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RENEWAL THEORY PROPERTIES OF ION CHANNELS

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The membranes of cells, such as those found in heart and nerve tissue, contain structures known as ion channels. The current produced by an ion channel in a cell membrane has been modelled as a finite state-space Markov process. Statistical analysis of ion channels has been largely limited to the case where a single channel is observed, or where the stimulus to the channel is so slight that only one channel at a time is emitting current. In many experiments, however, several channels are producing current simultaneously. Therefore only the superposition of the currents from an unknown number of channels can be observed. A hypothesis test is established which tests whether these channels operate independently and identically. If the null hypothesis is not rejected, confidence intervals for the mean emitting time and mean nonemitting time of individual channels and estimates of the distribution functions of the sojourns in emitting states and nonemitting states are obtained.

1. Introduction and statement of results.

1.1. *Molecular ion channels.* The membranes of cells, such as those found in heart and nerve tissue, contain molecular structures known as ion channels. These complex molecules may assume several configurations, some of which are capable of pumping ions across the cell membrane and producing a detectable unit of current. The amperage of this current, measured in picoamperes, is intrinsic to the molecular structure of the ion channel. In the absence of stimulus, the molecule produces no electrical current. In the presence of certain chemical or physical stimuli, the molecule may assume, first, transitional configurations which still do not produce any current and then either take a form which produces a unit of electrical current across the cell membrane or return to the resting state. The electrical output of an ion channel is thus an alternating sequence of random sojourns in the nonemitting and emitting states. The distributions of the lengths of sojourns in the emitting and nonemitting states, as well as the means of these distributions, are functions of the molecular bonding rates. These bonding rates and how they change with varying stimuli, are key to the understanding of many cellular functions and are the object of considerable research in the biological sciences [e.g., Horn (1984), Horn and Vandenberg (1984), Labarca, Rice, Fredkin and Montal (1985) and Neher (1983)].

Statistical analysis of ion channels has been largely limited to the case where a single channel is isolated and observed (a difficult task) or where the

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stimulus to the channel is so slight that only one channel at a time is emitting current. In many experiments the portion of cell membrane (a cell patch) contains an unknown number (e.g., 5 to 10) of ion channels and the stimulus is sufficiently strong that several channels are producing current simultaneously. Only the superposition of the currents from an unknown number of channels can be observed. If the ion channels have identical molecular structure, it follows that the superposed current is an integer multiple of the intrinsic current produced by one channel. Henceforth, we consider the current to be measured in integers. This paper uses this type of data to attack the problem of deciding whether or not the channels in a membrane are operating independently and, if so, estimating the distribution of the sojourn periods in emitting and nonemitting states.

1.2. *Single channel model.* The opening and closing of a pore (channel) in the cell membrane produces a series of pulses of electrical current. The temporal pattern is determined by the *gating mechanism*—the molecular structure of the channel. This pattern of activity may be quite complex. For example, the channel may exhibit *bursting* behaviour where the activity appears as separate bursts of rapidly oscillating current. Drug-induced currents in single ion channels of a cell membrane have been well described by Colquhoun and Hawkes (1977, 1981, 1982, 1983, 1987) as a finite state-space Markov process. The channel may be said to be either in *bound and open* (or *emitting*) states or in *unbound and closed* (or *nonemitting*) states. There are, moreover, several intermediate *bound but closed* (*nonemitting*) states. The finite state space is partitioned into two groups of states: the emitting states $\mathscr{A}$ and the nonemitting states $\mathscr{B}$. The simplest case is that of the alternating renewal process. Note that the 0–1 output process is not necessarily Markovian and that the sojourns in emitting or nonemitting states are not necessarily exponential. Such processes are known as *aggregated Markov* processes.

Studies of Fredkin, Montal and Rice (1985) and others support this representation of the gating mechanism of a single channel. The technique of single channel recording is described in Auerbach and Sachs (1983), Colquhoun and Sigworth (1983), Hamill, Marty, Neher, Sakmann and Sigworth (1981) and Sakmann and Neher (1983). The important problem of *time interval omission* (where sojourns in $\mathscr{A}$ or $\mathscr{B}$ shorter than a fixed value are missed by the recording machinery) has also attracted considerable attention [e.g., Blatz and Magleby (1986) and Ball and Sansom (1987, 1988)]. These papers take a Markovian approach. A recent paper of Milne, Yeo, Edeson and Madsen (1988) attacks the problem of time interval omission using renewal methods similar in spirit to that of this paper. A more general reference is the book of Hille (1984).

1.3. *Multiple ion channels.* When the channel patch contains several ion channels the output current process is the sum of the individual aggregated Markov processes. Frequently such data are discarded. The techniques referenced in Section 1.2 are not effective when investigating the gating mechanism at higher levels of stimulation where several channels emit current simultaneously. They are also ineffective in the case of nonidentical channels or when channels interact [e.g., stretch-activated channels in Sigurdson, Morris, Brezden and Gardner (1987)]. An accepted procedure has been to assume independent and identically distributed channels and fit the empirical cumulative distribution function for emitting and nonemitting periods to that predicted by a Markov chain model [e.g., Patlak and Horn (1982) and Kirber, Singer, Welsh, Fuller and Peura (1985)]. Dionne and Leibowitz (1982), Glasbey and Martin (1988), Horn and Lange (1983), Jackson (1985), Sine and Steinbach (1986) and Yeramian, Trautmann and Claverie (1986) have examined multichannel patches by different, essentially Markovian, techniques. The goal of this paper is to introduce a different approach to this problem, to provide a hypothesis test for independent and identical channels and to obtain useful estimates for the distribution of sojourn times. Our renewal-theoretic approach is also useful if Markovian models are not appropriate [see Nagy (1987)].

1.4. *Summary of approach and results.* Our approach to the problem of multiple ion channels adapts the following common heuristic process. Assume the channel patch contains c (unknown) independent and identical ion channels. Let p denote the ratio of (mean emitting time) to (mean emitting time + mean nonemitting time) for a single channel, and let $X(t)$ denote the total current being produced at time t. If the channels are independent, identical and stationary, the distribution of $X(t)$ is binomial. The probability $X(t) = s$ (s out of c are emitting) is

$$\binom{c}{s} p^s (1-p)^{c-s}.$$

Hence the observed proportions of time spent by $X(t)$ at different current levels can be fitted to a binomial density with parameters $\hat{c} \in \mathbb{N}$ and $\hat{p} \in [0,1]$. Estimate the unknown c and p by $\hat{c}$ and $\hat{p}$.

We construct random vectors $J(j)$, which represent the proportions of time spent by $X(t)$ at the different current levels $(0, 1, \dots, c)$ during the intervals $](j-1)u, ju]$, where u is the unit of time chosen for the analysis. From now on we omit reference to the unit time, u. If the channels are indeed independent and identical, then the expected value of each vector $J(j)$ will be

$$\left[\binom{c}{0} p^0 (1-p)^c, \binom{c}{1} p^1 (1-p)^{c-1}, \dots, \binom{c}{c} p^c (1-p)^0\right]'.$$

The entries of this vector are obtained from the binomial density of $X(0)$. We use renewal-theoretic arguments to show that $\{J(j)\colon j \geq 1\}$ is ϕ-mixing at a certain rate. Well-known results on weakly dependent sequences then allow us to prove a central limit theorem for $\{J(j)\colon j \geq 1\}$ and to state a χ^2 test for H_0: *The channels function independently and identically* versus H_1; *the occupation densities do not follow a binomial form*. The test requires a minimization of a quadratic form which yields consistent estimates of c and p. If H_0 is acceptable, additional arguments yield estimates for the mean sojourn times, confidence intervals for the mean sojourn times and estimates for the distributions of the sojourn lengths. There are a number of alternative mean vectors [cf., Glasbey and Martin (1988)] corresponding to different models. Our test can be adapted to test for these as well.

Section 2 gives the precise formulation and statement of our results. The proofs are presented in Section 3. We look at special cases and at examples in Section 4. In Example 2 we make some remarks on the time interval omission problem. In Examples 4 and 5 we look at two different alternatives to independent and identical channels. All estimators are designated by a carat, e.g., $\hat{F}$.

2. Statement of results.

2.1. *Model and notation.* Consider a single channel. Colquhoun and Hawkes (1982) modelled the gating mechanism by a finite state-space Markov process. We generalize to a finite state-space semi-Markov model. The state space is partitioned into the emitting states $\mathscr{A}$ and the nonemitting states $\mathscr{B}$. We emphasize, not the Markov property, but rather the renewal property of the model. For any state a, let P_{ab} denote the probability of transition to state b. This transition occurs after a sojourn of random length. Let π be the stationary probability measure of matrix P. If $a \in \mathscr{A}$, let θ_a denote the density of the time, starting from an arrival at a, until $\mathscr{B}$ is hit. Denote the mean of θ_a by μ_a. If $b \in \mathscr{B}$, let θ_b denote the density of the time, starting from an arrival at b, until $\mathscr{A}$ is hit. Denote the mean of θ_b by μ_b.

Consider the chain with kernel P in equilibrium and let S_n denote the state of this chain after n transitions. By stationarity,

$$P\{S_{n-1} \in \mathscr{B}, S_n = a\} = \sum_{b \in \mathscr{B}} \pi(b) P_{ba}.$$

Consequently, given that the embedded chain has jumped from $\mathscr{B}$, the probability it enters $\mathscr{A}$ at state a is

$$\frac{\sum_{b \in \mathscr{B}} \pi(b) P_{ba}}{\sum_{a \in \mathscr{A}} \sum_{b \in \mathscr{B}} \pi(b) P_{ba}}.$$

Similarly, given that the embedded chain has jumped from $\mathscr{A}$, the probability it enters $\mathscr{B}$ at state b is

$$\frac{\sum_{a \in \mathscr{A}} \pi(a) P_{ab}}{\sum_{b \in \mathscr{B}} \sum_{a \in \mathscr{A}} \pi(a) P_{ab}}.$$

Therefore, by regarding successive jumps from $\mathscr{A}$ to $\mathscr{B}$ or $\mathscr{B}$ to $\mathscr{A}$ as successive sojourns of a stationary semi-Markov process, the sojourn time at $a \in \mathscr{A}$ (respectively, $b \in \mathscr{B}$) has density

$$\frac{\sum_{b \in \mathscr{B}} \pi(b) P_{ba} \theta_a}{\sum_{a \in \mathscr{A}} \sum_{b \in \mathscr{B}} \pi(b) P_{ba}} \quad \left(\text{respectively, } \frac{\sum_{a \in \mathscr{A}} \pi(a) P_{ab} \theta_b}{\sum_{b \in \mathscr{B}} \sum_{a \in \mathscr{A}} \pi(a) P_{ab}} \right).$$

The sojourn time in $\mathscr{A}$ (respectively, $\mathscr{B}$) has distribution F (respectively, G) with density

$$\frac{\sum_{a\in\mathscr{A}}\sum_{b\in\mathscr{B}}\pi(b)P_{ba}\theta_a}{\sum_{a\in\mathscr{A}}\sum_{b\in\mathscr{B}}\pi(b)P_{ba}} \qquad \left(\text{respectively, } \frac{\sum_{b\in\mathscr{B}}\sum_{a\in\mathscr{A}}\pi(a)P_{ab}\theta_b}{\sum_{b\in\mathscr{B}}\sum_{a\in\mathscr{A}}\pi(a)P_{ab}}\right)$$

and mean μ_F (μ_G, respectively). Suppose that the cell patch contains c (c unknown) ion channels of the above structure. We can usually prescribe reasonable a priori bounds on c. For example, if the initial current observed is 8 units, we know that $c \geq 8$. The following tests of our model's independence assumption contain a parameter c^*. It should be chosen so that $P[X(0) > c^*] > 0$. As above, $X(t)$ denotes the total current being produced at time t and let $N(t)$ denote the number of departures from the set of emitting states $\mathscr{A}$ by time t (i.e., how many times did a channel turn off before t). We suppose that a single record of this process can be observed for a long period $[0, T]$, where T is very large with respect to μ_F and μ_G. Define

$$\begin{aligned}
I(s,t) &= I[X(t) = s] \quad \text{for } s = 0,1,2,\dots,c^*,\dots,c,\\
J(j,s) &= \int_{j-1}^{j} I(s,t)\,dt, \quad \text{for } s = 0,1,2,\dots,c^*,\dots,c,\ j \geq 1,\\
J(j) &= \langle J(j,s)\colon s = 0,1,2,\dots c^*,\dots,c\rangle,\\
J^*(j) &= \langle J(j,s)\colon s = 0,1,2,\dots c^*\rangle,\\
e_s &= \mathbb{E}J(1,s), \qquad e = \langle e_s\colon s = 0,1,2,\dots,c\rangle,\\
e^* &= \langle e_s\colon s = 0,1,2,\dots,c^*\rangle,\\
\hat{e}_s &= n^{-1}\sum_{j=1}^{n} J(j,s), \quad \text{for } s = 0,1,2,\dots,c, \qquad \hat{e} = \langle \hat{e}_s\rangle,\\
\hat{T}_n(r,s) &= \sum_{(i,j)\colon |i-j|<k(n)}^{n} n^{-1}(J(i,r) - \hat{e}_r)(J(j,s) - \hat{e}_s),
\end{aligned}$$

$$\left[0 < k(n) = o(\log n) \text{ nondecreasing}, \quad \text{e.g., } k(n) = \max\left(5, (\log n)^{3/4}\right)\right],$$

$$\begin{aligned}
T(r,s) = E(J(1,r)J(1,s) - e_r e_s) &+ \sum_{k=2}^{\infty} E((J(1,r) - e_r)(J(k,s) - e_s))\\
&+ \sum_{k=2}^{\infty} E((J(k,r) - e_r)(J(1,s) - e_s)),
\end{aligned}$$

$$\begin{aligned}
\hat{T}_n &= \left[\hat{T}_n(r,s)\colon r,s \in \{0,1,2,\dots,c^*\}\right],\\
T^* &= \left[T(r,s)\colon r,s \in \{0,1,2,\dots,c^*\}\right].
\end{aligned}$$

Furthermore, let $\tilde{e}(n) = \langle \tilde{e}_s\colon s \in 0,1,2,\dots,c^*\rangle$, where $\tilde{e}_s = \binom{\hat{c}}{s}\hat{p}^s(1-\hat{p})^{\hat{c}-s}$ and $(\hat{c}, \hat{p})$ are those constants $\hat{c}$, $0 \leq \hat{p} \leq 1$, which minimize the (for large n)

nonnegative quadratic form

$$Q_n(\tilde{e}) = \left[\sum_{j=1}^{n} (J^*(j) - \tilde{e})\right]^t \hat{T}_n^{-1} \left[\sum_{j=1}^{n} (J^*(j) - \tilde{e})\right].$$

2.2. *Hypothesis test.* The results of Section 3.1 yield that

$$n^{-1/2} \sum_{j=1}^{n} (J(j) - e) \to_{\mathscr{D}} N(0, T), \qquad \hat{T}_n \to T^*, \text{a.s.,}$$

and that T^* is an invertible and positive definite matrix. Further

$$\hat{T}_n^{-1/2}\left\{n^{-1/2} \sum_{j=1}^{n} (J^*(j) - e^*)\right\} \to_{\mathscr{D}} N(0, I)$$

and for n sufficiently large, $\hat{T}_n$ is invertible and positive definite. Here $\hat{T}_n^{1/2}$ is a matrix A for which $\hat{T}_n = AA^t$. Since $T_n^{-1} = (A^{-1})^t((A^{-1})^t)^t$, it is also a positive definite matrix. Now, as $n \to \infty$,

$$n^{-1}\left[\sum_{j=1}^{n} (J^*(j) - e^*)\right]^t \hat{T}_n^{-1} \left[\sum_{j=1}^{n} (J^*(j) - e^*)\right] \to_{\mathscr{D}} \chi^2(c^* + 1).$$

Here $\chi^2(n)$ denotes a χ^2 distribution with n degrees of freedom and $\chi^2(n, \alpha)$ is its upper 100α percentile. Under the null hypothesis of independent and identical ion channels,

$$e^* = \left\langle e_i = \binom{c}{i} p^i (1-p)^{c-i} : i \in 0, 1, 2, \dots, c^* \right\rangle.$$

Consequently, for large n, we have the approximate inequalities

$$\begin{aligned} &P\left[Q_n(\tilde{e}(n)) > n\chi^2(c^* + 1, \alpha)\right] \\ &\quad \le P\left[n^{-1}\left[\sum_{j=1}^{n} (J^*(j) - e^*)\right]^t \hat{T}_n^{-1} \left[\sum_{j=1}^{n} (J^*(j) - e^*)\right] > \chi^2(c^* + 1, \alpha)\right] \\ &\quad \approx \alpha. \end{aligned}$$

Finally, we state our hypothesis test for a given c^*. This test has a significance level of at most α:

H_0: The channels are independent and identical, i.e.,
the $e_s = \mathbb{E}J(1, s)$ are of the form $\binom{c}{s} p^s (1-p)^{c-s}$ for $0 \le s \le c^*$.
H_a: The expected values do not follow the pattern of a binomial density.

Reject H_0 if $(1/n)Q_n(\tilde{e}(n)) > \chi^2(c^* + 1, \alpha)$.

REMARK. Were the random vectors $J^*(j)$ independent instead of weakly dependent, the power of this test could be calculated by standard techniques. If the centering values e do not follow the pattern of binomial probabilities for any choice of c and p, then because T^{*-1} is positive definite,

$$\begin{aligned}\frac{1}{n}Q_n(\tilde{e}(n)) &\geq O_p(1) + 2n^{1/2}[(e^* - \tilde{e}(n))]^t\hat{T}_n^{-1}\left[n^{-1/2}\sum_{j=1}^{n}(J^*(j) - e^*)\right]\\ &\qquad + \min\left\{n\left[(e^* - \hat{e})\right]^t\hat{T}_n^{-1}\left[(e^* - \hat{e})\right] : \hat{e} \text{ of binomial form}\right\}\\ &\geq an + O_p(n^{1/2}) \quad \text{for some } a > 0.\end{aligned}$$

Remark. Here we fit the binomial density to the occupations of current levels 0 to c^*. The theory carries through for any other reasonable choice of current levels in the definition of $J^*(j)$.

Remark. The almost sure convergence of $\hat{T}_n$ to T^* has been rapid in the examples we have explored numerically. If we pick our unit time u so that with high probability there are several transitions observed in that period of time, then $k(n) = 5$ worked well in practice.

2.3. *Estimation.* We note that if the channels are independent and identical, the estimators $\hat{c}$ and $\hat{p}$ described above are consistent estimators of the parameters c and p. That is, $\hat{e}_s \to e_s$ almost surely and consequently $\hat{c} \to c$ and $\hat{p} \to p = \mu_F/(\mu_F + \mu_G)$. Without loss of generality we may assume all channels are in equilibrium at time 0 (since T is large, any initial period may be discarded).

Lemma 2.3.1. *The following hold almost surely*:

(a) $$\lim_{T\to\infty}\frac{1}{T}\int_0^T I(s,t)\,dt = \binom{c}{s}p^s(1-p)^{c-s},$$

(a′) $$\lim_{T\to\infty}\frac{1}{T}\int_0^T X(t)\,dt = cp,$$

(b) $$\lim_{T\to\infty}\frac{N(T)}{T} = \frac{cp}{\mu_F},$$

(c) $$\hat{\mu}_F = \frac{1}{N(T)}\int_0^T X(t)\,dt \textit{ is a consistent estimator of } \mu_F.$$

From this lemma and our previous consistent estimator of p follows a consistent estimator for μ_G. We can exploit the weak dependence structure of $J(j)$ to obtain confidence intervals for μ_F and μ_G. Define $\mathscr{J}(j)$ to be $\sum_{s=0}^{c} sJ(j,s)$.

Theorem 2.3.2.

$$T^{-1/2}\left\langle\int_0^T X(t)\,dt - \frac{cT\mu_F}{\mu_F + \mu_G},\quad N(T) - \frac{cT}{\mu_F + \mu_G}\right\rangle \to_{\mathscr{D}} N(0,\Sigma) \quad \textit{as } T \to \infty,$$

where Σ is the asymptotic 2×2 covariance matrix. Let $k = k(n)$ be the function defined in Section 2.1. *We have the following consistent estimates of the entries in Σ:*

$$\hat{\Sigma}_{11} = \frac{1}{T} \sum_{k=-k(n)}^{k(n)} \sum_{i} \left[\mathcal{J}(i) - \frac{1}{T} \sum_{j=0}^{T-1} \mathcal{J}(j) \right] \times \left[\mathcal{J}(i+k) - \frac{1}{T} \sum_{j=0}^{T-1} \mathcal{J}(j) \right], \tag{2.3.3}$$

$$\hat{\Sigma}_{22} = \frac{1}{T} \sum_{k=-k(n)}^{k(n)} \sum_{i} \left[N(i) - N(i-1) - \frac{1}{T} N(T) \right] \times \left[N(i+k) - N(i+k-1) - \frac{1}{T} N(T) \right], \tag{2.3.4}$$

$$\hat{\Sigma}_{12} = \frac{1}{T} \sum_{k=-k(n)}^{k(n)} \sum_{i} \left[N(i) - N(i-1) - \frac{1}{T} N(T) \right] \times \left[\mathcal{J}(i+k) - \frac{1}{T} \sum_{j=0}^{T-1} \mathcal{J}(j) \right]. \tag{2.3.5}$$

Since

$$\sqrt{T}(\hat{\mu}_F - \mu_F) = \frac{1}{\sqrt{T}} \left[\int_0^T X(t)\, dt - \mu_F N(T) \right] \frac{T}{N(T)},$$

and since $T/(N(T)) \to (\mu_F + \mu_G)/c$ almost surely, we have

THEOREM 2.3.6. *As $T \to \infty$, $\sqrt{T}(\hat{\mu}_F - \mu_F)$ converges in distribution to a centered normal distribution with variance*

$$\left(\frac{\mu_F + \mu_G}{c} \right)^2 \left[\Sigma_{11} + \mu_F^2 \Sigma_{22} - 2\mu_F \Sigma_{12} \right].$$

COROLLARY 2.3.7. *For the estimate of Σ given by* (2.3.3, 2.3.4, 2.3.5), *an approximate* $100(1-\alpha)\%$ *confidence interval for μ_F is*

$$\hat{\mu}_F \pm \frac{Z_\alpha}{\sqrt{T}} \left(\frac{\hat{\mu}_F + \hat{\mu}_G}{\hat{c}} \right) \left[\hat{\Sigma}_{11} + \hat{\mu}_F^2 \hat{\Sigma}_{22} - 2\hat{\mu}_F \hat{\Sigma}_{12} \right]^{1/2}.$$

COROLLARY 2.3.8. *For the estimate of Σ given by* (2.3.3, 2.3.4, 2.3.5), *applied to the processes $X'(t)$ = (number of channels off at time t) and $N'(t)$ = (number of departures from the nonemitting states, $\mathcal{B}$), an approximate* $100(1-\alpha)\%$ *confidence interval for μ_G is*

$$\hat{\mu}_G \pm \frac{Z_\alpha}{\sqrt{T}} \left(\frac{\hat{\mu}_F + \hat{\mu}_G}{\hat{c}} \right) \left[\hat{\Sigma}_{11} + \hat{\mu}_G^2 \hat{\Sigma}_{22} - 2\hat{\mu}_G \hat{\Sigma}_{12} \right]^{1/2}.$$

Here $\hat{\mu}_G$ is the analogous estimator to $\hat{\mu}_F$ determined from X' and N'. Note that $X'(t)$ is observable if c is known and that the values of $\hat{\Sigma}_{ij}$ are the same as those found in Theorem 2.3.6.

2.4. *Estimates of F and G.* Define $A_r(x)$ to be the conditional survival function of the time spent with r channels in $\mathscr{A}$, given that we start measuring from the moment the rth channel started emitting. Similarly, let $B_r(x)$ be the conditional survival function of the time spent with r channels in the emitting state, given we measure from the moment a channel stopped emitting. The $A_r(x)$ are estimated by $\hat{A}_r(x)$, the empirical conditional survival functions. Consider the number of times the process $X(t)$ goes from $r-1$ to r channels emitting and let $\hat{A}_r(x)$ represent the proportion of these which continue with r channels emitting for more than x units of time. Similarly, of those trajectories which decrease from $r+1$ channels emitting to r channels emitting, let $\hat{B}_r(x)$ represent the proportion which continue with these r channels emitting for more than x units of time. That these are consistent estimators of $A_r(x)$ and $B_r(x)$, respectively, follows from results on ergodic sequences. Then $1 - F(x)$ and $1 - G(x)$ can be estimated by [here $\hat{\mathscr{T}}(x)$ is an appropriate normalizing constant and $\hat{c}$ and $\hat{p}$ are the estimates from the hypothesis test]

$$\frac{\Sigma_{r=0}^{\hat{c}}\binom{\hat{c}}{r}\hat{p}^r(1-\hat{p})^{\hat{c}-r}r\hat{A}_r(x)}{(1-\hat{\mathscr{T}}(x))^{(\hat{c}-1)/\hat{c}}}\frac{1}{\hat{c}\hat{p}}$$

and

$$\frac{\Sigma_{r=0}^{\hat{c}}\binom{\hat{c}}{r}\hat{p}^r(1-\hat{p})^{\hat{c}-r}(\hat{c}-r)\hat{B}_r(x)}{(1-\hat{\mathscr{T}}(x))^{(\hat{c}-1)/\hat{c}}}\frac{1}{\hat{c}(1-\hat{p})}.$$

3. Proofs.

The proofs draw on recent results on the geometric ergodicity of Markov chains, well-known results from renewal theory and a central limit theorem for weakly dependent Banach-space-valued random variables. A key technique is the identification of a ϕ-mixing dependence structure in certain semi-Markov processes.

3.1. *Hypothesis test.* We have a system of c independent and identical ion channels, each of which may be described by an alternating process, i.e., $X_i(t) = 1$ or 0 when channel i is on or off (running at equilibrium). The first objective of this section is to establish a weak convergence theorem for the observable vector process

$$I(T) = \left\langle \int_0^T I(s,t)\,dt \colon s = 0,1,2,\dots,c \right\rangle, \quad \text{as } T \to \infty.$$

We will show that $I(T)$ may be expressed as a partial sum of $\{J(j)\}$, a ϕ-mixing sequence and then apply an invariance principle to derive the limiting distribution. It is awkward to show that the $\{J(j)\}$ are ϕ-mixing directly. Instead we will work on the random functions defined by $X(t)$ on the interval $]j-1, j]$ and prove that these Banach-space-valued random variables are ϕ-mixing. The second objective of this section is to use the above results to construct a hypothesis test on the independence assumption of our model when c is unknown.

Definition 3.1.1. ϕ-mixing. Let $\{Y_n\colon n \geq 1\}$ 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 $Y_a, Y_{a+1}, \dots, Y_b$. The sequence $\{Y_n\colon n \geq 1\}$ 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 $k, n \geq 1$.

To establish that the sequence of $\mathbb{R}^{c+1}$-valued random variables $\{J(j)\colon j = 1, 2, 3, \dots\}$ are ϕ-mixing, it suffices to show that for each channel ($i \in \{1, 2, \dots, c\}$), the sequence

$$\{\{I\{X_i(j+\tau) = 1\}\colon 0 < \tau \leq 1\}\colon j = 0, 1, 2, 3, \dots\} \tag{3.1.2}$$

is a ϕ-mixing sequence of random variables (which take their values in the space of right-continuous functions on $]0, 1]$ into $\{0, 1\}$). The ϕ-mixing property of (3.1.2) and the assumed independence of the ion channels imply that the sequence

$$\{\langle\{I\{X_i(j+\tau) = 1\}\colon 0 < \tau \leq 1\}\colon i = 1, 2, \dots, c\rangle\colon j = 0, 1, 2, \dots\} \tag{3.1.3}$$

is also ϕ-mixing. The overall mixing rate is bounded by the sum of the component mixing rates. For each fixed j, the variables of

$$\{\langle\{I\{X(j+\tau) = s\}\colon 0 < \tau \leq 1\}\colon s = 0, 1, 2, \dots, c\rangle\colon j = 0, 1, 2, \dots\} \tag{3.1.4}$$

are measurable functions of the corresponding variables of (3.1.3). Consequently the sequence (3.1.4) is ϕ-mixing with rate no worse than that of (3.1.3) (i.e., $\{y_i\colon i \geq 1\}$ ϕ-mixing $\Rightarrow$ $\{f(y_i)\colon i \geq 1\}$ is ϕ-mixing). Finally, the variables $\{J(j)\colon j = 1, 2, 3, \dots\}$ are measurable functions of the corresponding variables in (3.1.4). The ϕ-mixing property of (3.1.2) will be established in Lemma 3.1.5.

Lemma 3.1.5. *Consider the alternating process $X_1(t)$. Fix $a \in \mathscr{A}$. We identify returns to this state a as an embedded renewal process. Suppose that the distribution function, R, of the renewal periods is such that there exists a distribution function K with*

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

uniformly for all $s \geq 0$ *and all* $t \geq 0$ *with* $1 - K(t) > 0$. *Further, assume* R *is spread out, i.e., some convolution power of* R *has a nontrivial absolutely continuous component. For simplicity take* $R(1-) < 1$. *Assume also that for some* $s > 0$,

$$\int_0^\infty e^{st} K(dt) < +\infty.$$

Then the sequence $\{\{I\{X_1(j+\tau) = 1\}: 0 < \tau \leq 1\}: j \in \mathbb{Z}^+\}$ *is* ϕ*-mixing and* $\phi(n) = O(\rho^n)$, *for a* $\rho < 1$.

PROOF. We consider the embedded renewal process, with arrival times $\{S_n: n \in \mathbb{Z}\}$, where $S_n < 0$ for $n \leq 0$ and with interarrival distribution R. When S_1 has distribution g, we denote the associated probability measure by P_g. When the renewal process is stationary, we write P_ε (ε = equilibrium measure). To establish the lemma, we will prove that for some constants B and $\rho < 1$,

$$\sup_{f \in \mathscr{F}^n} |E(f | \mathscr{F}_0) - E_\varepsilon f| \leq B \rho^n. \tag{3.1.6}$$

Here E_ε is the expectation associated with P_ε,

$$\mathscr{F}_0 = \sigma\{\{I\{X_1(j+\tau) = 1\}: 0 < \tau \leq 1\}: j = -1, -2, \dots\},$$

$$\mathscr{F}^n = \sigma\{\{I\{X_1(j+\tau) = 1\}: 0 < \tau \leq 1\}: j = n, n+1, n+2, n+3, \dots\}$$

and $f \in \mathscr{F}^n$ means that $|f| \leq 1$ and f is $\mathscr{F}^n$-measurable. The excess process is defined as $V_t = \inf\{S_n - t: S_n \geq t\}$. We have by the Markov property of V_t that for all $f \in \mathscr{F}^n$,

$$\begin{aligned} E(f | \mathscr{F}_0) &= E(E(fI\{V_0 < n\} + fI\{V_0 \geq n\} | \mathscr{F}_0, V_0) | \mathscr{F}_0) \\ &= E(E(fI\{V_0 < n\} | V_0) | \mathscr{F}_0) + E(fI\{V_0 \geq n\} | \mathscr{F}_0). \end{aligned}$$

Moreover, by the Markov and renewal properties [where $L(x) = E(f | V_n = x)$]

$$\begin{aligned} E(fI\{V_0 < n\} | V_0) &= E(E(fI\{V_0 < n\} | V_0, V_n) | V_0) \\ &= E(E(f | V_0, V_n) I\{V_0 < n\} | V_0) \\ &= E(L(V_n) I\{V_0 < n\} | V_0). \end{aligned}$$

Now

$$\begin{aligned} E(f | \mathscr{F}_0) &= E(E(L(V_n) I\{V_0 < n\} | V_0) | \mathscr{F}_0) + E(fI\{V_0 \geq n\} | \mathscr{F}_0) \\ &= E(E(L(V_n) | V_0) | \mathscr{F}_0) + E(fI\{V_0 \geq n\} | \mathscr{F}_0) \\ &\quad - E(E(L(V_n) I\{V_0 \geq n\} | V_0) | \mathscr{F}_0). \end{aligned} \tag{3.17}$$

Now define $H(\omega, \cdot) = P[V_0 \in \cdot | \mathscr{F}_0](\omega)$ to be the conditional distribution of S_1. By hypothesis, $1 - H(\omega, [0, s]) \leq 1 - K(s)$ uniformly in ω and s. Recall that ε represents the equilibrium measure. Then Theorem 4.2 of Nummelin and Tuominen (1982) states that there exists a positive constant $\rho < 1$ such that

$$\| P_{\delta_0}[V_n \in \cdot\,] - \varepsilon(\cdot) \| = O(\rho^n).$$

Hence,

$$\| P_{\delta_x}[V_n \in \cdot\,] - \varepsilon(\cdot) \| = O(\rho^{[\![n-x]\!]}),$$

where $[\![n - x]\!]$ is the greatest nonnegative integer in $n - x$. Therefore

$$\int_0^\infty \| P_{\delta_x}[V_n \in \cdot\,] - \varepsilon(\cdot) \| H(dx) = O\left(\int_0^\infty \rho^{n-x} H(dx) \right) = O(\rho^n),$$

if ρ is close to 1. This holds uniformly for $H(\cdot) \in \{H(\omega, \cdot\,)\}$, since

$$\int_0^\infty e^{sx} H(dx) \le \int_0^\infty e^{sx} K(dx) < +\infty,$$

for some $s = -\ln \rho$ small. Finally, (3.1.7) and the above imply that

$$\begin{aligned}
&|E(f|\mathscr{F}_0) - E_\varepsilon(f)| \\
&\quad = |E(E(L(V_n)|V_0)|\mathscr{F}_0) + E(fI\{V_0 \ge n\}|\mathscr{F}_0) \\
&\qquad - E(E(L(V_n)I\{V_0 \ge n\}|V_0)|\mathscr{F}_0) - E_\varepsilon(f)| \\
&\quad \le \left| \int_0^\infty L(x) P_H[V_n \in dx] - \int_0^\infty L(x)\varepsilon(dx) \right| + 2|P[V_0 \ge n|\mathscr{F}_0]| \\
&\quad \le \left| \int_0^\infty L(x) P_H[V_n \in dx] - \int_0^\infty L(x)\varepsilon(dx) \right| + 2(1 - K(n)) \\
&\quad \le O(\rho^n).
\end{aligned}$$

The formal definition of ϕ-mixing follows from (3.1.6). □

REMARK. The condition in Lemma 3.1.5 that the renewal distribution R be controlled by a distribution K with finite moment generating function is satisfied when each channel is a finite state-space Markov process. Note also that Lemma 3.1.5 is valid even if there is only a single suitable renewal point a.

It is clear that $\|e\| \le 1$ and $\|J(j)\| \le 1$ in the Euclidean norm on $\mathbb{R}^{c+1}$. Under the conditions of our model and by Lemma 3.1.5, the sequence $\{J(j)$: $j \ge 1\}$ is stationary and ϕ-mixing with rate $\phi(n) \le K\rho^n$, for some constants $K > 0$ and $0 < \rho < 1$. Theorem 1 of Dehling (1983) or Proposition 2.1 of Kuelbs and Philipp (1980) now state that the central limit theorem holds for the sequence $\{J(j)$: $j \ge 1\}$. Specifically,

$$n^{-1/2} \sum_{j=1}^{n} (J(j) - e) \to_{\mathscr{D}} N(0, T),$$

where $N(0, T)$ denotes a $(c + 1)$-dimensional normal law on $\mathbb{R}^{c+1}$ with zero mean and the covariance matrix $T = \langle T(r,s)\rangle$ defined in Section 2.1. The series defining the components of T are necessarily convergent.

LEMMA 3.1.8. *Let* $\{Z_i: i \geq 1\}$ *be a* ϕ*-mixing stationary centered sequence of* $\mathbb{R}^d$*-valued random variables whose mixing coefficients* $\phi(k) \leq K\rho^k$ *for some* $0 < \rho < 1$ *and* $K \geq 1$. *Suppose also that* $\|Z_i\|_4 < \infty$. *Denote the ith coordinate of* Z_j *by* $Z_j^{(i)}$ *and for* $k \geq 0$, *define*

$$r_k^{(\mu,\nu)} = \mathbb{E}Z_1^{(\mu)}Z_{1+k}^{(\nu)} \quad \text{and} \quad \hat{r}_{kn}^{(\mu,\nu)} = \frac{1}{n-k}\sum_{i=1}^{n-k} Z_i^{(\mu)}Z_{i+k}^{(\nu)}.$$

For $k < 0$, *define* r_k *and* $\hat{r}_k$ *analogously. Set*

$$Y_n^{(\mu,\nu)} = \sum_{k=-k(n)}^{k(n)} \left(\hat{r}_{kn}^{(\mu,\nu)} - r_k^{(\mu,\nu)}\right)$$

and suppose that for the increasing sequence of positive integers $k(n)$, $k(n) \to \infty$ *and* $k(n) = o(\log(n))$ *as* $n \to \infty$. *Then for each* (μ, ν), *almost surely*

$$Y_n^{(\mu,\nu)} \to 0.$$

PROOF. The limiting value for $Y_n^{(\mu,\nu)}$ is finite by Theorem 1 of Dehling (1983). We will suppress the superscripts (μ,ν) in what follows. Let $\varepsilon_n \downarrow 0$ at a rate to be set later. For $q > 0$, Markov's inequality yields

$$\begin{aligned}
&P[|Y_n| > \varepsilon_n] \\
&\quad \leq (2k(n)+1)\max\{P[|\hat{r}_{kn} - r_k| > \varepsilon_n/(2k(n)+1)]: -k(n) \leq k \leq k(n)\} \\
&\quad \leq \max\left\{(2k(n)+1)^{q+1}\varepsilon_n^{-q}\mathbb{E}|\hat{r}_{kn} - r_k|^q: -k(n) \leq k \leq k(n)\right\} \\
&\quad \leq \max\left\{(2k(n)+1)^{q+1}\varepsilon_n^{-q}(n-k)^{-q}\mathbb{E}\left|\sum_{i=1}^{n-k} Y_{ki}\right|^q : -k(n) \leq k \leq k(n)\right\},
\end{aligned}$$

where $Y_{ki} = Z_iZ_{i+k} - \mathbb{E}Z_iZ_{i+k}$. The sequence $\{Y_{ki}: i \geq 1\}$ is a stationary ϕ-mixing sequence whose ϕ-mixing coefficients $\phi_k(\cdot)$ can be bounded as

$$\begin{aligned}
\phi_k(m) &\leq \phi(m-k) \quad &&\text{if } m > k, \\
\phi_k(m) &\leq 1 &&\text{if } m \leq k.
\end{aligned}$$

Define

$$\Phi_{ka}(b) = \sum\nolimits_{i=0}^{\infty}(i+1)^a(\phi_k(i))^b \quad \text{and} \quad \Psi_a(b) = \sum\nolimits_{i=0}^{\infty}(i+1)^a\rho^{bi}$$

for $a, b > 0$. Then

$$\begin{aligned}
\Phi_{ka}(b) &\leq \sum_{i=k+1}^{\infty}(i+1)^a(\phi(i-k))^b + \sum_{i=0}^{k}(i+1)^a \\
&\leq K^b\rho^{-kb}\Psi_a(b) < +\infty.
\end{aligned}$$

Theorem 5 of Doukhan and Portal (1983) now states that, for q a positive, even integer greater than or equal to 4,

$$\mathbb{E}\left|\sum_{i=1}^{n-k} Y_{ki}\right|^q \le K'(\phi_k, q)(q/2)n^{q/2},$$

where $K'(\phi_k, q)$ is a polynomial in $(\Phi_{k0}(\frac{1}{2}), \Phi_{k1}(\frac{1}{2}), \ldots, \Phi_{k(q-2)}(\frac{1}{2}))$ of degree at most q and whose coefficients depend only on q. Consequently, for C_q depending only on q,

$$K'(\phi_k, q) \le C_q \sum_{m=0}^{q-2} \left(\Phi_{km}(\tfrac{1}{2}) + \Phi_{km}^q(\tfrac{1}{2})\right).$$

Now

$$\begin{aligned}\mathbb{E}\left|\sum_{i=1}^{n-k} Y_{ki}\right|^q &\le (q/2)n^{q/2}C_q \sum_{m=0}^{q-2} \left(\Phi_{km}(\tfrac{1}{2}) + \Phi_{km}^q(\tfrac{1}{2})\right)\\ &\le (q/2)(\mathbb{K}n)^{q/2}\rho^{-qk(n)}C_q \sum_{m=0}^{q-2} \left(\Psi_m(\tfrac{1}{2}) + \Psi_m^q(\tfrac{1}{2})\right)\\ &\le M_{q,\rho}\rho^{-qk(n)/2}n^{q/2}\end{aligned}$$

and

$$P[|Y_n| > \varepsilon_n] \le M_{q,\rho}(2k(n)+1)^{q+1}\varepsilon_n^{-q}\rho^{-qk(n)/2}\left(\frac{n^{1/2}}{n - k(n)}\right)^q.$$

The Borel–Cantelli lemma yields our lemma if $\varepsilon_n = o(1)$ and

$$\sum (k(n))^{q+1}\varepsilon_n^{-q}\rho^{-qk(n)/2}\left(n^{1/2}/n - k(n)\right)^q < +\infty.$$

Set $\varepsilon_n = 1/k(n)$. If $k(n) = o(\log(n))$, the proof is complete. □

Setting $Z_i = J(i) - \mathbb{E}J(i)$ in Lemma 3.1.8 yields that, for $k(n)$ as above, as $n \to \infty$,

$$\sum_{k=-k(n)}^{k(n)} \hat{r}_{kn}^{(r,s)} \to T(r,s),$$

almost surely. For the test statistic, we replace $\mathbb{E}J(i)$ by $\hat{e}_i$. Let $\hat{T}_n(r,s)$ denote the statistic obtained after this substitution. Straightforward calculations yield that $\hat{T}_n(r,s)$ is a consistent estimator of $T(r,s)$.

3.2. *Proof of Lemma* 2.3.1. Each channel is a stationary semi-Markov process. By Cox (1962), the probability a channel is emitting at any time t is p. By the independence of the channels,

$$P[X(t) = s] = \binom{c}{s}p^s(1-p)^{c-s}.$$

Part (a) follows by the ergodic theorem, as does (a'). To show (b), let $N^i(t)$

count the number of departures from $\mathscr{A}$ for channel i before time t. By ergodicity,

$$\lim_{T\to\infty}\frac{N^i(T)}{T}=\frac{1}{\mu_F+\mu_G},$$

since $\mu_F+\mu_G$ is the mean cycle time. Since $N(t)=\Sigma_1^c N^i(t)$, (b) follows. Part (c) is easy. □

3.3. *Proof of Theorem* 2.3.2. Define $n(j)$ to be the number of $\tau\in\,]0,1]$ such that $X(j+\tau)-X((j+\tau)^-)=-1$. Thus $n(j)$ represents the number of departures from an emitting state in $\mathscr{A}$ between times j and $j+1$. Clearly

$$N(T)=\sum_{j=0}^{T-1}n(j)\quad\text{and}\quad\int_0^T X(t)\,dt=\sum_{j=0}^{T-1}\mathscr{I}(j).$$

Since $\{(n(j),\mathscr{I}(j)),\ j=0,1,2,\dots\}$ is a function of the variables of (3.1.4), it is also a ϕ-mixing sequence. The work of Sections 3.1 and 3.2 now yields the theorem. □

3.4. *Consistent estimators of F and G.* All channels are assumed to be in equilibrium. Define

$$\phi(x)=\int_x^\infty\frac{(1-F(s))}{\mu_F}\,ds,$$

$$\gamma(x)=\int_x^\infty\frac{(1-G(s))}{\mu_G}\,ds$$

and

$$\xi(x)=\frac{\int_x^\infty(1-F(s))\,ds+\int_x^\infty(1-G(s))\,ds}{\mu_F+\mu_G}.$$

Then

$$A_r(x)=(1-F(x))[\phi(x)]^{r-1}[\gamma(x)]^{c-r}=-\frac{\mu_F}{r}\frac{d}{dx}[[\phi(x)]^r][\gamma(x)]^{c-r}.$$

This follows since $r-1$ channels are on at the moment the rth channel started emitting. Each is in equilibrium. Given that the channel has just turned on, the density of the excess distribution is given by

$$\frac{\Sigma_{a\in\mathscr{A}}\Sigma_{b\in\mathscr{B}}\pi(b)P_{ba}\mu_a\int_s^\infty(\theta_a(t)/\mu_a)\,dt}{\Sigma_{a\in\mathscr{A}}\Sigma_{b\in\mathscr{B}}\pi(b)P_{ba}\mu_a}=\frac{1-F(s)}{\mu_F}.$$

Similarly, $c-r$ channels are off at the moment the rth started emitting. We make analogous arguments for $B_r(x)$ and obtain that

$$\frac{r}{\mu_F}A_r(x)+\frac{(c-r)}{\mu_G}B_r(x)=-\frac{d}{dx}\left[[\gamma(x)]^{c-r}[\phi(x)]^r\right]$$

is the density of the duration from an arbitrary point in time during which r channels are emitting until a transition. Consequently, since the probability that at an arbitrary point in time we have r channels emitting is $\operatorname{bin}(r) = \binom{c}{r}p^r(1-p)^{c-r}$, we have

$$-\frac{d}{dx}\sum_{r=0}^{c}\left[\operatorname{bin}(r)[\gamma(x)]^{c-r}[\phi(x)]^r\right] = -\frac{d}{dx}[\xi(x)]^c$$

is the density of the duration from an arbitrary point in time until a transition. Moreover,

$$\sum_{r=0}^{c}\left[\operatorname{bin}(r)[\gamma(x)]^{c-r}\left[-\frac{d}{dx}[\phi(x)]^r\right]\right] = c\left[[\xi(x)]^{c-1}\right]\left[\frac{1-F(x)}{\mu_F+\mu_G}\right]$$

and

$$\sum_{r=0}^{c}\left[\operatorname{bin}(r)\left[-\frac{d}{dx}[\gamma(x)]^{c-r}\right][\phi(x)]^r\right] = c\left[[\xi(x)]^{c-1}\right]\left[\frac{1-G(x)}{\mu_F+\mu_G}\right].$$

Consequently,

$$\begin{aligned}1-[\xi(x)]^c &= \int_0^x\sum_{r=0}^{c}\left[\operatorname{bin}(r)\left[\frac{r}{\mu_F}A_r(x)+\frac{(c-r)}{\mu_G}B_r(x)\right]\right]dx\\ &=: \mathscr{T}(x)\end{aligned}$$

may be estimated by

$$\begin{aligned}\hat{\mathscr{T}}(x) &:= \int_0^x\sum_{r=0}^{\hat{c}}\left[\binom{\hat{c}}{r}\left(\frac{\hat{\mu}_F}{\hat{\mu}_F+\hat{\mu}_G}\right)^r\left(\frac{\hat{\mu}_G}{\hat{\mu}_F+\hat{\mu}_G}\right)^{\hat{c}-r}\right]\\ &\qquad\times\left[\frac{r}{\hat{\mu}_F}\hat{A}_r(s)+\frac{(\hat{c}-r)}{\hat{\mu}_G}\hat{B}_r(s)\right]ds\\ &= \int_0^x\sum_{r=0}^{\hat{c}}\binom{\hat{c}}{r}\hat{p}^r(1-\hat{p})^{\hat{c}-r}\left[\frac{r}{\hat{\mu}_F}\hat{A}_r(s)+\frac{(\hat{c}-r)}{\hat{\mu}_G}\hat{B}_r(s)\right]ds.\end{aligned}$$

Also,

$$c[\xi(x)]^{c-1}\left[\frac{1-F(x)}{\mu_F+\mu_G}\right] = \sum_{r=0}^{c}\operatorname{bin}(r)\left[\frac{r}{\mu_F}A_r(x)\right].$$

This can be inverted to give the estimate for F stated in Section 2.4. The estimate for G follows similarly.

4. Special cases and examples.

4.1. *Consistent estimates of variances.* We give consistent estimates for σ_F, the standard deviation of F, and for σ_G, the standard deviation of G.

THEOREM 4.1.1. *Under the conditions of the independent and identical ion channels model and assuming independent emitting and nonemitting periods,*

$$\lim_{T\to\infty}\frac{1}{T}\operatorname{Var}(N(T)) = c\frac{(\sigma_F^2+\sigma_G^2)}{(\mu_F+\mu_G)^3}, \tag{4.1.2}$$

$$\lim_{T\to\infty}\frac{1}{T}\operatorname{Var}\int_0^T X(s)\,ds = c\left[\frac{\sigma_F^2}{\mu_F^2}+\frac{\sigma_G^2}{\mu_G^2}\right]\left[\frac{\mu_F^2\mu_G^2}{(\mu_F+\mu_G)^3}\right]. \tag{4.1.3}$$

PROOF. It suffices to consider each channel separately. Recall $N^i(t)$ represents the number of departures from the emitting state for channel i before time t. $N^i(t)$ counts the renewals of distribution $F * G$. Hence, by the central limit theorem for renewal processes [see Feller (1950)], (4.1.2) follows. Similarly, $\int_0^T X(s)\,ds$ represents the sum over c channels of independent periods of emission. Since the channels are independent and identical, $\operatorname{Var}\int_0^T X(s)\,ds$ is c times the variance associated with a single channel.

This is given in Cox (1962) and (4.1.3) follows. □

Consistent estimators for

$$\lim_{T\to\infty}\frac{1}{T}\operatorname{Var}(N(T)) \quad \text{and} \quad \lim_{T\to\infty}\frac{1}{T}\operatorname{Var}\int_0^T X(s)\,ds$$

are given in Section 3.1.

4.2. *Modified estimates of the distributions.* In the analysis of data containing large intervals during which no channels are emitting, the process of Section 2.4 may not yield good estimates for the distribution functions $G(t)$ for large t. This occurs in the motivating biological experiments of this paper when the ion channels are only very slightly stimulated. In this case it is useful to estimate $B_0(x)$ by the empirical conditional survival function $\hat{B}_0(x)$ and then invert directly. This yields the following estimate of $G(x)$:

$$\hat{G}(x) = 1 - \hat{B}_0(x)\left(1 - \int_0^x \frac{\hat{c}}{\hat{\mu}_G}\hat{B}_0(s)\,ds\right)^{1/\hat{c}-1}.$$

A similar reasoning yields the following alternative estimate for $F(x)$:

$$\hat{F}(x) = 1 - \hat{A}_1(x)\left[\int_x^\infty \frac{1-\hat{G}(s)}{\hat{\mu}_G}\,ds\right]^{1-\hat{c}}.$$

4.3. *Confidence intervals for mean on and off times.* The following specialization is useful in the motivating biological example of this paper. This theorem provides a confidence interval for μ_F when F is exponential. This would be the case for a Markov model having a single emitting state.

THEOREM 4.3.1. *If* $1 - F(x) = \exp(-x/\mu_F)$, *then*

$$\frac{N(T)^{3/2}}{\int_0^T X(s)\,ds}(\hat{\mu}_F - \mu_F)$$

converges weakly to a standard normal distribution. Here $\hat{\mu}_F = 1/(N(T))\int_0^T X(s)\,ds$.

PROOF.

$$\text{Let } \mathscr{N}(T) = \int_0^T \frac{1}{\mu_F} X(s)\,ds.$$

Since F is exponential with mean μ_F, its survival function is a constant $1/\mu_F$. Recall $N^i(t)$ counts the number of $\mathscr{A}$ to $\mathscr{B}$ transitions of channel i by time t. This is a stationary counting process, which by Theorem 7 of Brémaud (1981), has stochastic intensity

$$I\{\text{channel } i \text{ is on at time } t\}/\mu_F.$$

Since $N(t)$ is the sum of these $N^i(t)$, it has intensity $X(t)/\mu_F$ and so $N(T) - \mathscr{N}(T)$ is a martingale. For any time T and $0 \le \tau \le 1$, define

$$M_T(\tau) = N(\tau T) - \int_0^{\tau T} \frac{1}{\mu_F} X(s)\,ds = N(\tau T) - \frac{T}{\mu_F}\int_0^{\tau} X(Tu)\,du.$$

So $M_T(\tau)$ has intensity $TX(T\tau)/\mu_F$. Also define

$$\begin{aligned} H_T(\tau) &= T^{-1/2}, \\ g_T(\tau) &= c\tau/(\mu_F + \mu_G) \\ Y_T(\tau) &= \int_0^{\tau} H_T(u)\,dM_T(u). \end{aligned}$$

We will apply Theorem 2.1 of Aalen (1977) to $Y_T(\tau)$. We must verify the hypotheses of that result. Clearly as $T \to \infty$,

$$\begin{aligned} \int_0^{\tau} H_T^2(u)\frac{T}{\mu_F}X(Tu)\,du &= \frac{1}{T\mu_F}\int_0^{\tau T} X(u)\,du \\ &\to \frac{c\tau}{\mu_F}\frac{\mu_F}{(\mu_F + \mu_G)} \\ &= \frac{c\tau}{(\mu_F + \mu_G)} = g_T(\tau). \end{aligned}$$

Since the jumps of $N(\tau T)$ are of size 1, we conclude that as $T \to \infty$,

$$Y_T \to_{\mathscr{D}} \sqrt{\frac{c}{(\mu_F + \mu_G)}}\,W,$$

where W is a Wiener process. Specializing to the marginal at $\tau = 1$, we obtain that $T^{-1/2}(N(T) - \mathscr{N}(T))$ converges weakly to a normal distribution with mean 0 and variance $c/(\mu_F + \mu_G)$. Hence

$$\frac{N(T)^{3/2}}{\int_0^T X(s)\,ds}(\hat{\mu}_F - \mu_F)$$

$$= -\left(\frac{N(T)}{\int_0^T X(s)\,ds}\mu_F\right)\frac{\sqrt{N(T)}}{\mu_F}\left(\mu_F - \frac{1}{N(T)}\int_0^T X(s)\,ds\right)$$

$$= -\left(\frac{N(T)}{\int_0^T X(s)\,ds}\mu_F\right)\sqrt{\frac{T}{N(T)}}\,T^{-1/2}\left(N(T) - \int_0^T \frac{1}{\mu_F}X(s)\,ds\right)$$

converges weakly to a standard normal by Slutsky's lemma and the facts that

$$\left(\frac{N(T)}{\int_0^T X(s)\,ds}\mu_F\right) \to 1 \quad \text{almost surely}$$

and

$$\sqrt{\frac{T}{N(T)}} \to \sqrt{\frac{\mu_F + \mu_G}{c}} \quad \text{almost surely.} \qquad \square$$

4.4. *Five examples.* The hypothesis tests and estimation techniques of the previous sections were programmed in FORTRAN on an AMDAHL mainframe. A PASCAL version for the MacIntosh II was also used. Here we will apply these techniques to five sets of data: four simulated data sets and one set obtained from stretch-activated ion channels. The results of the hypothesis tests are summarized in Table 1. For certain examples we present $\hat{F}$ (and F if known) in Figures 1, 2 and 3.

EXAMPLE 1. Two-state Markov model. We simulated the data record of fou ion channels, where each channel is an alternating renewal process, i.e., a two-state model. The distribution of the emitting period for each channel is exponential with mean 0.1 and the distribution of the nonemitting state is exponential with mean 0.2. Figure 1 presents both the true exponential distribution function F and the estimated distribution function $\hat{F}$.

The estimated distribution function is a jagged nonmonotone line roughly following F. The fact that this estimate is not itself a distribution function follows from its definition in Section 2.4 as a weighted average of the observed survival functions $\hat{A}_r(x)$ divided by a continuous decreasing function of an integral in x. This integral $\mathscr{T}(x)$ was computed by interpolating the integrand numerically and is a continuous piecewise linear function, increasing in x. The $\hat{A}_r(x)$ are decreasing piecewise constant functions. Consequently, the estimate $\hat{F}$ will increase over a region where all the $\hat{A}s_r(x)$ are constant and $\mathscr{T}(x)$ increases. On the other hand, if one of the $\hat{A}_r$ shifts downward at x, the estimate will also decrease at that point.

The reader should also note that as x increases, the right tail of the distribution estimate is based on very few observations. Since each channel

TABLE 1

Observed and estimated values for five examples. Descriptive statistics and estimated values of parameters are presented for each of the five examples listed in Section 4.4. *Where independent and identical channels were simulated, the known values of the parameters are given in brackets below the corresponding estimates. For each case, the goodness-of-fit statistic of Section* 2.2 *is presented with the appropriate degrees of freedom.*

		Example 1	**Example 2**	**Example 3**	**Example 4**	**Example 5**
Observed values	Number of transitions	2700	9591	5686	8276	1285
	Number of data vectors	488	1000	822	1000	428
	Maximum current	4	5	9	5	3
	Minimum current	0	0	0	0	0
Estimates	$\hat{\mu}_F$	0.098	0.00097	2.33	0.02180	0.01938
(True)	(μ_F)	(0.100)	(0.00100)			
	$\hat{\mu}_G$	0.190	0.01971	5.33	0.15984	19.363
	(μ_G)	(0.200)	(0.0203)			
	$\hat{p}$	0.340	0.046	0.305	0.12	0.005
	(p)	(0.333)	(0.047)			
	$\hat{c}$	4	10	11	5	50
	(c)	(4)	(10)			
χ^2 statistic	Degrees of freedom	4	3	5	3	2
	Observed value	1.77	1.64	5.33	1.21	32.31

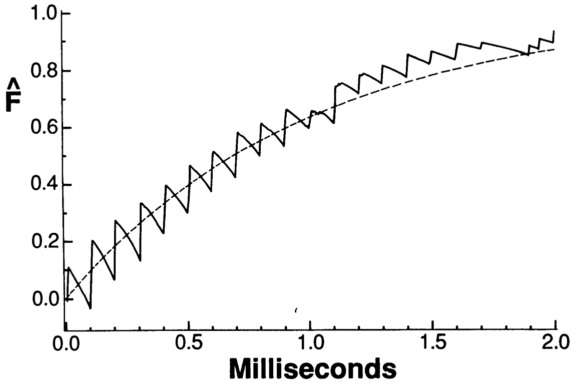

FIG. 1. *Distribution functions for Example* 1 *of Section* 4.4. *In Example* 1 *we considered an alternating renewal process. The distribution function of the length of an open period is known here to be exponential with mean* 1 *msec. This function is shown as a dashed line on the graph. The jagged, solid line is the estimate of the distribution function given by the formula of Section* 2.4.

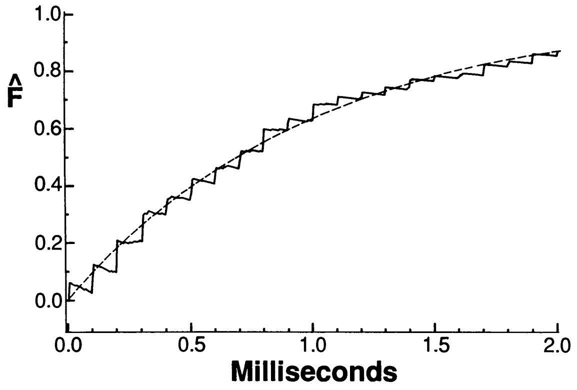

FIG. 2. *Distribution functions for Example* 2 *of Section* 4.4. *In Example* 2 *we considered data simulated according to the model of Colquhoun and Hawkes. The length of an open period in this case has an exponential distribution with mean* 2 *msec. This distribution function is shown as a dashed line on the graph. The jagged, solid line is the estimate of the distribution function given by the formula of Section* 2.4.

censors the observation of long sojourns by the other channels in either the emitting or the nonemitting states, very few long sojourns are observed. The right-tail behaviour of $\hat{F}$ can become very erratic. In particular, if the $\hat{A}_r(x)$ reach 0 before $\mathscr{T}(x)$ becomes 0, then $\hat{F}$ will decrease to 0. The authors have found in their simulated data sets that the estimated distribution function is generally reliable for x up to the minimum of $\hat{\mu}_F$ and $\hat{\mu}_G$. The effect is worst when the $\hat{\mu}_F$ and $\hat{\mu}_G$ are approximately equal; that is, when the censoring

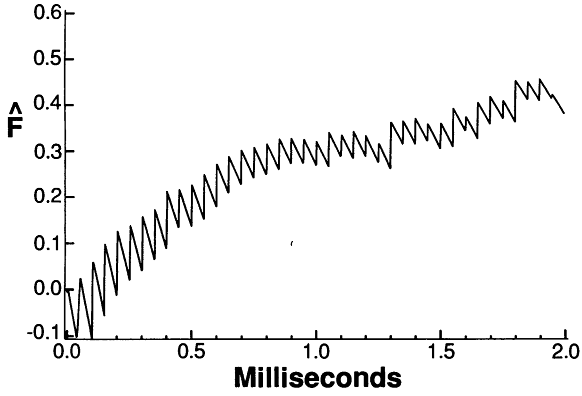

FIG. 3. *Distribution functions for Example* 3 *of Section* 4.4. *In Example* 3 *we considered experimental data on pond snails. The length of an open period of an ion channel is believed to have a mean value of approximately* 2.3 *msec. The jagged, solid line is the estimate of the unknown distribution function of the length of an open period for a single channel.*

effect is probably the greatest. The plots of Figures 1, 2 and 3 are restricted to the region where the estimated distribution function is not too wild.

EXAMPLE 2. Colquhoun and Hawkes four-state channels. We use the notation and constants of Colquhoun and Hawkes [(1982), page 233], who proposed a four-state linear Markov chain to model the behaviour of ion channels. We produced a record of 9591 transitions for 10 superimposed ion channels of this type. Only state AR is emitting. There are long sojourns in the off states and relatively long sojourns in the open state. The ready and tired states are very short.

$$\text{States:}\quad \underset{\text{very tired}}{T} \leftrightarrow \underset{\text{tired}}{AT} \leftrightarrow \underset{\text{ready}}{A_2T} \leftrightarrow \underset{\text{open, emitting}}{AR}$$

Generator for one channel:

$$\begin{bmatrix} -1073.66 & 1073.66 & 0 & 0 \\ 10^4 & -(536.83 + 10^4) & 536.83 & 0 \\ 0 & 2\times 10^4 & -(1.9\times 10^4 + 2\times 10^4) & 1.9\times 10^4 \\ 0 & 0 & 1000 & -1000 \end{bmatrix}$$

Transition matrix for the embedded chain:

$$\begin{bmatrix} 0 & 1 & 0 & 0 \\ 0.9491 & 0 & 0.0509 & 0 \\ 0 & 0.5128 & 0 & 0.4872 \\ 0 & 0 & 1 & 0 \end{bmatrix}$$

The sojourns in the open state are exponentially distributed with mean 0.001 sec. The density of the sojourns in the nonemitting states is

$$f(t) = 12.47\exp(-\lambda_6 t) + 236.2\exp(-\lambda_7 t) + 18750\exp(-\lambda_8 t),$$

where $\lambda_6 = 24.81$, $\lambda_7 = 11211$ and $\lambda_8 = 39376$.

As we indicated earlier, the time interval omission problem poses an important difficulty, particularly in the observation of $N(t)$. We introduced this into our data for a bandwidth of 0.050 msec. Opposing jumps in $X(t)$ closer together than the bandwidth were discarded. This censored data was subjected to further artificial censoring for bandwidths 0.075 and 0.100 msec. For each case, $\hat{\mu}_F$ and $\hat{p}$ were evaluated. The values of the $\hat{p}$ were erratic and could not be extrapolated back to bandwidth 0 with any confidence. However the values of the $\hat{\mu}_F$ were roughly linear. The value predicted for μ_F at bandwidth 0 by a least-squares line was 0.000962 sec. The actual value is 0.001.

EXAMPLE 3. Biological data. C. Morris and W. Sigurdson of the Department of Biology at the University of Ottawa kindly provided us with data on stretch-activated potassium ion channels in the heart and nerve cells of *Lymnaea stagnalis* (pond snails). The estimates are consistent with the results of previous experiments. The estimated distribution function [$\hat{F}(x)$ of Figure

3] is plotted only for x up to 2 msec. Beyond this point, the function became very erratic because of the censoring effect described in Example 1.

EXAMPLE 4. Nonidentical channels. We generated the data record of five independent ion channels where two channels have $p = \frac{1}{3}$ and three have $p = \frac{1}{2}$. Since the convolution of two binomials can be approximately binomial, our hypothesis test fails to pick up the fact that the channels are not all identical. This fact is of some practical importance, as a binomial fit to data is frequently employed in biological analysis. In fact, suppose that the channels had the same p, but different mean times μ_F and μ_G. Then the hypothesis test of this paper, or any test based upon the occupation densities, would not be able to distinguish between independent and identical channels and the case of different independent channels with the same p.

EXAMPLE 5. Dependent channels. We generated the data record of four ion channels where the current record of the fourth channel was a copy of the current record of the first channel. The first and fourth channels are clearly dependent processes. The test of this paper provides significant evidence against the hypothesis of independent and identical channels.

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