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On the use of time constants as estimates of mean lifetimes in single channel data analysis

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Abstract. For Markov models of single channel kinetics, a sojourn time in a class of states has a density function which is usually a linear combination of exponential densities. There are many instances in the single channel literature where the time constants of exponentials fitted to sojourn time data have been used as estimated mean sojourn times in individual states, though the two may be very different. In the present study the nature and magnitude of this difference in the case of a two state class is illustrated analytically and numerically. The time constants should be viewed at best as approximations, possibly poor, to the estimated mean sojourn times. Estimates of kinetic parameters cannot in general be obtained explicitly from the fitted parameters of the density alone. However, this is shown to be possible in some special cases and enables direct estimation of, for example, the channel opening rate constant β (or an upper limit to the estimate of β in the case of multiple channels) in standard sequential three or four state models of nicotinic receptor kinetics, using only the fitted parameters of the closed-time density.

Key words: Channel kinetics – Markov models – Sojourn times – Approximation – Eigenvalues

Introduction

In single channel data analysis, sojourn time data are usually fitted by a probability density function of a form determined by model assumptions. If the underlying channel kinetics are modelled by a Markov process satisfying mild conditions (see, for example, Colquhoun and Hawkes 1983 and Appendix 2 of Milne et al. 1988), the sojourn time (lifetime) density for a class of states is a linear combination of exponential densities, with the number of component densities equal to the number of kinetically distinct states in the class. The parameters (rate constants, reciprocal time constants) of the exponential densities correspond to the eigenvalues of a matrix whose diagonal elements are reciprocal mean sojourn times in individual states. It is well established (as in Colquhoun and Hawkes 1983, p. 174; Fredkin et al. 1985; and others) that the eigenvalues and diagonal elements are usually not identical, but the detailed relationship between the two in the single channel context has received little attention.

In practical single channel inference, the fitting of sojourn time data gives estimates of the rate constants of component exponential densities, whereas it is estimates of the reciprocal mean lifetimes that are usually required for interpretation of the data in terms of a proposed kinetic model. An assumption that fitted rate constants are equal or, implicitly, good approximations to estimated reciprocal mean lifetimes is sufficiently prevalent (see for example Sine and Steinbach 1984, 1986; Auerbach and Lingle 1987; Igusa and Kidokoro 1987; Hestrin et al. 1987; Bormann 1988; Papke et al. 1988; Jaramillo and Schuetze 1988; Sikdar et al. 1989) to warrant examination, since estimation of kinetic parameters based upon this assumption will usually be biased.

Here we discuss the theoretical relationship between eigenvalues and reciprocal mean lifetimes, and the errors that can arise when fitted time constants are used directly as estimates of mean lifetimes. For illustrative purposes, attention is restricted to a class containing just two states, that is, where the corresponding sojourn time density is bi-exponential. We show that although the approximations may often be adequate, they can under some conditions be very poor and lead to substantial errors with possible misinterpretation of data. Consequently, consideration should be given to the adequacy of such approximations if they are used (see for example Colquhoun and Sakmann 1985). In special cases, as we illustrate below, explicit estimation of kinetic parameters is possible, thus obviating the need for approximation. Throughout the following, the unit of time is arbitrary.

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Theory: eigenvalues and reciprocal mean lifetimes

As in Colquhoun and Hawkes (1982); Fredkin and Rice (1986); Milne et al. (1988); or Ball and Sansom (1988), the following development is based upon a finite state continuous-time homogeneous Markov process in equilibrium, where all states communicate. This can be represented by a transition rate matrix $\mathbf{Q}=[q_{ij}]$, where $q_{ij}\geq 0\ (i\neq j)$ is the transition rate from state i to state j and $d_i=-q_{ii}=\sum_{j\neq i} q_{ij}$.

We are concerned with sojourn times in a class $\mathscr{C}=\{1, 2\}$ of two neighbouring states for which all possible connections with other states are allowable. This is represented as follows:

$$\rightleftharpoons 1 \rightleftharpoons 2 \rightleftharpoons . \qquad \text{(Scheme 1)}$$

For $\mathscr{C}$, the submatrix of interest is

$$-\mathbf{Q}_{\mathscr{C}\mathscr{C}}=\begin{bmatrix} d_1 & -q_{12} \\ -q_{21} & d_2 \end{bmatrix} \qquad (1)$$

with states 1 and 2 having finite mean sojourn times d_1^{-1} and d_2^{-1} respectively. Without loss of generality the states are labelled so that $d_1\leq d_2$.

The (real) eigenvalues of $-\mathbf{Q}_{\mathscr{C}\mathscr{C}}$ are given by

$$\lambda_1, \lambda_2=\tfrac{1}{2}\{d_1+d_2\mp[(d_1+d_2)^2-4d_1 d_2 s]^{1/2}\} \qquad (2)$$

where the subscripts are chosen so that $\lambda_1\leq\lambda_2$, and

$$s=1-p_{12}p_{21}\ , \qquad (3)$$

where $p_{ij}=q_{ij}/d_i$, $j\neq i$, is the probability that a transition from state i is to state j. Then s can be interpreted as the success probability (that is, the probability of terminating a visit to $\mathscr{C}$) in the geometric distribution of the number of visits to one of the states in $\mathscr{C}$ during a sojourn in $\mathscr{C}$ which begins and ends in the other state. Except in the trivial case when $d_1=d_2$ and $s=1$ (which implies from (2) that $\lambda_1=\lambda_2$), the density function of a sojourn time in $\mathscr{C}$ is (from Colquhoun and Hawkes 1982, (1.32) and (1.33))

$$f(t)=C_1\lambda_1 e^{-\lambda_1 t}+C_2\lambda_2 e^{-\lambda_2 t}, \qquad (4)$$

where

$$C_1=\{\lambda_2-\pi_1(d_1-q_{12})-\pi_2(d_2-q_{21})\}/(\lambda_2-\lambda_1), \qquad (5)$$

$C_2=1-C_1$ (not necessarily greater than C_1), π_1 is the probability that a sojourn in $\mathscr{C}$ starts in state 1, and $\pi_2=1-\pi_1$.

It can be seen from (2) that

$$\lambda_1+\lambda_2=d_1+d_2\ , \quad \lambda_1\lambda_2=d_1 d_2 s\ , \qquad (6)$$

and consequently

$$0\leq\lambda_1\leq d_1\leq d_2\leq\lambda_2\leq d_1+d_2\ . \qquad (7)$$

Clearly λ_1 and λ_2 depend not only on d_1 and d_2 but also on q_{12} and q_{21} through their product. Except when $s=0$, in which case exit from $\mathscr{C}$ is not possible, both λ_1 and λ_2 are strictly positive. They are distinct whenever $s<1$ (that is, when both q_{12} and q_{21} are positive). Furthermore, $\lambda_1=d_1$ and $\lambda_2=d_2$ if and only if $s=1$. (In this last case, at least one of p_{12} and p_{21} is zero, and it is not possible to loop between the states during a given sojourn in $\mathscr{C}$; further, at least one type of sojourn in $\mathscr{C}$ consists of a single sojourn in one of the two states. If both p_{12} and p_{21} are zero, the states cannot be neighbours.) When d_1 is small so is λ_1, and λ_2 is then close to d_2. The difference between an eigenvalue and a reciprocal mean sojourn time is given from (2) by

$$\varepsilon=d_1-\lambda_1=\lambda_2-d_2=\tfrac{1}{2}\{[(d_1+d_2)^2-4d_1 d_2 s]^{1/2}-(d_2-d_1)\}\ , \qquad (8)$$

which decreases monotonically from d_1 to zero as s increases from zero to 1. The behaviour of λ_1 and λ_2 as s varies is illustrated graphically in Fig. 1.

Inference: approximation errors

In practice, the density (4) is fitted to $\mathscr{C}$-sojourn time data giving estimates $\hat{\lambda}_1$, $\hat{\lambda}_2$ and $\hat{C}_1$ of λ_1, λ_2 and C_1. A formal approach to inference might involve estimation of the four parameters d_1, d_2, q_{12} and q_{21}; in general four estimating equations would be needed (five if π_1 in (5) were also included). A full likelihood method could be used when the successive sojourn times in $\mathscr{C}$ are independent (and might serve as a useful approximation in the dependent case); though analytically complicated this could be implemented numerically. However, the approach in many published studies is less formal and attempts to recover information on d_1 and d_2 on the basis of the eigenvalue estimates $\hat{\lambda}_1$ and $\hat{\lambda}_2$ alone.

Let $\hat{\lambda}_1+\hat{\lambda}_2=b$ and $\hat{\lambda}_1\hat{\lambda}_2=c$. Then (6) become

$$d_1+d_2=b\ , \quad d_1 d_2=c/s\ , \qquad (9)$$

and solving these gives estimates

$$\hat{d}_1, \hat{d}_2=\tfrac{1}{2}[b\mp(b^2-4c/\hat{s})^{1/2}]\ , \quad (\hat{d}_1\leq\hat{d}_2) \qquad (10)$$

provided an estimate $\hat{s}$ of s is available. For $\hat{d}_1$, $\hat{d}_2$ to be real requires

$$\hat{s}\geq 4c/b^2\ , \qquad (11)$$

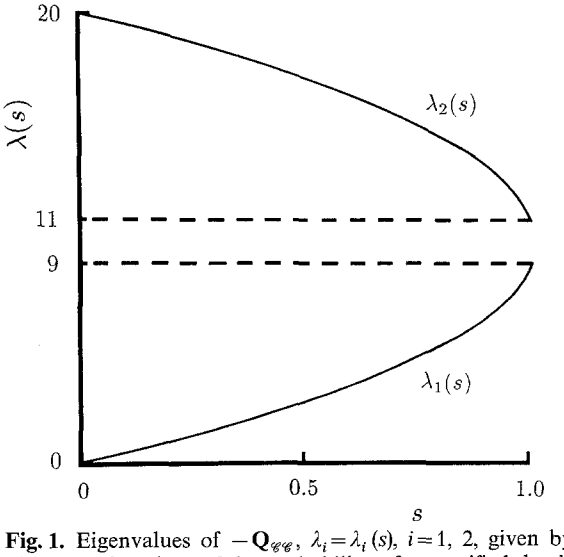

Fig. 1. Eigenvalues of $-\mathbf{Q}_{\mathscr{C}\mathscr{C}}$, $\lambda_i=\lambda_i(s)$, $i=1, 2$, given by (2), are plotted as functions of the probability s for specified d_1, d_2. Values used in this case were $d_1=9$ and $d_2=11$

an inequality which enters only when the values of $\hat{\lambda}_1$, $\hat{\lambda}_2$ and $\hat{s}$ are estimates that may not be constrained to satisfy this by the estimation procedure.

The common assumption that $\hat{\lambda}_1$ is equal or approximately equal to $\hat{d}_1$ results in an error of $\hat{\varepsilon}=\hat{d}_1-\hat{\lambda}_1=\hat{\lambda}_2-\hat{d}_2$, which can be expressed from (2) or (10) as

$$\hat{\varepsilon}=\tfrac{1}{2}\{(\hat{\lambda}_2-\hat{\lambda}_1)-[(\hat{\lambda}_2-\hat{\lambda}_1)^2-4\hat{\lambda}_1\hat{\lambda}_2(1-\hat{s})/\hat{s}]^{1/2}\}, \qquad (12)$$

and has an upper limit of $(\hat{\lambda}_2-\hat{\lambda}_1)/2$. The error $\hat{\varepsilon}$ decreases (*i*) as $\hat{s}$ increases (see Fig. 1), and (*ii*) as $\hat{\lambda}_1/\hat{\lambda}_2$ decreases for fixed b and $\hat{s}$. These effects are evident in the numerical examples of Table 1, which shows relative errors of up to 90% in terms of d_1 (or 900% in terms of the observed quantity $\hat{\lambda}_1$). In summary, good estimates of d_1 and d_2 can only be obtained from knowledge of s as well as λ_1 and λ_2; without some information on the value of s considerable care should be exercised in using the fitted rate constants $\hat{\lambda}_i$ as estimates of the reciprocal mean lifetimes d_i.

The value of $\hat{C}_1$ places some restriction on the value of $\hat{s}$ and hence on $\hat{\varepsilon}$; for example, if $\hat{\lambda}_1=1$, $\hat{\lambda}_2=19$ and information on the value of $\hat{C}_1$ is not used, the estimated error for $\hat{d}_1$ and $\hat{d}_2$ may be up to $\varepsilon_1=\varepsilon_2=90\%$ (see Table 1), but if $\hat{C}_1=0.9$, $\hat{s}=0.28$ and the estimated probability $\hat{\pi}_1$ of a sojourn in $\mathscr{C}$ starting in state 1 is 0.125, we have $\hat{\varepsilon}=3.33$, $\varepsilon_1=77\%$, and $\varepsilon_2=21\%$. Furthermore, whenever $\hat{C}_1<0.5$ a simple restriction on $\hat{\varepsilon}$ can be demonstrated. Letting $u=\hat{C}_1\hat{\lambda}_1+\hat{C}_2\hat{\lambda}_2$ we have, corresponding to a rearrangement of (5)

$$\hat{\pi}_1\hat{q}_{1*}+\hat{\pi}_2\hat{q}_{2*}=u \qquad (13)$$

where $q_{1*}=d_1-q_{12}$, $q_{2*}=d_2-q_{21}$. Thus $u\leq\hat{d}_2$ and it follows that

$$\hat{\varepsilon}=\hat{\lambda}_2-\hat{d}_2\leq\hat{\lambda}_2-u<(\hat{\lambda}_2-\hat{\lambda}_1)/2$$

whenever $\hat{C}_1<0.5$ (that is $u>b/2$), in which case $\hat{s}\geq c/[u(b-u)]$. Again, if $\hat{\lambda}_1=1$ and $\hat{\lambda}_2=19$, and it is known that $\hat{C}_1=0.05$, we have $u=18.1$ and $\hat{s}\geq 0.5525$, with $\hat{\varepsilon}\leq 0.9$, $\varepsilon_1\leq 47\%$ and $\varepsilon_2\leq 5\%$.

Explicit parameter estimation

As remarked above, information on the error $\hat{\varepsilon}$ depends on the value of $\hat{s}$; even a rough estimate of s should yield estimates of d_1 and d_2 better than $\hat{d}_1=\hat{\lambda}_1$, $\hat{d}_2=\hat{\lambda}_2$. The parameter s cannot be estimated from $\hat{\lambda}_1$ and $\hat{\lambda}_2$, nor from these together with $\hat{C}_1$ (that is, from the fitted parameters of the density function) except in special cases. One such case is

$$\rightleftharpoons 1 \rightleftharpoons 2, \qquad \text{(Scheme 2)}$$

a reduction of Scheme 1 where exit from and entry into $\mathscr{C}$ via state 2 are disallowed (the labelling no longer implying that $d_1\leq d_2$), so $q_{21}=d_2$ and $\pi_1=1$. Then the fitted parameters provide explicit estimates for d_1, d_2 and s, it following from (3), (5) and (9) that

$$\hat{d}_2=\hat{\lambda}_1\hat{\lambda}_2/(\hat{C}_1\hat{\lambda}_1+\hat{C}_2\hat{\lambda}_2), \quad \hat{d}_1=\hat{\lambda}_1+\hat{\lambda}_2-\hat{d}_2,$$
$$\hat{s}=\hat{\lambda}_1\hat{\lambda}_2/(\hat{d}_1\hat{d}_2). \qquad (14)$$

Table 1. Errors in estimating d_1 and d_2 from λ_1 and λ_2 for Scheme 1. Effect of the estimates $\hat{\lambda}_1$, $\hat{\lambda}_2$ and $\hat{s}$ on the error $\hat{\varepsilon}=\hat{d}_1-\hat{\lambda}_1(=\hat{\lambda}_2-\hat{d}_2)$ determined from (12). An asterisk indicates the minimum value (11) which $\hat{s}$ can take if only $\hat{\lambda}_1$ and $\hat{\lambda}_2$ are specified. The effects of holding any two of the sum $\hat{\lambda}_1+\hat{\lambda}_2$, product $\hat{\lambda}_1\hat{\lambda}_2$ and $\hat{s}$ constant as the other varies can be seen. Relative errors (with respect to the desired value) were calculated as $\varepsilon_1=100\hat{\varepsilon}/(\hat{\lambda}_1+\hat{\varepsilon})$, and $\varepsilon_2=100\hat{\varepsilon}/(\hat{\lambda}_2-\hat{\varepsilon})$

$\hat{\lambda}_1$	$\hat{\lambda}_2$	$\hat{s}$	$\hat{\varepsilon}$	ε_1 (%)	ε_2 (%)
1	19	0.19*	9.000	90.0	90.0
		0.39	1.839	64.8	10.7
		0.59	0.766	43.4	4.2
		0.79	0.285	22.2	1.5
		0.99	0.011	1.1	0.1
5	15	0.75*	5.000	50.0	50.0
		0.79	2.750	35.5	22.4
		0.89	1.034	17.1	7.4
		0.99	0.076	1.5	0.5
9	11	0.99*	1.000	10.0	10.0
		0.994	0.366	3.9	3.4
		0.999	0.051	0.6	0.5
1	20	0.99	0.011	1.1	0.1
2	10	0.99	0.025	1.3	0.3
4	5	0.99	0.281	6.6	6.0

Table 2. Estimation of d_1, d_2 and s for Scheme 2. Estimates of d_1, d_2 and s were obtained using (14) for a range of fitted density functions based on (4). Note that in Scheme 2 the labelling of states does not imply that $d_1\leq d_2$

$\hat{\lambda}_1$	$\hat{\lambda}_2$	$\hat{C}_1$	$\hat{d}_1$	$\hat{d}_2$	$\hat{s}$
1	19	0.1	18.90	1.10	0.9103
		0.5	18.10	1.90	0.5525
		0.9	13.21	6.79	0.2119
		0.95	10.00	10.00	0.1900
		0.99	3.90	16.10	0.3027
		0.999	1.34	18.66	0.7620
5	15	0.1	14.64	5.36	0.9561
		0.5	12.50	7.50	0.8000
		0.9	7.50	12.50	0.8000
		0.99	5.29	14.71	0.9633
9	11	0.01	10.98	9.02	0.9997
		0.5	10.10	9.90	0.9901
		0.99	9.02	10.98	0.9995

Table 2 presents solutions for $\hat{d}_1$, $\hat{d}_2$ and $\hat{s}$ from (14) based on a range of fitted density functions. Even modest changes in the coefficients of the bi-exponential density result in marked shifts in the values of $\hat{d}_1$ and $\hat{d}_2$. In particular, the finding that sojourn times can be fitted with a bi-exponential density having well separated parameters ($\hat{\lambda}_1\ll\hat{\lambda}_2$) does not necessarily indicate that the two underlying kinetic states have commensurately unequal mean lifetimes; they may even be equal. Nor can it be assumed that if one state really is short-lived, it must be the first visited (that is state 1 of Scheme 2). Though this conclusion may be suggested by other evidence, such as bursting behaviour, it can be confirmed by solution of (14) using the estimate $\hat{C}_1$.

It is worth noting that the transition rate $q_{1*}=d_1-q_{12}=d_1 s$ out of class $\mathscr{C}$ is, from (13) or (14), simply estimated by $\hat{q}_{1*}=u$, a result which extends naturally to a class of more than two states. That is, for any class $\mathscr{C}$ containing n states where entry to and exit from $\mathscr{C}$ can occur via only one state (say state 1), the transition rate out of $\mathscr{C}$ can be estimated by $\hat{q}_{1*}=\sum_{i=1}^{n}\hat{C}_i\hat{\lambda}_i$. Thus, in the four state sequential model of nicotinic or GABA receptor agonist binding and isomerization,

$$3 \rightleftharpoons 2 \rightleftharpoons 1 \rightleftharpoons 4, \qquad \text{(Scheme 3)}$$

where $\mathscr{C}=\{1, 2, 3\}$ is a class of closed states and state 4 is open, the channel opening rate constant $\beta=q_{14}$ can be estimated *explicitly* from the closed-time density (see Madsen and Edeson 1988, for a survey of β estimates). This may be a useful alternative, particularly in systems where bursting is not evident, to the widely applied method based on the eigenvalue approximation for d_1 and an estimate of the number of gaps per burst (represented, for example, by (12) and (13) of Colquhoun and Sakmann 1985). Moreover, $\sum_{i=1}^{3} C_i\lambda_i$ is independent of agonist concentration (as can be seen from (3.75) of Colquhoun and Hawkes 1981, where $f_{34}(0)=\beta$) and so in principle is $\hat{q}_{14}$, whereas the standard estimation procedure assumes zero concentration (Colquhoun and Hawkes 1981, (3.9)).

As always, the quality of the estimation (for both methods) is dependent on the appropriateness of the model; thus it is assumed that the experimental record arose from a patch containing only one channel and that desensitization is not present. If the number of channels in the patch is unknown, $v=\sum_{i=1}^{3}\hat{C}_i\hat{\lambda}_i$ represents (again in principle) an upper limit to $\hat{\beta}$. In the case of desensitization, if the real underlying mechanism is described by Scheme 3 with states 2 and 3 normal closed, state 1 open, and state 4 desensitized (or blocked), the closed-time density will reflect sojourns in state 4 as well as states 2 and 3. One eigenvalue (say λ_3) will equal d_4 and $C_3=p_{14}$ Then $\beta=q_{21}=\left[\sum_{i=1}^{2} C_i\lambda_i\right]\Big/(1-C_3)$ and the estimation error in assuming desensitization is not present will be $v-\hat{\beta}=\hat{C}_3(\hat{\lambda}_3-v)/(1-\hat{C}_3)$. Thus v will be greater than (less than) $\hat{\beta}$ whenever $\hat{d}_4=\hat{\lambda}_3$ is greater than (less than) v, for example when $\hat{\lambda}_3$ is greater than (less than) both $\hat{\lambda}_1$ and $\hat{\lambda}_2$. If the probability that an open channel closes to the desensitized state is low this error will tend to be small.

Some comparisons of estimates given by the two methods are possible where published studies provide sufficient information to calculate v. These suggest that differences may often be relatively small, but can also be appreciable. For example, Hestrin et al. (1987) give a value for $\hat{\beta}$ of 1587 s^{-1}, 16% higher than v calculated from their data on p 450; Sine and Steinbach (1986) estimate β to be 14 764 s^{-1} (suberyldicholine data, brief closure assumption), which is 51% higher than v obtained from their data of Fig. 2. How these differences should be interpreted, and the relative merits of the two estimation methods in terms of approximation errors, selection of bursts from the experimental record, unknown number of channels, possible desensitization, limited time resolution and digitization rate remain to be studied.

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