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On the superposition of currents from ion channels

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SUMMARY

We derive a number of statistical properties of the superposition of several independent channels contributing to a patch-clamp recording. Failure of these properties indicates dependence of the channels and may suggest the nature of interactions. We show how properties such as dwell-time distributions of the individual channels may be determined from those of the superposition in the case that the channels are independent.

1. INTRODUCTION

Patch-clamp recordings of ion channels often reveal the presence of more than one channel. There may be multiple channels of the same type or mixtures of different types of channels. The constituent channels may or may not be independent. The presence of multiple channels complicates the analysis of such recordings substantially. In this paper we examine several types of superpositions and, under the assumption of independence, derive properties of the superposition which are quantitatively testable. We also show that if independence holds, the dwell-time distributions of the constituent channels can be recovered from the superposition.

The paper is organized as follows: In §2 we introduce certain probability distributions which are equivalent to dwell-time distributions but which are much more tractable to work with in the context of superpositions. In §3 we consider several cases of superpositions of independent channels: identical channels with only two conductance levels ('on' and 'off'), identical channels with more than two conductance levels, and finally non-identical channels. In each of these cases we show how the one- and two-dimensional dwell-time distributions can be recovered from the superposition. In the course of these derivations a number of interesting properties of the dwell-time distributions at the various levels of the superposition are developed. In §4 we suggest methods of estimating relevant probability distributions from a finite record. A closing summary and discussion of open problems is presented in §5.

2. BASIC DEFINITIONS

In this section we define certain probability distributions related to and equivalent to more commonly used dwell-time distributions. As we will see in the next section, the former are more natural to use in the analysis of superpositions. Here we focus on a single ion channel which, for simplicity of exposition and notation, we assume has either zero or unit conductance, although the analysis is basically unchanged for a channel with multiple conductance levels. We will let $X(t)$ denote the conductance at time t. We will assume throughout that the process $X(t)$ is stationary.

The probability distribution that will be particularly useful to us in considering superpositions is

$$g_o(t) = P(X(s) = 1, 0 \leqslant s \leqslant t), \quad (1)$$

the unconditional probability that the channel is open for a length of time t regardless of when it opened or when it closed. Saying this in another way, $g_o(t)$ is the probability that during a randomly placed interval of length t the channel is open. The definition implies that $g_o(t)$ is a non-increasing function. It also follows that

$$g_o(0) = P(X(0) = 1) = \pi_o,$$

the probability that the channel is open. Note that

$$\frac{g_o(t)}{\pi_o} = P(X(s) = 1, 0 \leqslant s \leqslant t \,|\, X(0) = 1).$$

We define the closing rate of the channel as

$$\zeta_c = \lim_{h\to 0^+} \frac{1}{h} P(X(0) = 1 \,\&\, X(h) = 0)$$

$$= \lim_{h\to 0^+} \frac{1}{h} P(X(h) = 0 \,|\, X(0) = 1) P(X(0) = 1),$$

and the opening rate is defined similarly. When there are only two conductance levels (zero and one), $\zeta_c = \zeta_o = \zeta$. Under reasonable assumptions, $\zeta = -g_o'(0)$. To establish this we will assume the smoothness condition

$$P(X(s) = 1, 0 \leqslant s \leqslant h \,|\, X(0) = 1) = P(X(h) = 1) \,|\, X(0) = 1 + o(h).$$

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This condition essentially stipulates that transitions do not occur too rapidly. Then

$$\lim_{h\to 0^+}\frac{1}{h}(g_o(h)-g_o(0))$$
$$=\lim_{h\to 0^+}\frac{1}{h}\left[\frac{P(X(s)=1, 0\leqslant s\leqslant h)}{P(X(0)=1)}-1\right]\times P(X(0)=1)$$
$$=\lim_{h\to 0^+}\frac{1}{h}[P(X(h)=1\,|\,X(0)=1)+o(h)-1]\times P(X(0)=1)$$
$$=-\zeta,$$

where the last step follows from

$$P(X(h)=1\,|\,X(0)=1)-1=-P(X(h)=0\,|\,X(0)=1).$$

We now relate the g functions to more commonly used dwell-time distributions. Let $F_o(t)$ be the cumulative distribution function for an open dwell-time (the probability that the dwell time is less than or equal to t) and let

$$\bar{F}_o(t)=1-F_o(t)=P(T>t)$$

denote the survival function. g_o and $\bar{F}$ are related by a fundamental result in the theory of stationary point processes (Cox & Miller 1965):

$$\frac{g_o(t)}{\pi_o}=\frac{1}{\mu_o}\int_t^\infty \bar{F}_o(u)\,du.$$

Here μ_o is the mean open dwell time. This result has been used in the study of superpositions by Dabrowski *et al.* (1989) and Yeo *et al.* (1989). From this it follows that

$$\bar{F}_o(t)=-\frac{\mu_o}{\pi_o}g_o'(t). \tag{2}$$

and since $\bar{F}_o(0)=1$, we have

$$\mu_o=-\frac{g_o(0)}{g_o'(0)}=\frac{\pi_o}{\zeta}. \tag{3}$$

Differentiating equation (2) we find probability density function of an open dwell time,

$$f_o(t)=-\frac{g_o''(t)}{g_o'(0)},$$

from which it follows that $g_o''(t)\geqslant 0$, or that $g_o(t)$ is convex.

The underlying state of the channel is frequently modelled as a stationary Markov process with a finite number of states, the 'open states' being conducting and the 'closed states' being non-conducting. We denote by Q the matrix of infinitesimal transition probabilities (kinetic rates) and partition Q as Q_{oo}, Q_{oc}, Q_{co} and Q_{cc} as in Colquhoun & Hawkes (1981). Let ρ be the row vector of equilibrium probabilities for the underlying process (ρ_i is the probability that the process is in state i) and let ρ be partitioned as ρ_o and ρ_c. Then

$$g_o(t)=\rho_o\,e^{Q_{oo}t}\,\mathbf{1},$$

where $\mathbf{1}$ is a vector of ones. The probability that the channel is open is $\pi_o=\rho_o\mathbf{1}$. The opening or closing rate is

$$\zeta=\rho_o Q_{oc}\mathbf{1}=\rho_c Q_{co}\mathbf{1}=-\rho_o Q_{oo}\mathbf{1}=-\rho_c Q_{cc}\mathbf{1}.$$

These identities follow as ρ and $\mathbf{1}$ are left and right eigenvectors of Q with eigenvalue 0.

We will make use of two-dimensional g functions defined in the following way: $g_{co}(s,t)$ is the probability that the channel opens at time $t=0$, having been previously closed for a duration of time at least s and is subsequently open for a duration of time at least t. If S denotes the time that the channel is closed before opening, and T the time that channel is open, then,

$$g_{co}(s,t)=\zeta P(S>s\ \&\ T>t). \tag{4}$$

This two-dimensional function is related to the one-dimensional functions by

$$g_{co}(0,t)=-g_o'(t)=\zeta\bar{F}_o(t), \tag{5}$$
$$g_{co}(s,0)=-g_c'(s)=\zeta\bar{F}_c(s). \tag{6}$$

Also

$$g_{co}(0,0)=-g_c'(0)=\zeta. \tag{7}$$

In the case that the successive open and closed dwell times are independent, as in an alternating renewal process,

$$g_{co}(s,t)=\zeta\bar{F}_c(s)\,\bar{F}_o(t).$$

If the process is time reversible, then $g_{co}(s,t)=g_{oc}(t,s)$ and the joint density of a closed and following open time is given by

$$f_{co}(s,t)=\frac{1}{\zeta}\frac{\partial^2}{\partial s\,\partial t}g_{co}(s,t).$$

Since the probability structure of a Markovian model is determined by the two-dimensional open–closed and closed–open probability density functions (Fredkin *et al.* 1983), it is also determined by the two-dimensional g functions.

3. THE SUPERPOSITION OF INDEPENDENT CHANNELS

In this section we use the functions g defined in the previous section to characterize the superposition of n independent channels as a stochastic process:

$$S(t)=X_1(t)+X_2(t)+\ldots+X_n(t).$$

(a) *Identical bi-level channels*

We first consider the case in which each channel has two conductance levels, zero and one. Let $g_c(t)$ and $g_o(t)$ be defined as above and consider

$$G_k(t)=P(S(u)=k, 0\leqslant u\leqslant t).$$

$G_k(t)$ is the probability that, during a randomly placed time interval of length t, k of the n channels are open and $n-k$ are closed. The G_k are the analogues for the superposition process of the g's for a single channel. $G_k(0)$ is the probability that there are k channels open in the superposition, and $-G_k'(0)$ is the rate at which level k of the superposition is entered (which equals the rate at which it is exited).

We will see that many properties of the superposition process are most simply expressible in terms of $G_k(t)$, $k = 0, 1, \ldots, n$. In particular, because the channels are independent,

$$G_k(t) = \binom{n}{k} g_o(t)^k \, g_c(t)^{n-k}. \tag{8}$$

From the results of the previous section we see that the cumulative distribution function and the probability density function of the dwell times of the superposition at level k can be obtained from differentiating $G_k(t)$. Doing so yields the results reported in Dabrowski *et al.* (1989) and Yeo *et al.* (1989). Observe that if the open and closed dwell-time densities are sums of exponentials, the resulting expressions will be quite unwieldy, containing a large number of exponentials with rates being linear combinations of the rate parameters occurring in the dwell-time densities.

From equation (3), the average duration of an excursion in level k is (Yeo *et al.* 1989)

$$\begin{aligned}\mu_k &= -\frac{G_k(0)}{G_k'(0)} \\ &= -\left(\frac{\mathrm{d}}{\mathrm{d}t}\ln G_k(0)\right)^{-1} \\ &= \left(\frac{k}{\mu_o} + \frac{n-k}{\mu_c}\right)^{-1} \\ &= \frac{\mu_o \mu_c}{k\mu_c + (n-k)\,\mu_o}.\end{aligned} \tag{9}$$

These expressions are useful in assessing the biases incurred if an analysis is based on an incorrect supposition of the number of channels. Suppose, for example, that $n = 2$, but that since doublets are never observed in a recording, the analysis proceeds as if $n = 1$. Then the average duration of level one dwell times will be taken as the average open time:

$$\mu_1 = \frac{\mu_o \mu_c}{\mu_c + \mu_o} = \mu_o \left(\frac{\mu_c}{\mu_o + \mu_c}\right).$$

If the average closed dwell time is much larger than the average open time, μ_1 will differ little from μ_o, so there will only be a small amount of bias in the estimate of μ_o. However, because from equation (9), $\mu_0 = \mu_c/2$, the mean closed dwell time will be seriously underestimated.

Continuing with the case of $n = 2$, the survival function for a level one dwell time is

$$\begin{aligned}\bar{F}_1(t) &= \frac{G_1'(t)}{G_1'(0)} \\ &= \bar{F}_o(t)\, g_c(t) + \bar{F}_c(t)\, g_o(t),\end{aligned}$$

which follows after some algebra, using the relations $g_o(0) + g_c(0) = 1$ and $g_c'(0) = g_o'(0)$. Suppose that the channel is predominately closed and that the closed times tend to be long; then for small t, $1 \approx g_c(t) \gg g_o(t)$ so that $\bar{F}_1(t) \approx \bar{F}_o(t)$. In this situation, an analysis unaware of the existence of two channels in the patch would be expected to estimate a survival function for the open dwell time that was reasonably accurate for small t, but that was increasingly biased for large t. The relation between the survival function at level zero and the actual closed dwell-time survival function is given by

$$\begin{aligned}\bar{F}_0 &= \frac{G_0'(t)}{G_0'(0)} \\ &= \bar{F}_c(t)\, \frac{g_c(t)}{g_c(0)}.\end{aligned}$$

From this expression we see that $\bar{F}_0(t)$ decays more rapidly than does $\bar{F}_c(t)$, so that inferences about the closed-time distribution will be more severely biased than those about the open-time distribution.

The probabilities $G_k(0)$ have been widely used in the biophysical literature to examine the hypothesis of identical independent channels (for example, see Jackson (1985), Thorn & Martin (1987) and Glasbey & Martin (1988)). Because $g_o(0)$ and $g_c(0)$ are the probabilities that a channel is open and closed respectively, $g_o(0) + g_c(0) = 1$, and equation (8) implies that the $G_k(0)$ are a binomial distribution. The relative frequencies of times spent at levels $k = 0, 1, \ldots, n$ have been compared with those probabilities. A similar comparison can be done for each t: from the binomial expansion, the probability that the conductance of the superposition does not change during a length of time t is

$$\begin{aligned}G_+(t) &= \sum_{k=0}^{n} G_k(t) \\ &= (g_o(t) + g_c(t))^n.\end{aligned}$$

Define $H_k(t)$ to be the probability that superposition is at level k for a length of time at least t given that it does not change level during that time:

$$\begin{aligned}H_k(t) &= \frac{G_k(t)}{G_+(t)} \\ &= \binom{n}{k}\left(\frac{g_o(t)}{g_o(t)+g_c(t)}\right)^k \left(1 - \frac{g_o(t)}{g_o(t)+g_c(t)}\right)^{n-k}.\end{aligned} \tag{10}$$

From this expression we see that the model of independent identical channels implies that the $H_k(t)$ are a binomial distribution.

The single-channel functions $g_c(t)$ and $g_o(t)$ can be recovered from the $G_k(t)$: because the mean of a binomial distribution with n trials and success probability p is np,

$$\frac{g_o(t)}{g_o(t)+g_c(t)} = \frac{1}{n}\sum_{k=0}^{n} kH_k(t),$$

and thus

$$\begin{aligned}g_o(t) &= \frac{g_o(t)+g_c(t)}{G_+(t)}\,\frac{1}{n}\sum_{k=0}^{n} kG_k(t) \\ &= \frac{1}{nG_+(t)^{(n-1)/n}}\sum_{k=0}^{n} kG_k(t).\end{aligned} \tag{11}$$

The relation between $g_o(t)$ and the $G_k(t)$ is thus fairly direct. We note that in particular

$$g_o(0) = \frac{1}{n}\sum_{k=0}^{n} kG_k(0), \tag{12}$$

is the probability that a constituent channel is open, as $G_+(0) = 1$. An expression similar to equation (11) can be obtained for $g_c(t)$:

$$g_c(t) = \frac{1}{nG_+(t)^{(n-1)/n}}\sum_{k=0}^{n}(n-k)\,G_k(t).$$

The dwell-time densities can be obtained by differentiation, but it is not clear to us that there is any particular advantage, other than conventionality, for using these as basic objects rather than g_o and g_c. Note that for a Markov model $f_o(t)$ and $g_o(t)$ are both sums of the same exponentials, differing only in amplitudes.

We now turn to the two-dimensional G functions, the analogues of the functions g_{oc} and g_{co} introduced in the previous section. Consider $G_{k,k+1}(s,t)$: in terms of the constituent channels, this is the probability that (i) one channel undergoes a transition from closed to open, (ii) k channels are open throughout an interval of length $s+t$, and (iii) $n-k-1$ channels are closed throughout the interval. We thus have

$$G_{k,k+1}(s,t) = ng_{co}(s,t)\binom{n-1}{k}g_o(s+t)^k\,g_c(s+t)^{n-k-1}. \tag{13}$$

Similarly,

$$G_{k,k-1}(s,t) = ng_{oc}(s,t)\binom{n-1}{k-1}g_o(s+t)^{k-1}\,g_c(s+t)^{n-k}.$$

In particular, $G_{k,k+1}(0,0)$ is the rate at which the superposition makes transitions from level k to level $k+1$:

$$G_{k,k+1}(0,0) = n\zeta\binom{n-1}{k}\pi_o^k(1-\pi_o)^{n-k-1}.$$

Summing equation (13) from $k=0$ to $n-1$ we find

$$\begin{aligned} G_{++}(s,t) &= ng_{co}(s,t)\,(g_o(s+t)+g_c(s+t))^{n-1} \\ &= ng_{co}(s,t)\,G_+(s+t)^{(n-1)/n}. \end{aligned} \tag{14}$$

From this expression we see that $g_{co}(s,t)$ can be found from $G_{++}(s,t)$ and $G_+(s+t)$. $g_{oc}(s,t)$ can be determined similarly. Thus the joint closed–open and open–closed dwell-time probability density functions can be found from statistics of the superposition process. As remarked above, under mild assumptions on a Markov model, these joint density functions determine the entire probability law of the single-channel processes, and as a consequence the entire probability law of the single-channel processes can be determined from the superposition (that this is true may also be seen from the closing remarks of Fredkin & Rice 1987).

As a special case of equation (14), at $s=t=0$ we have $G_{++}(0,0) = ng_{co}(0,0)$ which is n times the average opening rate. This result makes intuitive sense since $G_{++}(0,0)$ is the rate at which the superposed process jumps to a higher level and each such jump is the contribution of a single channel opening. From this expression and from equation (12) we see that the mean open time of a single channel can be found from the superposition by

$$\mu_o = \frac{\sum_{k=0}^{n} kG_k(0)}{G_{++}(0,0)}. \tag{15}$$

This relation was also noted by Dabrowski *et al.* (1990), with quite different notation, in the case of an alternating renewal process. This simple expression would appear to be quite useful as it only depends weakly on n, which may not be known accurately. The numerator and denominator can be estimated easily from a multi-channel recording, and even if the recording contains no excursions to high levels, this is presumably the case because the corresponding $G_k(0)$ are quite small and thus have little effect on the numerator.

Let S be the duration of a closing and T the duration of the subsequent opening of a constituent channel. Thus from equations (4) and (14), we have

$$\begin{aligned} P(S>s\ \&\ T>t) &= \frac{ng_{co}(s,t)}{n\zeta} \\ &= \frac{G_{++}(s,t)}{G_{++}(0,0)\,G_+(s+t)^{(n-1)/n}}. \end{aligned}$$

The survival function of an open time can be obtained by evaluating this expression at $s=0$ giving

$$\bar{F}_o(t) = \frac{G_{++}(0,t)}{G_{++}(0,0)\,G_+(t)^{(n-1)/n}}. \tag{16}$$

It is noteworthy that if n is large, it need not be known with great precision to use these expressions to provide estimates of the bivariate and univariate survival functions. In fact, for large n, the exponent $(n-1)/n$ can be replaced by 1 with little consequent bias. Leibowitz & Dionne (1984), for example, report experiments in which the number of acetylcholine receptors in a patch at a neuromuscular junction is of the order 100–1000.

When normalized to sum to one, the values of $G_{k,k+1}(s,t)$ are related as binomial probabilities. Let

$$\begin{aligned} H_{k,k+1}(s,t) &= \frac{G_{k,k+1}(s,t)}{G_{++}(s,t)} \\ &= \binom{n-1}{k}\left(\frac{g_o(s+t)}{g_o(s+t)+g_c(s+t)}\right)^k \\ &\quad\times\left(1-\frac{g_o(s+t)}{g_o(s+t)+g_c(s+t)}\right)^{n-k-1}. \end{aligned} \tag{17}$$

From this expression we see that the values of $H_{k,k+1}(s,t), k = 0,1,\ldots,n-1$, are binomial probabilities, providing another testable consequence of the model of independent identical channels. Furthermore, these probabilities only depend on s and t through $s+t$. The case $s=t=0$ yields

$$H_{k,k+1}(0,0) = \binom{n-1}{k}\pi_o^k(1-\pi_o)^{n-k-1}, \tag{18}$$

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which expresses the intuitively obvious fact that the relative rates at which transitions are made to higher levels are related as binomial probabilities.

Comparing equations (17) and (10), we see that the model also predicts interesting relations between the H functions with two arguments and those with one, for example:

$$\frac{1}{n-1}\sum_{k=0}^{n-1} kH_{k,k+1}(s,t) = \frac{g_o(s+t)}{g_o(s+t)+g_c(s+t)} = \frac{1}{n}\sum_{k=0}^{n} kH_k(s+t). \quad (19)$$

(*b*) *Identical multi-level channels*

Non-degenerate case

Extension of the previous section to the superposition of independent identical channels with more than two conductance levels is straightforward if the conductance levels are incommensurate. Let each channel have $s+1$ conductance levels, $Y_0 = 0, Y_1, \ldots, Y_s$. The conductance state of n channels is characterized by an $s+1$ dimensional vector of integers $\nu = (\nu_0 \ldots \nu_s)$, where ν_k is the number of channels in conductance level k†. By incommensurate we mean that an ideal observation of the conductance $Y_{\text{obs}} = \nu \cdot Y$ determines the integer vector ν uniquely. Specifically if ι is a vector of integers then $\iota \cdot Y = 0$ implies that $\iota_k = 0, k = 1, \ldots, s$. In the next section we will describe some of the complications that ensue when this assumption of incommensurability fails.

We define $G_\nu(t)$ to be the probability that the n-channel system is in the state characterized by ν during a random time interval of duration t. Then, as in the case of bi-level channels,

$$G_\nu(t) = \frac{n!}{\nu!} g(t)^\nu,$$

where $g_k(t)$ is the probability that a given channel is in conductance state Y_k during a random time interval of duration t, the quantity analogous to $g_c(t)$ and $g_o(t)$ in the previous section. As we have done before, we introduce the probability $G_+(t)$ that the n-channel system makes no transitions during a random time interval of duration t,

$$G_+(t) = \sum_\nu G_\nu(t) = |g(t)|^n,$$

and the conditional probability of state ν, given that no transitions occur,

$$H_\nu(t) = \frac{G_\nu(t)}{G_+(t)}.$$

The $H_\nu(t)$ are a multinomial distribution.

If the n-channel system is in state ν during a random time interval of duration t, the fraction of channels in conductance level k is ν_k/n, which provides an estimate of the one channel conditional probability $g_k(t)/|g(t)|$ for conductance level k. Weighting this estimate with the probability of state ν, $H_\nu(t)$, we arrive at the estimate

$$\frac{g(t)}{|g(t)|} = \sum_\nu H_\nu(t)\, \nu/n$$

or

$$g(t) = \frac{G_+(t)^{1/n}}{n} \sum_\nu H_\nu(t)\, \nu = \frac{\sum_\nu G_\nu(t)\, \nu}{nG_+(t)^{(n-1)/n}}$$

for the vector of one-channel probabilities $g(t)$, in analogy with equation (11). This is, in fact, the maximum likelihood estimator.

We turn now to the two time functions for one or many channels. For one channel, we define $g_{ij}(s,t)$ as the probability that the channel makes a transition from conductance level Y_i to level Y_j at time $t = 0$, having been previously in level Y_i for a duration of time at least s and is subsequently in level Y_j for a duration of time at least t. For $s = t = 0$, we obtain the $Y_i \rightarrow Y_j$ transition rate ζ_{ij}:

$$g_{ij}(0,0) = \zeta_{ij},$$

and the analogues of equations (5) and (6) are

$$g_{ij}(0,t) = \zeta_{ij}\bar{F}_j(t), \quad (20)$$

$$\sum_i g_{ij}(0,t) = -g'_j(t), \quad (21)$$

$$g_{ij}(s,0) = \zeta_{ij}\bar{F}_i(s), \quad (22)$$

$$\sum_j g_{ij}(s,0) = -g'_i(s), \quad (23)$$

where $\bar{F}_i(t)$ is the survival function for level Y_i. If we define π_i to be the equilibrium probability of level Y_i, so that $\pi_i = g_i(0)$, and μ_i to be the mean dwell time in level Y_i, it follows from equations (20–23) and

$$\mu_i = \int_0^\infty \bar{F}_i(t)\,\mathrm{d}t,$$

that

$$\mu_i = \frac{\pi_i}{\sum_j \zeta_{ji}} = \frac{\pi_i}{\sum_j \zeta_{ij}},$$

which are equivalent because the rate of entry into level Y_i, $\sum_j \zeta_{ji}$, must equal the rate of exit from that level, $\sum_j \zeta_{ij}$.

To discuss the two time many channel functions, let us define the $s+1$ component vectors ϵ^i to have ith component unity and all other components zero. Let κ be any vector of non-negative integers with $|\kappa| = n-1$. Define $G^\kappa_{ij}(s,t)$ to the probability that some channel makes a transition $Y_i \rightarrow Y_j$ at time $t = 0$, the system of channels having been previously in the state described by the vector $\kappa + \epsilon^i$ for a duration of time at least s and is subsequently in the state described by the vector $\kappa + \epsilon^j$ for a duration of time at least t. Then

$$G^\kappa_{ij}(s,t) = nG_\kappa(s+t)\, g_{ij}(s,t) = \frac{n!}{\kappa!} g(s+t)^\kappa g_{ij}(s,t),$$

† In this section the following standard mathematical notation will be used in connection with vectors of integers: $|\nu| = \Sigma_k \nu_k$, $\nu! = \Pi_k \nu_k!$, and, if x is any real vector, $x^\nu = \Pi_k x_k^{\nu_k}$ and $\nu \cdot x = \Sigma_k \nu_k x_k$.

the analogue of equation (13). The quantities $G^{\kappa}_{ij}(s,t)$ can be directly estimated from observations, as we discuss in §4. To estimate $g_{ij}(s,t)$, define

$$G_{ij}(s,t) = \sum_{\kappa} G^{\kappa}_{ij}(s,t) = n|g(s+t)|^{n-1}\, g_{ij}(s,t)$$
$$= nG_{+}(s+t)^{n-1/n}\, g_{ij}(s,t), \qquad (24)$$

the analogue of equation (14). $G_{ij}(s,t)$ is the probability of a $Y_i \rightarrow Y_j$ transition at 0, say, such that there are no other transitions during the time interval $(-s,t)$.

It is interesting to note that, for $s=t=0$, these relations become

$$G^{\kappa}_{ij}(0,0) = \frac{n!}{\kappa!}\pi^{\kappa}\zeta_{ij}$$

and

$$G_{ij}(0,0) = n\zeta_{ij}, \qquad (25)$$

so that

$$\frac{G^{\kappa}_{ij}(0,0)}{G_{ij}(0,0)} = \frac{|\kappa|!}{\kappa!}\pi^{\kappa},$$

a multinominal distribution independent of the pair ij. Moreover

$$\sum_j G_{ij}(0,0) = n\sum_j \zeta_{ij} = \frac{n\pi_i}{\mu_i}$$
$$= \frac{\Sigma_{\nu} G_{\nu}(0)\, \nu_i}{\mu_i},$$

so that, in terms of observable quantities,

$$\mu_i = \frac{\Sigma_{\nu} G_{\nu}(0)\,\nu_i}{\Sigma_j G_{ij}(0,0)}, \qquad (26)$$

the analogue of equation (15).

The bivariate survival function of a dwell time at level i and a subsequent dwell time at level j is

$$\bar{F}_{ij}(s,t) = \frac{g_{ij}(s,t)}{\zeta_{ij}}.$$

From equations (24) and (25) we have

$$\bar{F}_{ij}(s,t) = \frac{G_{ij}(s,t)}{G_{ij}(0,0)\, G_{+}(s+t)^{(n-1)/n}}.$$

The univariate survival functions can be found by summing equation (20) over i and use equations (24) and (25) to give

$$\bar{F}_j(t) = \frac{\Sigma_i G_{ij}(0,t)}{G_{+}(t)^{(n-1)/n}\,\Sigma_i G_{ij}(0,0)}. \qquad (27)$$

Again, as in the previous section, we see that equation (26) does not depend strongly on n and that equation (27) is insensitive to the value of n when n is large.

Degenerate case

In the case that some of the conductance levels are commensurate, the superposition of independent, identical channels each having more than two conductance levels does not lead to relations quite so neat as those of the previous section. Rather than carrying out the analysis in the greatest generality, we will consider a particular, but non-trivial case. Suppose that each of n independent, identical channels has three conductance levels: zero, one, and two. (Labarca *et al.* (1985) consider a chloride channel which has this character.) Note that, unlike the incommensurate case, there is considerable ambiguity in such a superposition; for example a current of level three may arise from three channels being open with unit conductance or from one being open at level two and one being open at level one. None the less, we will see that the statistics of the individual channels can be recovered from those of the superposition. The superposition thus has possible conductances $k = 0, 1, \ldots, 2n$ with

$$G_k(t) = \Sigma' \frac{n!}{n_0!\,n_1!\,n_2!} g_0(t)^{n_0} g_1(t)^{n_1} g_2(t)^{n_2},$$

where Σ' denotes the sum over n_0, n_1, n_2 such that $n_0+n_1+n_2 = n$ and $n_1+2n_2 = k$ (n_0, n_1, and n_2 are the numbers of channels at levels zero, one and two). Let $H_k(t) = G_k(t)/G_{+}(t)$ as before, where $G_{+}(t) = \Sigma_k G_k(t)$.

In §3*a*, viewing the $H_k(t)$ as a binomial distribution made it possible to express the g functions in terms of the mean of that distribution and thus ultimately in terms of the G functions. Here we will use a similar approach, but as the $H_k(t)$ are not a binomial distribution, the relations are not so simple. We will see that in order to recover the g functions, it will be necessary to use the mean and variance of the distribution. To motivate the analysis, suppose that V is a random variable taking on values $v = 0, 1, 2$ with probabilities p_0, p_1, p_2. The mean of V is

$$\mu = p_1 + 2p_2,$$

and

$$E(V^2) = p_1 + 4p_2 = \sigma^2 + \mu^2.$$

Let $T = V_1 + V_2 + \ldots + V_n$ where the V_i are independent and identically distributed as V. Then T has mean and variance given by

$$\mu_T = n\mu$$
$$\sigma^2_T = n\sigma^2.$$

The probabilities p_1 and p_2 can be expressed in terms of μ_T and σ^2_T as

$$p_1 = n^{-1}(2\mu_T - \sigma^2_T - n^{-1}\mu^2_T), \qquad (28)$$
$$p_2 = (2n)^{-1}(-\mu_T + \sigma^2_T + n^{-1}\mu^2_T), \qquad (29)$$

and $p_0 = 1 - p_1 - p_2$.

Now the distribution defined by the $H_k(t)$ is the same as the distribution of T above. Because every value of $g_0(t)^{n_0} g_1(t)^{n_1} g_2(t)^{n_2}$ with $n_0+n_1+n_2 = n$ enters into $G_{+}(t)$ with multiplicity given by the corresponding multinomial coefficient, we have

$$G_{+}(t) = (g_0(t) + g_1(t) + g_2(t))^n.$$

The analogues to the p_i above are

$$p_i(t) = \frac{g_i(t)}{g_0(t)+g_1(t)+g_2(t)}$$

which can be solved for in terms of the mean and variance of the distribution defined by the $H_k(t)$ using equations (28) and (29). Having found the $p_i(t)$ the $g_i(t)$ are found as

$$g_i(t) = G_+(t))^{1/n}\, p_i(t).$$

Thus the dwell-time distributions of the constituent channels can be recovered from the superposition.

The two-dimensional G functions determine the two-dimensional g functions. To see this, consider $G_{0,1}(s,t)$: one channel makes a $0 \rightarrow 1$ transition and the remaining $n-1$ are closed, giving

$$G_{0,1}(s,t) = nG_0^{(n-1)}(s+t)\, g_{01}(s,t),$$

where $G_0^{(n-1)}(s+t)$ is the G function for a superposition of $n-1$ channels; $G_0^{(n-1)}(s+t)$ can be expressed in terms of the one-dimensional g functions which have shown how to determine above. Similarly, we have the equations,

$$G_{k,k+1}(s,t) = nG_k^{(n-1)}(s+t)\,(g_{01}(s,t)+g_{12}(s,t)),\qquad k = 1,\ldots,2n-2$$

and

$$G_{2n-1,2n}(s,t) = nG_k^{(n-1)}(s+t)\, g_{12}(s,t).$$

The functions $g_{01}(s,t)$ and $g_{12}(s,t)$ can be found by solving this overdetermined system of $2n$ equations. Finally,

$$G_{k,k+2}(s,t) = nG_k^{(n-1)}(s,t)\, g_{02}(s,t), \quad k = 0,\ldots,2n-2$$

from which $g_{02}(s,t)$ can be determined as

$$g_{02}(s,t) = \frac{1}{n}\frac{\sum_{k=0}^{2n-2} G_{k,k+2}(s,t)}{\sum_{k=0}^{2n-2} G_k^{(n-1)}(s+t)}.$$

(*c*) *Non-identical channels*

To see some of the issues that arise in this case, consider an example of two channels in which channel I has conductance levels zero and Y_1 and channel II has conductance levels zero and $Y_2 \neq Y_1$. The superposition thus has levels zero, Y_1, Y_2, and Y_1+Y_2, which we denote by $k = 0, 1, 2, 3$. Then

$$G_0(t) = g_c^{(1)}(t)\, g_c^{(2)}(t)$$
$$G_1(t) = g_o^{(1)}(t)\, g_c^{(2)}(t)$$
$$G_2(t) = g_c^{(1)}(t)\, g_o^{(2)}(t)$$
$$G_3(t) = g_o^{(1)}(t)\, g_o^{(2)}(t),$$

where $g^{(1)}$ and $g^{(2)}$ are the g functions for channels I and II. Examining these equations, we see that if

$$\tilde{g}_c^{(1)}(t) = \rho(t)\, g_c^{(1)}(t)$$
$$\tilde{g}_o^{(1)}(t) = \rho(t)\, g_o^{(1)}(t)$$
$$\tilde{g}_c^{(2)}(t) = \rho(t)^{-1}\, g_c^{(2)}(t)$$
$$\tilde{g}_o^{(2)}(t) = \rho(t)^{-1}\, g_o^{(2)}(t)$$

for any $\rho(t) > 0$, the G functions are unchanged. All solutions for g functions in terms of the observable G functions are of this form.

Because $\tilde{g}_c^{(i)}(0)+\tilde{g}_o^{(i)}(0) = g_c^{(i)}(0)+g_o^{(i)}(0) = 1$, $i = 1,2$, we must have $\rho(0) = 1$. Consequently, the equilibrium probabilities for the channels are uniquely determined as

$$g_c^{(1)}(0) = G_0(0)+G_2(0)$$
$$g_c^{(2)}(0) = G_0(0)+G_1(0).$$

We next show that the ambiguity can be resolved by considering the two-dimensional g functions, which must satisfy equations like

$$G_{01}(s,t) = \tilde{g}_{co}^{(1)}(s,t)\, \tilde{g}_c^{(2)}(s+t) = \tilde{g}_{co}^{(1)}(s,t)\, \frac{g_c^{(2)}(s+t)}{\rho(s+t)},$$

where $G_{01}(s,t)$ is observable and $\tilde{g}_c^{(2)}(s+t)$ is found from the one-dimensional G. Because

$$G_{01}(s,t) = g_{co}^{(1)}(s,t)\, g_c^{(2)}(s+t),$$

we must have

$$\tilde{g}_{co}^{(1)}(s,t) = \rho(s+t)\, g_{co}^{(1)}(s,t)$$

and similarly

$$\tilde{g}_{co}^{(2)}(s,t) = \rho(s+t)^{-1}\, g_{co}^{(2)}(s,t).$$

We then have

$$\tilde{g}_{co}^{(1)}(s,0) = \rho(s)\, g_{co}^{(1)}(s,0) = -\rho(s)\, g_c^{(1)\prime}(s)$$

by equation (5). We must also have

$$\tilde{g}_{co}^{(1)}(s,0) = -\tilde{g}_c^{(1)\prime}(s) = -\rho'(s)\, g_c^{(1)}(s) - \rho(s)\, g_c^{(1)\prime}(s).$$

Equating these two expressions for $\tilde{g}_{co}^{(1)}(s,0)$ we find

$$\rho'(s)\, g_c^{(1)}(s) = 0.$$

Unless $g_c^{(1)}(s) = 0$, in which case $\tilde{g}_c^{(1)}(s) = g_c^{(1)}(s)$, we must have $\rho'(s) = 0$. This together with the condition $\rho(0) = 1$ implies that $\rho(s) = 1$. Therefore, there is no ambiguity after all.

Let us see then how we can recover information from the two-dimensional G functions. We have

$$G_{01}(s,t) = g_{co}^{(1)}(s,t)\, g_c^{(2)}(s+t) \tag{30}$$
$$G_{02}(s,t) = g_{co}^{(2)}(s,t)\, g_c^{(1)}(s+t) \tag{31}$$
$$G_{12}(s,t) = g_{co}^{(2)}(s,t)\, g_o^{(1)}(s+t) \tag{31}$$
$$G_{23}(s,t) = g_{co}^{(1)}(s,t)\, g_o^{(2)}(s+t). \tag{33}$$

Evaluating these at $s = t = 0$ we have

$$G_{01}(0,0) = \zeta^{(1)}\pi_c^{(2)}$$
$$G_{02}(0,0) = \zeta^{(2)}\pi_c^{(1)}$$
$$G_{12}(0,0) = \zeta^{(2)}\pi_o^{(1)}$$
$$G_{23}(0,0) = \zeta^{(1)}\pi_o^{(2)}.$$

These equations yield

$$\zeta^{(1)} = G_{01}+G_{23}$$
$$\zeta^{(2)} = G_{02}+G_{12}$$
$$\pi_c^{(1)} = G_{02}/(G_{02}+G_{12})$$
$$\pi_c^{(2)} = G_{01}/(G_{01}+G_{23})$$

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where we have suppressed the arguments $(0,0)$ of the Gs. The closed (and hence open) probabilities and the opening and closing rates for each of the channels can thus be simply computed. From these we can find the mean dwell times:

$$\mu_c^{(1)} = \frac{G_{02}}{(G_{02}+G_{12})\,(G_{01}+G_{23})}$$

$$\mu_c^{(2)} = \frac{G_{01}}{(G_{02}+G_{12})\,(G_{01}+G_{23}}).$$

$\mu_o^{(1)}$ and $\mu_o^{(2)}$ are given by similar expressions (the numerators G_{02} and G_{01} are replaced by G_{12} and G_{23}).

Although we have shown that the two-channel functions of the superposition uniquely determine the one-channel functions of the constituents, we have not yet given a constructive procedure for actually finding the latter. The uniqueness argument used the two-dimensional functions of the superposition, so it is reasonable to look to these for the desired construction. By using equations (5) and (6) we have the following eight equations:

$$G_{01}(s,0) = -g_c^{(1)\prime}(s)\, g_c^{(2)}(s)$$
$$G_{02}(s,0) = -g_c^{(2)\prime}(s)\, g_c^{(1)}(s)$$
$$G_{12}(s,0) = -g_c^{(2)\prime}(s)\, g_o^{(1)}(s)$$
$$G_{23}(s,0) = -g_c^{(1)\prime}(s)\, g_o^{(2)}(s)$$
$$G_{01}(0,s) = -g_o^{(1)\prime}(s)\, g_c^{(2)}(s)$$
$$G_{02}(0,s) = -g_o^{(2)\prime}(s)\, g_c^{(1)}(s)$$
$$G_{12}(0,s) = -g_o^{(2)\prime}(s)\, g_o^{(1)}(s)$$
$$G_{23}(0,s) = -g_o^{(1)\prime}(s)\, g_o^{(2)}(s).$$

These may be solved for $g_o^{(1)}(s)$, for example, as follows: We have

$$\frac{g_c^{(1)\prime}(s)}{g_o^{(1)\prime}(s)} = \frac{G_{01}(s,0)}{G_{01}(0,s)} = \frac{G_{23}(s,0)}{G_{23}(0,s)} \equiv a(s)$$

$$\frac{g_c^{(1)}(s)}{g_o^{(1)}(s)} = \frac{G_{02}(s,0)}{G_{12}(s,0)} = \frac{G_{02}(0,s)}{G_{12}(0,s)} \equiv b(s).$$

Solving the second equation for $g_c^{(1)}(s)$ and substituting into the second gives

$$b'(s)\, g_o^{(1)}(s) + b(s)\, g_o^{(1)\prime}(s) = a(s)\, g_o^{(1)\prime}(s)$$

or

$$\frac{g_o^{(1)\prime}(s)}{g_o^{(1)}(s)} = \frac{b'(s)}{a(s)-b(s)}.$$

By using the initial condition $g_o^{(1)}(0) = \pi_o^{(1)}$, which we have already shown to be constructable from the two-dimensional G functions, the solution of this differential equation is

$$g_o^{(1)}(s) = \pi_o^{(1)} \exp\left(\int_0^s \frac{b'(u)}{a(u)-b(u)}\,\mathrm{d}u\right). \quad (34)$$

Having found the one-dimensional g functions, the two-dimensional gs can be determined from the equations (30–33) for the $G_{ij}(s,t)$. Thus, the one- and two-dimensional dwell-time distributions of the component processes can be uniquely determined from the statistics of the superposition.

4. ESTIMATION

In this section we present some proposals for estimating the G functions of the superposition, in terms of which the g functions of the constituent processes can be determined and the plausibility of a model of independent channels can be examined, using the various relations developed in the previous sections. $G_k(t)$ is the probability that the superposition is at level k during a randomly placed interval of length t. We propose estimating this probability from a finite record $S(u)$, $0 \leqslant u \leqslant T$, by sliding a window of width t along the record. Let

$$I_k(t,u) = \begin{cases} 1 & \text{if } S(v) = k, u \leqslant v \leqslant u+t \\ 0 & \text{otherwise.} \end{cases}$$

Then $E(I_k(t,u)) = P(I_k(t,u) = 1) = G_k(t)$. $G_k(t)$ is then estimated by

$$\hat{G}_k(t) = \frac{1}{T-t}\int_0^{T-t} I_k(t,u)\,\mathrm{d}u. \quad (35)$$

Because the expectation may be taken inside the integral, $E(\hat{G}_k(t)) = G_k(t)$. Upon examination of equation (35), we see that the estimate may be more simply expressed. Consider the contribution of the ith entrance to level k to the integral (35). If this sojourn is of duration $T_i < t$, there will be no contribution whereas if $T_i > t$, the contribution will be $T_i - t$. We can thus express equation (35) as

$$\hat{G}_k(t) = \frac{1}{T-t}\sum_{i=1}^{n_k} (T_i - t)_+, \quad (36)$$

where n_k is the number of sojourns at level k and

$$(T_i - t)_+ = \begin{cases} T_i - t & \text{if } T_i > t \\ 0 & \text{otherwise.} \end{cases}$$

For equations (35) and (36) to be identical we need to use the convention in equation (36) that if the superposition is at level k at time $t = 0$, T_1 is defined to be the observed duration from $t = 0$, and a corresponding convention holds if the superposition is at level k at the end of the record.

For some purposes it may be preferable to modify the estimate as

$$\tilde{G}_k(t) = \frac{1}{T}\sum_{i=1}^{n_k} (T_i - t)_+. \quad (37)$$

The bias of this estimate is negligible if $t \ll T$, and the estimate has the advantages that $\tilde{G}_k(t)$ is a piecewise linear function that changes slope when $t = T_i$ for some i, decreases monotonically and is convex. Its derivative is thus a decreasing step function, which is the natural form for an estimate of a survival function (see equation (2)). $\tilde{G}_k(t)$ may be computed efficiently for all t by first sorting the T_i. $\hat{G}_k(0)$ is the proportion of time that the superposition is at level k, and $\tilde{G}_k(t) = 0$ for t larger than the maximum T_i.

Under assumptions of ergodicity, it can be shown that $\hat{G}_k(t)$ converges to $G_k(t)$ at $T \to \infty$. We will not pursue more detailed discussion of the statistical behaviour of $\hat{G}_k(t)$ at this time. However, we note that the values of this random function at different *t*s are strongly correlated.

We have seen in a previous section that under various scenarios the *g* functions can be determined from the G_k, as in equation (11), for example. These relations can be used to form estimates of the *g* functions in terms of the $\hat{G}_k$.

Dwell-time distributions are frequently fit by sums of exponentials to explore the plausibility of various kinetic models. Such forms could be fit either to the *g* functions which have been estimated from the $\hat{G}_k$ or directly to the $\hat{G}_k$. Although the former is less direct, it has the advantage of separating the rate constants of the open states from those of the closed states, which may be more numerous. (Note that $G_k(t)$ is a sum of exponentials with rates given by sums and multiples of the eigenvalues of Q_{oo} and Q_{cc}.)

The two-dimensional *G* functions may be estimated in a simple way. Note that whereas one-dimensional *G* functions are proportional to integrals of survival functions (2), two-dimensional *G* functions are proportional to bivariate survival functions (4). We are thus led to the natural estimate

$$\hat{G}_{k,k+1}(s,t) = \frac{N(s,t)}{T-t-s}, \tag{38}$$

where $N(s,t)$ is the number of $k \rightarrow k+1$ transitions preceded by a duration at least *s* at level *k* and followed by a duration at least *t* at level $k+1$. Again, it may be desirable to modify this estimate slight,

$$\tilde{G}_{k,k+1}(s,t) = \frac{N(s,t)}{T}, \tag{39}$$

The estimate $\tilde{G}_{k,k+1}(s,t)$ is then a step function. We note that mean dwell times can be estimated in a relatively straightforward way via relations such as (15). We have also seen that when *n* is large, survival functions can be estimated without precise knowledge of *n* from the two-dimensional *G* functions via relations like (16).

5. CONCLUDING REMARKS

In this paper we have developed a number of quantitative and testable properties of superpositions of independent channels. For example, in the case of independent identical bi-level channels, we have shown that the $H_k(t)$ are a binomial distribution for all *t* and in the non-degenerate case of independent identical multi-level channels, the $H_\nu(t)$ are a multinomial distribution for all *t*. For independent non-identical channels we have seen that the *G* functions satisfy certain relations. If the constituent channels of a superposition interact, these interactions should be evident from the failure of some of these properties to hold and perhaps insight into the character of the interactions can be gained from the nature of the deviations. It is conceivable, from example, that one or more channels being open for a given length of time affects the opening rates of the remaining closed channels. Deviations from relations such as (17) may result. We note that Yeramian *et al.* (1986) also use two-time probabilities, different from ours, in a desire to improve upon the discriminatory power of tests based only on stationary probabilities.

In all our examples it has proved possible in theory to recover the statistics of individual channels from those of the superposition. The practical limitations of the various procedures remain to be explored. In particular, we note that our formulae for recovery entail knowing *n*, the number of channels in the patch, although as we have noted, some estimates are insensitive to the value of *n*. Also, the sampling properties of the estimates of the *G* and *g* functions are not clear. As an example, the sampling properties of an estimate based on the relation (34) may be rather complicated. The bootstrap (Efron & Tibshirani 1986) may be a useful tool in assessing sampling fluctuations.

We have shown that the two-dimensional dwell-time distributions of the constituent channels can be recovered from the superposition. For an aggregated Markov model, these distributions determine the entire probability law of the channel process (Fredkin & Rice 1986). For a more general model of a stationary sequence of dwell times, it is an open question whether the complete probability laws of the constituent channels can be recovered from the superposition.

Our primary tools in this analysis have been the *g* and *G* functions, which are especially well suited to analysing the one- and two-dimensional distributions of the superposition and its constituents. These tools complement those of Colquhoun & Hawkes (1990) who make use of the two-channel *Q* matrix to derive expressions for probabilities of various runs of single and double openings.

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