PARAMETER ESTIMATION IN A REACTION-DIFFUSION MODEL FOR SYNAPTIC TRANSMISSION AT A NEUROMUSCULAR JUNCTION

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ABSTRACT. A reaction-diffusion system with nonlinear time-dependent boundary conditions is developed to model the uptake and release of neural transmitter at an invertebrate neuromuscular junction. The system of differential equations is transformed into an equivalent normalized, nonlinear integrodifferential equation problem which is solved using standard techniques for nonlinear Volterra integral equations. A statistical procedure for estimating the model parameters by fitting the model to specific experimental data is described and the goodness of fit of the model to the data in the presence of correlated errors is assessed.

1. Introduction. In some species, one of the basic mechanisms by which a transmitter molecule moves at a neuromuscular junction is free diffusion. A presynaptic action potential stimulates the release into the synaptic cleft of a quantum of transmitter molecules such as glutamate which may be contained in one or more vesicles. These transmitter molecules then diffuse across the cleft to bind reversibly with receptors located on the postsynaptic membrane. When sufficient receptor sites are occupied, channels open and a miniature endplate current (MEPC) is generated. Recently, considerable effort has been devoted to the development of quantitative models for the miniature endplate currents generated at the vertebrate neuromuscular junction and this has led to an understanding of several of the underlying mechanisms involved including the kinetics of channel opening and the enzymatic degradation of the transmitter \[1, 4, 11, 16, 17, 18, 21, 22, 23, 29\]. In contrast to the vertebrate MEPC, the mechanisms underlying the evoked synaptic potential in invertebrates such as the crayfish, for example, are poorly understood \[2, 3\]. In particular, it is not known whether the free transmitter is enzymatically degraded or merely absorbed by specialized cells or even if the kinetics at the receptor site involve multiple reversible reactions.

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The majority of the data available consists of excitatory postsynaptic currents (EPSC’s) measured extracellularly at the crayfish neuromuscular junction using a loose patch clamp technique [2]. It is assumed that spatial effects at the postsynaptic membrane are small, as the loose patch electrode records all of the individual channel currents simultaneously. However, it is possible that the currents may not add in a linear fashion. For example, during depolarization, the membrane potential shifts towards the reversal potential and the channels which open late during a quantal event would be expected to contribute less because of a reduction in the driving force of the current. Also, a loose patch electrode will pick up only a fraction of the total current from each channel due to an imperfect seal between the electrode and the synapse. This is assumed to be approximately 50% but equal at each channel and was taken into account by doubling the raw data. Details of the recording arrangement and synapse size and location can be found in [30]. The time course of four such measurements is displayed in Figure 1. Each trace consists of 400 observations collected by digitizing a continuous 40 millisecond recording at .1 msec per observation. There are three large records and one small response, the latter being discernible from noise as an extended excursion above the baseline. The ordinate is the number of open channels on the postsynaptic membrane, assuming a current of 8 pico Amps per open channel. The time origin coincides with the time at which an oscilloscope sweep was triggered. The large positive and negative spikes at 5–7 msec are stimulus artifacts indicating the time at which a stimulus was applied, and the small deflections at 13–15 msec mark the entry of the action potential into the synaptic region. Only after this point is transmitter released in response to the stimulus. The postsynaptic responses begin with latencies of about 18–20 msec, and are signaled by a rapid rise in current flow followed by a more gradual decline to baseline. The amplitude, latency, and possibly even the shape of the response appear to vary from trace to trace.

The magnitude of the experimental and/or measurement noise is apparent from the initial and latter portions of the traces. A statistical analysis of the noise shows that it is Gaussian but correlated, and any attempt at modeling the response must take this correlation into account insofar as statistical properties of the estimated model are concerned.
The objective of this present work is to develop and analyze a simple one-space dimensional reaction-diffusion model for the neural transmission process at the crayfish neuromuscular junction with the goals of recovering the variable and parameter values in the model from the EPSC data and assessing the goodness of fit of the model in the presence of correlated errors. The model developed here represents a marked departure from those employed in other MPEC studies [21, 22, 23, 29] in that the flux of transmitter across the boundaries has been incorporated.
into the boundary conditions directly rather than by specifying source and sink terms located near the boundaries. While this approach more accurately reflects the transport processes involved, it does introduce the added complexity of nonlinear time-dependent boundary conditions in the defining equations. The model is first normalized to ensure that the process is described only in terms of independent dimensionless parameters and variables. The application of a nonlinear regression model to obtain "best estimates" of the unknown parameter values requires repeated solution of the model for each adjustment in the estimated values for these quantities. Consequently, the direct application of numerical solution schemes at this point is neither feasible nor desirable. Instead, the system of differential equations is transformed into an equivalent nonlinear integrodifferential equation problem which is concerned only with events at the receptor site. Removing the spatial component of the problem not only simplifies the numerical computation but also serves to isolated those parameters which can be most accurately estimated from the EPSC data. A numerical procedure for solving the integrodifferential equation is developed based on a well-known collocation scheme for nonlinear Volterra integral equations [5, 6] and is applied to the statistical analysis of the data displayed in Figure 1. The results obtained are summarized in Table 1 and Figure 2.

<table>
<thead>
<tr>
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TABLE 1. Estimated parameters and goodness of fit $p$-values.
FIGURE 2. The solid lines are the data and fitted model (smooth curve). The dashed lines are the residuals.
2. The mathematical model. We begin by assuming that the synaptic cleft is homogeneous and that the diffusion process from the presynaptic membrane in the \( yz \) plane at \( x = 0 \) to the postsynaptic membrane in the \( yz \) plane at \( x = h \) is uniform over each \( yz \) cross section. We also assume that the transmitter is released uniformly across the presynaptic membrane and that the receptors are uniformly distributed over the postsynaptic membrane. The simple one-space dimensional, reaction-diffusion model for the transmission process is then given by

\[
\begin{align*}
(1) \quad & \frac{\partial u_1}{\partial t} = D[\partial^2 u_1 / \partial x^2] - \alpha u_1, \quad 0 < x < h, \quad t > 0 \\
(2) \quad & u_1(x, 0) = 0, \quad u_2(0) = 0, \quad 0 < x < h \\
(3) \quad & -D \frac{\partial u_1(0, t)}{\partial x} = c_0 \delta(t), \quad t > 0 \\
(4) \quad & -D \frac{\partial u_1(h, t)}{\partial x} = d u_2(t)/dt = k_1 (R - u_2(t)) u_1(h, t) - k_2 u_2(t), \quad t > 0
\end{align*}
\]

where \( u_1 (x, t) \) (moles metre\(^{-3}\)) is the concentration of free transmitter at \( x \) at time \( t \) after release, \( u_2(t) \) (moles metre\(^{-2}\)) is the concentration of the bound transmitter at the postsynaptic membrane, \( D \) (metre\(^2\)sec\(^{-1}\)) is the diffusion coefficient, \( R \) (moles metre\(^{-2}\)) is the initial surface concentration of receptors and \( c_0 \) (moles sec metre\(^{-2}\)) is the instantaneous flux of transmitter across the presynaptic membrane at time \( t = 0 \). Here a single-stage reversible reaction with nonnegative rate constants \( k_1 \) (metre\(^3\) sec mole\(^{-1}\)) and \( k_2 \) (sec\(^{-1}\)) has been assumed for the interaction of the transmitter and receptor whereby one molecule of transmitter binds reversibly with one molecule of receptor to open a channel in the postsynaptic membrane. Thus, the flux of the transmitter across the membrane equals the rate at which receptor sites become occupied at \( x = h \). The standard quadratic mass action relation is used to model the forward component of the biomolecular reaction. There is as yet no evidence of enzymatic degradation of glutamate [27], which is the excitatory transmitter at the crayfish neuromuscular junction. Two postulated retrieval mechanisms are direct uptake into the presynaptic terminal and uptake into neighboring glial cells with subsequent
transport to the presynaptic neuron, but the relative contributions of the mechanisms are unknown [25]. The kinetics of glutamate uptake have been studied in the salamander and in synaptosomes [27]. A suitable model modification to reflect uptake at the presynaptic site would include a term such as $-\gamma u_1(0, t)$ on the righthand side of boundary condition (3), and in a radially symmetric three-dimensional synapse, a similar term at the radial boundary to reflect uptake by glial cells. Eccles and Jaeger found that diffusion alone would be sufficient to account for the decay of postsynaptic current in a three-dimensional model [10]. In our one-dimensional model we assume only that the rate of disappearance of free transmitter in the cleft is proportional to its concentration, with constant of proportionality $\alpha (\sec^{-1})$. While $h$ and $D$ can be estimated with some degree of accuracy, little is known regarding the parameters $c_0, \alpha, k_1, k_2$ and $R$. By identifying $h$, $D$ and $R$ as the primary variables and setting

$$\xi = x/h, \quad \tau = (D/h^2)t, \quad u(\xi, \tau) = (h/R)u_1(x(\xi), t(\tau)), \quad v(\tau) = u_2(t(\tau))/R$$

$$C_1 = c_0/R, \quad K_1 = (h/D)Rk_1, \quad K_2 = (h^2/D)k_2,$$

$$\beta = (h^2/D)\alpha.$$ 

(1)-(4) can be expressed in terms of the independent dimensionless quantities $\{\xi, \tau, u, v, C_1, K_1, K_2, \beta\}$

$$\partial u/\partial \tau = \partial^2 u/\partial \xi^2 - \beta u, \quad 0 < \xi < 1, \quad \tau > 0$$

$$u(\xi, 0) = 0, \quad v(0) = 0, \quad 0 < \xi < 1$$

$$-\partial u(0, \tau)/\partial \xi = (h^2/D)C_1\delta((h^2/D)\tau), \quad \tau > 0$$

$$-\partial u(1, \tau)/\partial \xi = dv(\tau)/d\tau = K_1(1-v(\tau))u(1, \tau) - K_2v(\tau), \quad \tau > 0.$$

The space and time scalings are now natural for this problem. $\xi$ denotes the distance as a fraction of cleft width, $\tau$ is given in multiples of the mean transit time $h^2/(2D)$ across the synaptic cleft and $v(\tau)$ is the proportion of receptors in the bound state at time $\tau$. The form
of system (6)–(9) is deceptively simple. There are some well-known difficulties which can be encountered in the analysis of systems with nonlinear boundary conditions. Indeed, it has been shown [19] that even in the elementary case where $C_0 = 0$ and (9) is replaced by the boundary condition $\partial u(1, \tau)/\partial x = g(u(1, \tau))$, there are large classes of smooth functions $g$ for which this system does not have a unique global solution. $g(z) = |z|^{2\alpha+1} h(z)$ with $\alpha > 0$ and $h$ monotone increasing is an example of such a class.

3. The integrodifferential equation. Here problem (6)–(9) is transformed into an equivalent nonlinear integrodifferential equation in $v(t)$. Let $U(\xi, s) = L\{u(\xi, \tau)\}$ where $L$ denotes the usual Laplace transform. Taking the Laplace transform of (6) gives from (7)

$$U_{\xi\xi}(\xi, s) - (s + \beta)U(\xi, s) = 0, \quad s > 0$$

which has the general solution

$$U(\xi, s) = A(s) \cosh(\xi(s + \beta)^{1/2})$$

$$+ B(s)(s + \beta)^{-1/2} \sinh(\xi(s + \beta)^{1/2}).$$

(10)

Since

$$U_\xi(\xi, s) = A(s)(s + \beta)^{1/2} \sinh(\xi(s + \beta)^{1/2}) + B(s) \cosh(\xi(s + \beta)^{1/2}),$$

the boundary condition (8) becomes

$$U_\xi(0, s) = B(s) = L\{\partial u(0, \tau)/\partial \xi\}$$

$$= L\{-((h^2/D)C_1\delta((h^2/D)\tau))\} = -C_1$$

while at $\xi = 1$,

(11) $U_\xi(1, s) = A(s)(s + \beta)^{1/2} \sinh((s + \beta)^{1/2}) - C_1 \cosh((s + \beta)^{1/2}).$

Solving for $A(s)$ in (11) gives

$$A(s) = U_\xi(1, s)/((s + \beta)^{1/2} \sinh((s + \beta)^{1/2}))$$

$$+ C_1(s + \beta)^{-1/2} \coth((s + \beta)^{1/2})$$

(12)
and substituting (12) into (10) yields at $\xi = 1$

$$
U(1, s) = U_{\xi}(1, s)(s + \beta)^{-1/2} \coth((s + \beta)^{1/2}) \\
+ C_1(s + \beta)^{-1/2} \cosh^2((s + \beta)^{1/2})/\sinh((s + \beta)^{1/2}) \\
- C_1(s + \beta)^{-1/2} \sinh((s + \beta)^{1/2}) \\
= U_{\xi}(1, s)(s + \beta)^{-1/2} \coth((s + \beta)^{1/2}) \\
+ C_1(s + \beta)^{-1/2} \csch((s + \beta)^{1/2}) \\
= \mathbf{L}\{-dv(\tau)/d\tau\}(s + \beta)^{-1/2} \coth((s + \beta)^{1/2}) \\
+ C_1(s + \beta)^{-1/2} \csch((s + \beta)^{1/2}).
$$

(13)

Now $u(1, \tau)$ in (9) can be recovered from (13) using the translation and convolution properties of the inverse transform $\mathbf{L}^{-1}$ to give

$$
u(1, t) = \mathbf{L}^{-1}\{\mathbf{L}\{-dv(\tau)/d\tau\}(s + \beta)^{-1/2} \coth((s + \beta)^{1/2})\} \\
+ C_1 \mathbf{L}^{-1}\{(s + \beta)^{-1/2} \csch((s + \beta)^{1/2})\}
$$

(14)

$$
g(\tau) = -e^{-\beta \tau} \theta_3(0|i\pi \tau) \quad \text{and} \quad f(\tau) = e^{-\beta \tau} \theta_4(0|i\pi \tau)
$$

(15)

and $\theta_3(0|i\pi \tau)$ and $\theta_4(0|i\pi \tau)$ are the Jacobi theta functions

$$
\theta_3(0|i\pi \tau) = \sum_{n=-\infty}^{\infty} (1/(\pi \tau)^{1/2}) e^{-n^2/\tau}
$$

(16)

and

$$
\theta_4(0|i\pi \tau) = \sum_{n=-\infty}^{\infty} (1/(\pi \tau)^{1/2}) e^{-(n-(1/2))^2/\tau}.
$$

(17)

g(\tau) is a weakly singular kernel of convolution type and is $L^1$ summable on $(0, \infty)$. Substituting (14) into (9) yields the equivalent integrodifferential equation problem

$$
dv(\tau)/d\tau = K_1(1-v(\tau)) \left[ \int_0^\tau g(\tau-s)dv(s)/ds \; ds + C_1 f(\tau) \right] - K_2 v(\tau),
$$

$$
v(0) = 0
$$

(18)
which depends explicitly only on the four dimensionless parameters $K_1$, $C_1$, $K_2$ and $\beta$.

4. Numerical solution. Setting $z(\tau) = dv(\tau)/d\tau$ in (18) yields the following system of nonlinear Volterra integral equations

\begin{align*}
    v(\tau) &= \int_0^\tau z(s) \, ds, \\
    z(\tau) &= K_1(1-v(\tau)) \left[ \int_0^\tau g(\tau-s)z(s) \, ds + C_1f(\tau) \right] - K_2v(\tau)
\end{align*}

for which standard methods [12, 13, 20, 24] are available for establishing the existence of a unique continuous solution $(v(\tau), z(\tau)) = (v(\tau), dv(\tau)/d\tau)$ defined for all $\tau \in [0, \infty)$. While the arguments are somewhat lengthy and will not be presented here, the result follows from the $L^1$ summability of the singular kernel and standard comparison theorems which ensure the uniform boundedness of $v(\tau)$ and $dv(\tau)/d\tau$ based on the sharp estimates $\theta_3(0|\pi \tau) \leq 1 + 1/(\pi \tau)^{1/2}$ and $\theta_4(0|\pi \tau) \leq 1$ for $\theta_3$ and $\theta_4$ given by (16) and (17), respectively [7]. Indeed, it follows immediately from (18) that $v(\tau) < 1$ for all $\tau > 0$ and that the righthand side of the second equation in (19) satisfies a uniform Lipschitz condition. The Green's function $K(\xi; \eta, \tau)$ for the differential operator in (6)--(9) is given by

$$K(\xi; \eta, \tau) = \begin{cases} 
    e^{-\beta\tau}(4\pi\tau)^{-1/2} \sum_{n=-\infty}^{\infty} \{ e^{-(\xi-\eta+2n)^2/4\tau} + e^{-(\xi+\eta-2n)^2/4\tau} \} H(\tau), \\
    e^{-\beta\tau}[1 + \sum_{n=1}^{\infty} 2e^{-(n\pi)^2\tau} \cos(n\pi\xi) \cos(n\pi\eta)]H(\tau), \\
    \tau \leq \tau_0, \\
    \tau > \tau_0,
\end{cases}$$

where $H$ is the usual Heaviside function and $\tau_0$ is a fixed positive constant. The series in $K(\xi; \eta, \tau)$ converges absolutely and uniformly on $0 \leq \xi \leq 1$, $0 < \tau \leq T$, and the unique global solution $u(\xi, \tau)$ of (6)--(9) is given by

$$u(\xi, \tau) = C_1K(\xi; 0, \tau) - \int_0^\tau K(\xi; 1, \tau - s)(dv(s)/ds) \, ds.$$
The Jacobi theta functions are rapidly converging series which permit the implementation of the efficient and stable collocation algorithms for nonlinear Volterra equations described in [5, 6].

5. Statistical analysis. We now consider the estimate of the unknown model parameters and the associated issue of assessing goodness of fit. A single data trace consists of $T$ observations $\{X(t), t = 0, \ldots, T - 1\}$, which are assumed to follow the statistical model

$$X(t) = u(t|\theta) + \varepsilon(t), \quad t = 0, \ldots, T - 1$$

or, equivalently, after applying (5),

$$Y(\tau) = X(\tau)/R = v(\tau|\theta) + \varepsilon(\tau), \quad \tau = 0, \ldots, T - 1$$

where $\theta$ is the vector of unknown parameters to be estimated. The term $\varepsilon(\tau)$ incorporates noise generated by the recording systems, currents resulting from random ion fluxes across the postsynaptic membrane, and errors in the model specification. Because the errors are not entirely model based, they are included in an additive fashion rather than as a model component. To accommodate the observed correlation in the noise, the error series $\{\varepsilon(\tau), \tau = 0, \ldots, T - 1\}$ is assumed to be a zero mean stationary series with power spectrum $f_{\varepsilon\varepsilon}(\lambda)$. Thus (20) is a nonlinear regression model with a transient signal and correlated errors. A direct nonlinear least squares approach to parameter estimation for related models is discussed by Hasan [14], including the estimation of standard errors and the construction of confidence intervals. However, a much simplified analysis is available after transformation to the frequency domain. Let

$$d_Y^{(T)} \left( \frac{2\pi k}{T} \right) = \frac{1}{2\pi} \sum_{\tau=0}^{T-1} Y(\tau) e^{-i2\pi k\tau/T}$$

denote the discrete Fourier transform of $\{Y(\tau), \tau = 0, \ldots, T - 1\}$ at frequency $2\pi k/T$, and define $d_v^{(T)}(2\pi k/T|\theta)$ and $d_\varepsilon^{(T)}(2\pi k/T)$ to be the corresponding transforms of $v$ and $\varepsilon$. Transforming (20) leads to

$$d_Y^{(T)} \left( \frac{2\pi k}{T} \right) = d_v^{(T)} \left( \frac{2\pi k}{T} | \theta \right) + d_\varepsilon^{(T)} \left( \frac{2\pi k}{T} \right)$$
which is again a nonlinear regression model, but in this case the errors \( d_{i}^{(T)}(2\pi k/T), \quad k = 1, \ldots, K \) are asymptotically independent complex normal random variables with mean 0 and variance parameter \( 2\pi Tf_{\varepsilon\varepsilon}(0) \), under general mixing conditions [8]. Therefore (21) is a nonlinear regression model with approximately independent, identically distributed errors and standard statistical methods of testing and estimation are appropriate [26]. In particular, the log likelihood of the transformed data is

\[
\log L = \sum_{k=1}^{K} \frac{|d_{i}^{(T)}(2\pi k/T) - d_{0}^{(T)}(2\pi k/T|\theta)|^2}{4\pi Tf_{\varepsilon\varepsilon}(0)} + JK \log 2.
\]

Maximum likelihood estimates are calculated by maximizing (22) with respect to \( \theta \), and as this maximization is independent of \( f_{\varepsilon\varepsilon}(0) \), the maximum likelihood estimates are equivalent to nonlinear least squares estimates.

After estimating model parameters, it is desirable to have an objective criterion for assessing the quality of fit. Such a criterion is generally related to the sum of squared deviations of the data from the fitted curve, and to be useful, the sampling distribution of the criterion should be understood. One measure of goodness of fit is immediate from the form of the likelihood (22). From the asymptotic distribution of \( d_{i}^{(T)}(2\pi k/T) \), it follows that if \( v(.|\theta) \) is the correct model and all parameters are known then

\[
\sum_{k=1}^{K} \frac{|d_{i}^{(T)}(2\pi k/T) - d_{0}^{(T)}(2\pi k/T|\theta)|^2}{2\pi Tf_{\varepsilon\varepsilon}(0)}
\]

has an approximate \( \chi^2 \) distribution with \( 2K \) degrees of freedom, with the degrees of freedom being reduced by 1 for each independently estimated parameter. It follows from standard statistical distribution theory that if an independent estimate \( \phi(0) \) of \( f_{\varepsilon\varepsilon}(0) \) is available such that \( \phi(0)/f_{\varepsilon\varepsilon}(0) \) has a \( \chi^2 \) distribution with \( M \) degrees of freedom, then

\[
F = \frac{M}{2K - \dim{\theta_{\text{ind}}}} \sum_{k=1}^{K} \frac{|d_{i}^{(T)}(2\pi k/T) - d_{0}^{(T)}(2\pi k/T|\theta_{\text{ind}})|^2}{\phi(0)}
\]

will have an \( F \) distribution with \( 2K - \dim{\theta_{\text{ind}}} \) numerator and \( M \) denominator degrees of freedom, where \( \dim{\theta_{\text{ind}}} \) is the number of
independently estimated parameters of the model. A goodness of fit test for the model $v$ is based on a comparison of (23) with a suitable ordinate of the $F$ distribution. The formal null hypothesis tested is that $v(\cdot|\theta_{\text{ind}})$ is a satisfactory model and the $p$-value is the probability, assuming $v(\cdot|\theta_{\text{ind}})$ to be the correct model, that the test statistic $F$ will be larger than the observed value. Small $p$-values are taken as evidence that the model does not accurately reflect the processes producing the given data.

6. The parameter estimation. The object here is to determine the values of the seven parameters \{\(h, R, c_0, D, \alpha, k_1, k_2\)\} in (1.4) which minimize the discrepancy between the data $X(t)$ and $u_2(t)$ and while the numerical computation has been reduced to the calculation of $\nu(\tau)$ and just four dimensionless parameters $K_1$, $C_1$, $K_2$ and $\beta$, the values for $h$, $R$, $c_0$ and $D$ must still be taken into account when fitting this data. Because of transformation (5), it is equivalent to solve for the parameters \{\(h, R, C_1, D, \beta, K_1, K_2\)\} which minimize the discrepancy between the data $X(t)$ and $\nu(\tau)$. The dimension of the problem can be partially reduced by assuming a value for one of the parameters. Based on anatomical observations, the cleft height $h$ can be fixed at 70 nanometers [30]. For each data set in Figure 1, the likelihood (22) is then maximized over the remaining six parameters, with each adjustment of the individual parameter values in the iteration process requiring the solution of equation (18). Clearly, it would require a prohibitive amount of computer time if it were necessary to solve system (1)-(4) numerically for both $u_1(x, t)$ and $u_2(t)$ at each iteration of each of the six parameter values. The results obtained are illustrated in Figure 2. The three traces in each plot correspond to data, fitted values and residuals (data-fitted). Parameter estimates for the nondimensionalized system are included in Table 1 and can easily be transformed to estimates for the original model using (5). Because the likelihood is nonlinear in the parameters, convergence of an iterative maximization scheme is to a local maximum. The starting iterates for $K_1$ and $K_2$ were based on reported rate constants for the first stage reaction of sensitized receptor and transmitter [9] ($k_1 = 2 \times 10^8$ metre$^3$ sec mole$^{-1}$ and $k_2 = 2 \times 10^5$ sec$^{-1}$). The initial value for $C_1$ was based on a vesicular content of 6000 transmitter molecules [3], and the starting value for $\beta$ derived from an assumed exponential decay of
\( \nu(\tau) \) for large \( \tau \) [22]. Adjustment of the parameter \( D \) requires some care because it determines through (5) the relationship between the time step \( \Delta \tau \) in the numerical solution procedure and the observed time step \( \Delta t = 0.1 \) msec in the data. In the minimization procedure a grid of 25 values of \( D \) ranging from \( 0.5 \times 10^{-10} \) metre\(^2\) sec\(^{-1}\) to \( 12.5 \times 10^{-10} \) metre\(^2\) sec\(^{-1}\) was tested at each combination of the other parameters. The estimates in Table 1 agree well with the range of values \( (2.5 - 5) \times 10^{-10} \) metre\(^2\) sec\(^{-1}\) calculated in [15].

In several cases the magnitude of the residuals is approximately the same in the principle region of the response as in the tail, indicating a satisfactory fit. In calculating the goodness of fit \( F \) statistic, the estimate of \( f_{ee}(0) \) was based on the final 10 msec (100 observations) of the associated data trace. As these observations are approximately 4 msec removed from the response, it seems reasonable to assume that the two are statistically independent. The value \( K = 10 \) was used throughout leading to an \( F \) statistic with 14 numerator and 20 denominator degrees of freedom. The associated \( p \)-values indicate satisfactory fits for data sets I, III and IV, and as is seen in Figure 2, the misfit for data set II is largely in the tail of the response. The magnitudes of the estimated rate constants are reasonably uniform over each of the responses. \( R \), the number of receptors, and \( C_1 \), the relative proportion of released transmitter molecules, are more variable, and differ substantially for trace IV.

8. Discussion. A one-space dimensional reaction-diffusion model for the uptake and release of neural transmitter at the crayfish neuromuscular junction was developed and successfully fitted to four experimental data sets. The transformation of the original system of differential equations (1)-(4) into an equivalent normalized, nonlinear integrodifferential equation problem provided a fast and efficient means of solution which reduced the focus of the problem to those events at the receptor site for which data was available. A statistical procedure for fitting the model to the data was outlined and the goodness of fit in the presence of correlated errors was assessed. The \( p \)-values obtained for the response sets I, III and IV indicate a satisfactory fit while the low \( p \)-value for set II can be explained by a poor fit over the latter and less relevant range of observations of that data set. The magnitudes of the six parameter values computed \( \{ R, C_1, D, \beta, K_1, K_2 \} \) are
reasonably uniform over all four of the response sets.

The boundary integral formulation and statistical analysis developed here can be generalized to higher space dimensions and extended to include multiple nonlinear reaction kinetics at the receptor sites. We believe that improved parameter estimates and even better data fits can be achieved using a three-dimensional model and multiple reversible reaction mechanisms such as those proposed in [23]. Investigations in this direction are currently under way and should lead to a better understanding and more accurate modelling of the uptake and release of transmitter at an invertebrate neuromuscular junction.

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