Y_n: n \geq 1\}$ be a sequence of random variables taking values in a separable Banach space. Let $\mathscr{M}_a^b$ denote the σ-field generated by the variables $Y_a, Y_{a+1}, \ldots, Y_b$. The sequence $\{Y_n: n \geq 1\}$ is said to be ϕ-mixing with rate $\phi(\cdot)$ if for some $\phi(n) \downarrow 0$,

$$|P[A \cap B] - P[A]P[B]| \leq \phi(n) P[A]$$

for all $A \in \mathscr{M}_1^k$, $B \in \mathscr{M}_{k+n}^\infty$ and $k, n \geq 1$.

The following three theorems summarize the work of Dabrowski, McDonald and Rösler (1990), and form the foundation for our statistical procedures. Theorem 11 places the problem in the context of ϕ-mixing processes. The literature on mixing processes is quite well developed [cf. Philipp (1986)], and good sufficient conditions for invariance principles are available [e.g., Dehling (1983) and Kuelbs and Philipp (1980)]. Dabrowski, McDonald and Rösler (1990) apply these results to obtain weak convergence of the desired statistics. The continuous mapping theorem completes the proofs of our theorems. Theorem 12(a) provides the necessary weak convergence for our tests on the independence of channels, and Theorem 12(b) sets the stage for our tests of stationarity. Theorem 13 is the critical innovation which allows us to calculate the mean number of downsteps necessary for the application of Theorem 12(a).

THEOREM 11. *The sequence of random functions (taking values in the space of right-continuous functions on* $]0, 1]$ *into* $\{0, 1\}$)

$$\left\{\left\{I\{X^i(j+\tau) = 1\}: 0 \leq \tau \leq 1\right\}: j \in \mathbb{Z}^+\right\}$$

is ϕ*-mixing with rate* $\phi(n) = O(\rho^n)$ *for* $\rho < 1$, ρ *not depending on* i.

Since measurable functions of a sequence of ϕ-mixing sequence are again ϕ-mixing, we may apply the known results on weakly dependent random variables to various statistics based on the variables of Theorem 11, for example, $X(t)$, and $N(t)$.

THEOREM 12. *Let* $\{Y_1, Y_2, \dots\}$ *be a stationary* ϕ*-mixing sequence of* $\mathbb{R}^m$*-valued random variables such that* $\phi(n) \le K\rho^n$, $\exists\ 0 < \rho < 1$, $K \ge 1$ *and* $\mathbb{E}\|Y_1\|^4 < +\infty$. *Let* $k(n)$ *be a nondecreasing sequence of positive integers such that* $k(n) = o(\log n)$. *Define*

$$T(s,t) = \operatorname{Cov}(Y_1(s), Y_1(t)) + 2\sum_{j=2}^{\infty} \operatorname{Cov}\big(Y_1(s), Y_j(t)\big), s,t \in \{1,2,\dots,m\},$$

$$\bar{Y}_n = n^{-1}\sum_{i=1}^{n} Y_i$$

and

$$T_n(s,t) = n^{-1}\sum \big(Y_i(s) - \bar{Y}_n(s)\big)\big(Y_j(t) - \bar{Y}_n(t)\big), \qquad s,t \in \{1,2,\dots,m\}.$$

This last sum ranges over $1 \le i, j \le n$ *for which* $|i - j| \le k(n)$.

(a) *Then* $T_n \to T$ *a.s.*, $n^{1/2}(\bar{Y}_n - \mathbb{E}(Y_1)) \to_{\mathscr{D}} \mathscr{N}(0,T)$, *a multivariate normal, and if* T *is invertible*, $n(\bar{Y}_n - \mathbb{E}(Y_1))^T T_n^{-1}(\bar{Y}_n - \mathbb{E}(Y_1)) \to_{\mathscr{D}} \chi^2_m$, *a* χ^2 *distribution with m degrees of freedom.*

(b) *Take* $m = 1$ *above. Define random* $C[0,1]$*-valued functions,* f_n, *for* $n \ge 1$ *by*

$$f_n(t) = \begin{cases} 0, & \textit{if } t = 0, \\ (nT_n)^{-1/2}\left(\sum_{i=1}^{r} Y_i - r\bar{Y}_n\right), & \textit{if } t = r/n \textit{ for some } r \in \{1,2,\dots,n\}, \\ \textit{linear}, & \textit{otherwise}. \end{cases}$$

Then $T_n \to T$ *a.s.*, $f_n \to_{\mathscr{D}} B$, *a standard Brownian motion, and if* $g_n(t) = f_n(t) - tf_n(1)$, $g_n \to_{\mathscr{D}} B_b$, *a standard Brownian bridge.*

THEOREM 13. *For the model of independent channels given in Section* 2.1 *satisfying Condition* 9, *and in steady state*:

(a)
$$\mathbb{E}M_{js} = \sum_{|\mathscr{I}|=s}\left[\left(\prod_{m\in\mathscr{I}} p^m\right)\left(\prod_{n\in\mathscr{I}^c}(1-p^n)\right)\right] =: e_s$$

for $s \in \{1,2,\dots,c\}$;

and

(b)
$$\mathbb{E}N_{js} = \sum_{|\mathscr{I}|=s}\left[\left(\prod_{m\in\mathscr{I}} p^m\right)\left(\prod_{n\in\mathscr{I}^c}(1-p^n)\right)\left(\sum_{m\in\mathscr{I}}(\mu_F^m)^{-1}\right)\right] =: f_s$$

for $s \in \{1,2,\dots,c\}$.

Here $\mathscr{J}$ *denotes a subset of* $\{1,2,\dots,c\}$, $|\mathscr{J}|$ *denotes the cardinality of* $\mathscr{J}$, *and* $p^i = \mu_F^i/(\mu_F^i + \mu_G^i)$.

Proof. Channel i has probability p^i of emitting current at an instant t, independently of the other channels. Consequently, the chance that s channels are emitting current at t can be easily computed to be e_s. Part (a) now follows from stationarity of the process.

Fix a channel, i. Denote the successive states of the embedded chain by $\{S_k^i\colon k \ge 0\}$ and the successive sojourn times by $\{T_k^i\colon k \ge 0\}$. For any $t \ge 0$ define

$$S^i(t) = S_k^i \quad \text{if} \sum_{j=1}^{k-1} T_j^i \le t < \sum_{j=1}^{k} T_j^i$$

and

$$Z^i(t) = t - \sum_{j=1}^{k-1} T_j^i.$$

Thus $S^i(t)$ denotes the state of channel i at time t, and $Z^i(t)$ is the age at t [i.e., the length of time since $S^i(t)$ was entered]. By stationarity, $P[S^i(t) \in \mathscr{A}^i] = p^i$. Also (recall the notation of Section 2.2)

$$P\big[S^i(t) = a, Z^i(t) \in du \,|\, S^i(t) \in \mathscr{A}^i\big] = \rho^i(a)\frac{\Theta_a^i(u)}{\mu_a^i}\,du$$

and

$$P\big[S^i(t) = b, Z^i(t) \in du \,|\, S^i(t) \in \mathscr{B}^i\big] = \delta^i(b)\frac{\Theta_b^i(u)}{\mu_b^i}\,du,$$

where $\Theta_a^i(u) = \int_u^\infty \theta_a^i(s)\,ds$ and $\Theta_b^i(u) = \int_u^\infty \theta_b^i(s)\,ds$. Recall that $N^i(t)$ is the point process which counts the number of transitions from $\mathscr{A}^i$ to $\mathscr{B}^i$. By Theorem 7 of Chapter 2 in Brémaud (1981), we see that a compensator for $N^i(t)$ is

$$A^i(t) = \int_0^t \sum_{a \in \mathscr{A}^i} I\{S^i(u) = a\}\frac{\theta_a^i(Z^i(u))}{\Theta_a^i(Z^i(u))}\,du.$$

Consequently, $N^i(t) - A^i(t)$ is a martingale. Fix $s \in \{1,2,\dots,c\}$ and define $f(t) = I\{X(t-) = s\}$. Hence

$$\int_0^T f(t)\,d\big[N^i(t) - A^i(t)\big]$$

is also a martingale, and has expectation 0. If $\mathscr{N}_s(t)$ = the number of downward transitions in $]0, T]$ from s, then

$$\begin{aligned}
\mathbb{E}\mathcal{N}_s(T) &= \mathbb{E}\left(\int_0^T f(t)\, d\left[\sum_{i=1}^{c} N^i(t)\right]\right)\\
&= \int_0^T \mathbb{E}\left[I\{X(t-) = s\} \sum_{a\in\mathcal{A}^i} I\{S^i(t) = a\} \frac{\theta_a^i(Z^i(t))}{\Theta_a^i(Z^i(t))}\right] dt\\
&= \int_0^T \sum_{|\mathcal{J}|=s} \left[\mathbb{E}\left[\prod_{i\notin\mathcal{J}} \{I\{S^i(t) \in \mathcal{B}^i\}\} \prod_{i\in\mathcal{J}} \{I\{S^i(t) \in \mathcal{A}^i\}\} \right.\right.\\
&\qquad\qquad \left.\left. \times \sum_{i\in\mathcal{J}} \sum_{a\in\mathcal{A}^i} I\{S^i(t) = a\} \frac{\theta_a^i(Z^i(t))}{\Theta_a^i(Z^i(t))}\right]\right] dt\\
&= \int_0^T \sum_{|\mathcal{J}|=s} \left[\prod_{i\notin\mathcal{J}} (1 - p^i) \prod_{i\in\mathcal{J}} p^i \right.\\
&\qquad\qquad \left. \times \sum_{i\in\mathcal{J}} \sum_{a\in\mathcal{A}^i} \mathbb{E}\left[I\{S^i(t) = a\} \frac{\theta_a^i(Z^i(t))}{\Theta_a^i(Z^i(t))} \middle| S^i(t) \in \mathcal{A}^i \right]\right] dt\\
&= \Upsilon \quad (\text{say}).
\end{aligned}$$

But

$$\begin{aligned}
&\mathbb{E}\left[I\{S^i(t) = a\} \frac{\theta_a^i(Z^i(t))}{\Theta_a^i(Z^i(t))} \middle| S^i(t) \in \mathcal{A}^i \right]\\
&\qquad = \frac{1}{p^i}\mathbb{P}[S^i(t) = a]\,\mathbb{E}\left[\frac{\theta_a^i(Z^i(t))}{\Theta_a^i(Z^i(t))} \middle| S^i(t) = a \right]\\
&\qquad = \frac{1}{p^i}\mathbb{P}[S^i(t) = a] \int \frac{\theta_a^i(z)}{\Theta_a^i(z)} \frac{\Theta_a^i(z)}{\mu_a^i}\, dz\\
&\qquad = \frac{1}{\mu_a^i p^i} \frac{\sum_{b\in\mathcal{B}^i} \pi(b)\mathcal{P}_{ba}\mu_a^i}{\mu_F^i + \mu_G^i}.
\end{aligned}$$

Therefore, since $p^i = \mu_F^i/(\mu_F^i + \mu_G^i)$,

$$\begin{aligned}
\Upsilon &= T \sum_{|\mathcal{J}|=s} \left[\prod_{i\notin\mathcal{J}} (1 - p^i) \prod_{i\in\mathcal{J}} p^i \sum_{i\in\mathcal{J}} (\mu_F^i)^{-1} \left[\sum_{a\in\mathcal{A}^i} \sum_{b\in\mathcal{B}^i} \pi(b)\mathcal{P}_{ba} \right]\right]\\
&= T \sum_{|\mathcal{J}|=s} \left[\prod_{i\notin\mathcal{J}} (1 - p^i) \prod_{i\in\mathcal{J}} p^i \sum_{i\in\mathcal{J}} (\mu_F^i)^{-1} \right] \quad \text{as desired.} \qquad \square
\end{aligned}$$

Since the vectors $\{\gamma_j\colon j \geq 1\}$ are functions of the ϕ-mixing variables of (11), they are also ϕ-mixing. Clearly $\mathbb{E}\|M_{sj}\|^4 \leq 1$, and from renewal theory

$$\begin{aligned}\mathbb{E}\|N_{sj}\|^4 &\leq \mathbb{E}\left[\sum_{i=1}^{c} \left[N^i(j) - N^i(j-1)\right]\right]^4\\ &\leq c^4 \sum_{i=1}^{c} \mathbb{E}\left[N^i(j) - N^i(j-1)\right]^4 < +\infty.\end{aligned}$$

Under Condition 9, we may apply Theorem 12 to obtain the asymptotic normality of

$$n^{-1/2} \sum_{j \leq n} \left(\langle M_j, N_j\rangle - \mathbb{E}\langle M_j, N_j\rangle\right). \tag{14}$$

The values for $\mathbb{E}\langle M_j, N_j\rangle$ are given by Theorem 13. This establishes the hypothesis tests on independence of Section 2.2 when the mean is known. In such a case the degrees of freedom of the χ^2 statistic would be $|\mathscr{M}| + |\mathscr{N}|$. If the $3K$ parameters (c^k, p^k and μ_F^k) were all continuous, then standard techniques would yield $|\mathscr{M}| + |\mathscr{N}| - 3K$ as the number of degrees of freedom for the limiting χ^2 statistic. Here, however, the c^k are discrete, and in (1) we are minimizing over a space which is the union of an infinite number of distinct smooth manifolds. As T becomes large, only one of these yields the minimum, and we are in fact minimizing over $2K$ continuous parameters. This yields $|\mathscr{M}| + |\mathscr{N}| - 2K$ degrees of freedom for (1).

4.2. *Confidence intervals for the lag covariances of Section* 2.2. If $\{Y_i\}$ is first-order stationary,

$$\begin{aligned}C_n^{(\kappa)} - \operatorname{Cov}(Y_1, Y_{1+\kappa}) &= \frac{1}{n-\kappa}\left(\sum_{j=1}^{n-\kappa} Y_j Y_{j+\kappa} - \mathbb{E}Y_1 Y_{1+\kappa}\right)\\ &\quad + \mathbb{E}Y_1\left(\mathbb{E}Y_{1+\kappa} - \bar{Y}_a\right) + \bar{Y}_b\left(\mathbb{E}Y_1 - \bar{Y}_a\right).\end{aligned}$$

We may define the ϕ-mixing sequence of vector-valued random variables $Z_i = \langle Y_i Y_{i+\kappa}, -Y_{i+\kappa}, -Y_i\rangle$ with mean vector $e_z = \langle \mathbb{E}Y_i Y_{i+\kappa}, -\mathbb{E}Y_{1+\kappa}, -\mathbb{E}Y_1\rangle$. Theorem 1 of Dehling (1983) or Proposition 2.1 of Kuelbs and Philipp (1980) yields that

$$\theta_{n-\kappa} := (n-\kappa)^{1/2}\left[\frac{1}{n-\kappa}\sum_{j=1}^{n-\kappa} Z_j - e_z\right] \to N(0, T) \quad \text{in distribution}.$$

Here T is the appropriate covariance matrix. We note that with probability 1, $\gamma_{n-\kappa} = \langle 1, \mathbb{E}Y_1, \bar{Y}_b\rangle \to \langle 1, \mathbb{E}Y_1, \mathbb{E}Y_1\rangle^T = \gamma$, and that consequently

$$\gamma_{n-\kappa}^T \theta_{n-\kappa} \to_{\mathscr{D}} N(0, \gamma^T T \gamma). \tag{15}$$

But $(n-\kappa)^{-1/2}\gamma_{n-\kappa}^T \theta_{n-\kappa} = C_n^{(k)} - \operatorname{Cov}(Y_1, Y_{1+\kappa})$. Now approximate hypothesis tests and confidence intervals may be constructed from (15) and Theorem 12.

REMARK 16. A fundamental lemma [cf. Ibragimov (1962)] for ϕ-mixing sequences states that if $p^{-1} + q^{-1} = 1$, $|\mathbb{E}\xi\eta - \mathbb{E}\xi\mathbb{E}\eta| \leq 2\phi^{1/p}(n)\|\xi\|_p\|\eta\|_q$, where ξ is $\sigma(Y_1, \ldots, Y_k)$-measurable, η is $\sigma(Y_{k+n}, Y_{k+n+1}, \ldots)$-measurable, $\|\xi\|_p < \infty$, and $\|\eta\|_q < \infty$. We can apply this to $\operatorname{Cov}(Y_1, Y_{1+\kappa})$ when $\|Y_1\|_4 < \infty$ and when we have independent channels to obtain that

$$|\operatorname{Cov}(Y_1, Y_{1+\kappa})| \ll \rho^{3\kappa/4} \quad \text{for some } 0 < \rho < 1.$$

If the Y_i are almost surely bounded [e.g., $Y_j = \int_{j-1}^{j} X(t)\,dt$], $|\operatorname{Cov}(Y_1, Y_{1+\kappa})| \ll \rho^{\kappa}$ for some $0 < \rho < 1$. This permits a rough judgement of the validity of our model from a plot of the lag covariance at lag κ versus the lag κ. The graph should decrease exponentially.

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DEPARTMENT OF MATHEMATICS
UNIVERSITY OF OTTAWA
585 KING EDWARD
OTTAWA, ONTARIO
CANADA K1N 6N5